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Plant resources in the control of the key food pests Andean potato weevils (*Premnotrypes* spp.) and coffee berry borer (*Hypothenemus hampei*): a systematic review

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Abstract. Potato and coffee crops grapple with pronounced challenges posed by pests, notably the Andean potato weevils (*Premnotrypes* spp.) and the coffee berry borer (*Hypothenemus hampei*). These pests inflict significant economic repercussions on both smallholder and commercial farmers by disrupting essential food supply chains. This review delves into the insecticidal potentials of botanical resources against these pests. Notably, extracts and essential oils (EOs) from native flora or commercially available in each affected country have compelled efficacy against *Premnotrypes vorax*, *P. latithorax* and *Hypothenemus hampei*, such as *Schinus molle* L. and *Schinus terebinthifolia* Raddi, respectively. Through rigorous laboratory tests, on-site evaluations, and cost-effectiveness assessments, there emerges a strong advocacy for these botanical solutions. They present not only a sustainable countermeasure to these pests but also a greener alternative to conventional pesticides, thereby potentially reducing the environmental degradation and health concerns synonymous with chemical pesticides. The shift towards natural pesticides, especially those derived from plants like S. molle and S. terebinthifolia, is not only environmentally strategic but also economically prudent, aligning with both market trends and long-term sustainability goals.

Key words: sustainability, pest management, insecticidal activity, essential oils, plant extracts.

INTRODUCTION

Damage to agricultural produce inflicted by pests during growth or storage phases markedly diminishes global food supplies. Such losses intensify food scarcity challenges, particularly in regions already grappling with low human development indices (Savary et al., 2019). The United Nations reports that approximately 13% of food is lost from harvest to retail, with an estimated 17% of global food production going to waste, including 11% in households, 5% in food service, and 2% in retail (United Nations, 2022). Potatoes are grown on both a small and large scale in different countries around the world, especially China and India (Asia), Ukraine and Russia (Europe), Egypt and Algeria (Africa), and the USA and Peru (America), being an essential component of traditional foods and fast food chains worldwide scope (Devaux et al., 2021; FAO, 2022). In addition, potatoes also have nutraceutical potential, with some varieties known for their high antioxidant activity (Gil-Rivero et al., 2019). However, the presence of agricultural pests dramatically reduces the supply of this product, affecting the supply to consumers and harming suppliers. Among the main potato pests are those of the genus *Premnotrypes* (*Curculionidae*), which in turn are coleopterans known as Andean potato weevils (El gorgojo de los Andes), affecting plantations located mainly in Colombia, Venezuela, Bolivia, Ecuador and Peru (Bragard et al., 2020). These beetles feed on the roots of plants, which can lead to a reduction in the productivity and quality of crops. In addition, *Premnotrypes* larvae cause internal damage to potato tubers (*Solanum tuberosum*), making them unsuitable for human or animal consumption (Pérez-Álvarez et al., 2010). These pests are restricted to the highlands of the tropical Andes, so that the adults feed on leaves by making cuts on the leaflets edges, whereas the larvae have subterranean cycle and feed on tubers. Moreover, the biological cycle is univoltine and synchronized with the potato phenology (Loayza-Huillca et al., 2023).

As another example, coffee (*Coffeea arabica*) stands as one of the world's most extensively enjoyed beverages, so its seed has been introduced and well adapted to several continents, in which today there are major coffee grains exporting countries, such as Brazil, Colombia and Honduras (Americas), Vietnam, Indonesia and India (Asia), and Ethiopia and Uganda (Africa), so that the global production reached 168.5 million 60-kilogram bags as of 2021/2022, moving about US$ 88.3 billion (FAO, 2023; Triolo et al., 2023; WPR, 2023). However, local plantations are severely affected by the coleopteran *Hypothenemus hampei* (*Scolytidae*), also known as coffee berry borer (La broca del café), which can cause significant damage to coffee beans, reducing the quality and yield of the harvest (Abewoy, 2022). The damage to the coffee occurs when the female beetle bores a hole into the fruit and eventually into the seed, creating builds galleries for reproduction, followed by larval feeding on the endosperm (Vega et al., 2015; Damon, 2000).

Currently, for the control of these two pests, pesticides of synthetic origin are mostly used, for example, carbofuran and endosulfan, respectively, which, despite being efficient, have already shown the emergence of resistance, in addition to the residual effects on the environment and consumers (Montesdeoca & Gallegos, 2009). Carbofuran is related to human erythrocyte membrane toxicity (Sharma et al., 2012), as well as acetylcholinesterase inhibition caused by occupational exposition (Gammon et al., 2012), and an acute pancreatitis case (Rizos et al., 2004), whereas endosulfan is associated with harmful side effects on humans, causing male infertility due to testicular atrophy and reduced sperm count (Sebastián & Raghavan, 2015). Due to these causes, more sustainable control measures have been advocated, which includes agricultural practices covered by the current term Integrated Pest Management (IPM). Recent articles exemplify the use of these practices to benefit plantations, such as the intercropping practice adhesion (Lepse & Zeipina, 2023), crop rotation for natural pest predators installation (Talgre et al., 2023), the use of pest controlling fungi as *Beauveria* and *Trichoderma* genus (Mahfudz et al., 2019; Novikova et al., 2021), as well as the use of botanical resources with insecticidal activity, such as essential oils (EOs) and plant extracts (Mawussi et al., 2009; Jankevica et al., 2018; Smith & Perfetti, 2020), which can be used directly or through formulations that increase their efficiency, as is the case of nanoemulsions (Echeverria & Albuquerque, 2019). In this sense, it is essential to increase the number of studies with local plants and/or with high availability, including their products and their periodic compilation, to better channel the information. Therefore, this review aims to analyze the various studies on the use of products of plant origin as a sustainable alternative insecticide against the pests *Premnotrypes vorax*, *P. latithorax* and *Hypothenemus hampei*, which are of agricultural importance in several countries.

PLANT RESOURCES WITH INSECTICIDAL ACTIVITY

Against *Premnotrypes* spp

Schinus molle L. is a very distributed species in South America, mainly in the Peruvian territory. Its aqueous extract from leaves (one year old) obtained by hydrosol steam drag method presented insecticidal activity against several stages of *Premnotrypes vorax* development, so 5% concentration caused 50% mortality in adults in 24 h. Moreover, the extract at the concentration of 10% caused 25% inhibition of egg hatching at 24 h. Although the mortality rates did not surpass 50% in adults in 24 hours, using *S. molle* extract could be considered a reasonable alternative for managing this pest (López et al., 2017). Moreover, the EO extracted from *S. molle* leaves was tested by fumigation for its impact on adult and larval mortality, larval hatching inhibition, and antifeedant activity. Results showed that an 8% concentration yielded a 36.67% mortality rate for adults and larvae, 94.78% antifeedant activity, and 80% larval hatching inhibition within 24 hours (Peña Caiza, 2018). Field tests were conducted to assess the EO's commercial potential. Applying a 15% dilution of *S. molle*'s EO yielded promising outcomes, including a reduction in the incidence (30.07%) and severity (17.07%) of white worms, and it led to the growth of plants with larger tuber diameters (4.88 cm) and higher yields (5.35 kg treatment^{-1}). Furthermore, the treatment showed a favorable cost-benefit ratio, with net gains of approximately 0.42 times the amount invested (Cortez Villarroel, 2018).

In another investigation regarding EOs from *Coriaria thymifolia* Humb. & Bonpl. ex Willd. (aerial parts), *Clinopodium tomentosum* (Kunth) Govaerts (leaves and flowers), and *Euphorbia helioscopia* L. (aerial parts) exhibited effective activity against *P. vorax* larvae. They were analyzed at concentrations ranging from 0.5% to 2% throughout 24 to 72 hours. Of particular note were the EOs from *C. tomentosum* and *E. helioscopia* at a 2% concentration, which led to 100% larval mortality within 24 hours. The EO from *C. thymifolia* achieved a similar result in 48 hours (Urquizo Nachimba, 2017).

The Andean species, *Minthostachys spicata* (Benth.) Epling and *Clinopodium bolivianum* (Benth.) Kuntze were tested against *Premnotrypes latithorax* due to the considerable larvicidal activity the EOs derived from their aerial parts exhibited. After 24 hours, the LC_{50} and LC_{90} values for *M. spicata* were found to be 0.040 and 0.095 $\mu L\ cm^{-2}$, respectively, whereas for *C. bolivianum* , these values were 0.088 and 0.248 $\mu L\ cm^{-2}$. The main compounds in *M. spicata* EO were identified as pulegone, isomenthone, and menthone, whereas isomenthone, thymol, and menthone were the predominant compounds in *C. bolivianum* EO (Solís-Quispe et al., 2018).

Additionally, the leaf oils from *C. bolivianum* and *Eucalyptus globulus* Labill. were found to have insecticidal activity against *P. vorax*. These oils were tested in the field at a 6 $mL\ L^{-1}$ concentration to evaluate their effects in natural conditions. The EO from *C. bolivianum* achieved a population reduction of 55.8%, while the EO from *E. globulus* led to a reduction of 36.6%. The damage inflicted by the larvae was found to be 3,988.98 and 4,672.49 $kg\ h^{-1}$ for *C. bolivianum* and *E. globulus*, respectively, so that the treatments were applied at the time of emergence and, later, at the pre-flowering time. The primary constituents of *C. bolivianum* EO were identified as isomenthone, carvacrol, and 1,8-cineole, while the EO from *E. globulus* mainly contained *α*-pinene and 1,8-cineole (eucalyptol) (Rojas Roque, 2007).

Research by Baldeón Ordóñez highlighted the insecticidal effects of EOs from three species of *Tagetes* against *P. vorax*: *T. minuta*, *T. terniflora*, and *T. zypaquirensis*. When tested on the larval stage at concentrations ranging from 0.2% to 1.0%, *T. zypaquirensis* EO displayed the most effective activity after seven days of treatment with a 0.8% concentration, resulting in a 77.77% mortality rate. After 21 days, *T. minuta* and *T. zypaquirensis* EOs resulted in 100% mortality, while *T. terniflora* achieved 88.89% mortality (Baldeón Ordóñez, 2012). Lastly, garlic (*Allium sativum* L.) extract (at 20%) and oil (500, 1,000, and 1,500 ppm) were tested against adult *P. vorax* by applying them to potato foliage over an eight-day period. The oil treatment resulted in a high mortality rate (96% – 100%), while the extract treatment yielded a mortality rate of less than 50% (Romero Alvino, 2021).

Against *Hypothenemus hampei*

Numerous EOs have been found to exhibit substantial insecticidal activity against *Hypothenemus hampei*. For instance, the aerial parts EO from *Ocimum canum* Sims resulted in a lethal dose (LD_{50}) of 320 ppm over 24 hours against adult Coleoptera. The oil's primary constituents were the monoterpenoids (4-terpineol and linalool) (Mawussi et al., 2012). Similarly, an emulsion of *Aeollanthus pubescens* Benth. EO demonstrated an LD_{50} of 220 ppm within 24 hours, with linalool and fenchone as the predominant compounds (Mawussi et al., 2009).

Mendesil et al. (2012) research evaluated the insecticidal activity of eleven EOs derived from various species against *Hypothenemus hampei*, of which six EOs exhibited notable efficacies. *Thymus vulgaris* L., *Ruta chalepensis* L., *Chenopodium ambrosioides* L., and *Cymbopogon nardus* (L.) Rendle induced a mortality rate between 80% and 90% within 24 hours. Meanwhile, *Mentha spicata* L. led to a 60% mortality rate within the same timeframe, whereas *Aloysia* sp. yielded an 87.5% mortality rate within 48 hours. These experiments were conducted by immersing samples (1% in ethanol) in filter paper (Mendesil et al., 2012).

The EO from the leaves of the sandbank plant *Schinus terebinthifolia* Raddi also exhibited insecticidal activity, resulting in 100% mortality in a surface contamination test at a 1% concentration over 24 hours. Using the same concentration and timeframe, the EO demonstrated 97.5% mortality in a direct contact test (Santos et al., 2013). Other Brazilian species such as *Pogostemon cablin* (Blanco) Benth. has also shown promising activity against *Hypothenemus hampei*. Its EO had an LD_{50} and LD_{90} of 19.20 (14.50–24.50) and 141.00 (85.00–318.00) μg mg^{-1} of insect, respectively, after 24 hours. For the EO-based emulsion, these values were 28.60 and 96.90 μg mg^{-1} of insect, respectively. The lethal time was approximately 10 minutes. Tests conducted via direct contact revealed changes in female development, including modifications in reproduction and feeding, increased walking activity, and histopathological alterations in the midgut. The oil's primary compounds were patchoulol, *α*-guaiene, and *α*-bulnesene (Santos et al., 2022).

Eucalyptus resinifera Sm. (Myrtaceae) also demonstrated insecticidal solid activity against adult females, especially in a fumigation test. The leaf EO achieved an LC_{50} of 64.72 μL L^{-1} of air after 24 hours, which was more efficient than the positive control (garlic extract) that presented an LC_{50} above 700 μL L^{-1} of air. The LT_{50} value (lethal time causing 50% mortality) was approximately 4 minutes. The oil's main compounds were 1,8-cineole (59.3%), *p*-cymene (12.9%), and *α*-pinene (9.7%), with a high yield of 2.45%. These three substances, mainly responsible for the biological activity, exhibited synergistic effects. The biological activity of the EO and the compound mixture was statistically equal and far more efficient than the individual compounds or a two-compound mixture (Reyes et al., 2019).

Lastly, the aerial parts EO from *Minthostachys mollis* Griseb. (0.5%) resulted in 100% pest mortality within 18 hours. Its main compounds (menthone, pulegone, and caryophyllene) were also evaluated. Only pulegone achieved 100% mortality in 72 hours, whereas menthone and caryophyllene resulted in 85% and 50% mortality, respectively. Interestingly, the insecticidal activity was higher using the immersion method compared to the spray method, resulting in a mortality rate of only 52% at the identical concentration (Benites et al., 2018; Calle-Álvarez et al., 2004).

Moreover, a formulation comprising neem oil (*Azadirachta indica* A. Juss.) and *d*-limonene was tested against *Hypothenemus hampei* by filter paper contact, direct contact, and aspersion in a coffee plantation. When assessed for insecticidal activity, the formulation, diluted to a 1.12% concentration in water, caused 63.34% and 100% mortality rates after 48 hours, via filter paper contact and direct contact, respectively. In the field, the formulation reduced the *Hypothenemus hampei* population by 62.4% after 60 days (with treatments applied every 20 days), compared to the negative control (Brito et al., 2021).

Additional formulations combining various components have also demonstrated activity against *Hypothenemus hampei*, with coconut (*Cocos nucifera* L.) oil being a common major compound used in concentrations between 2% – 5%. The formulation containing coconut shell wood vinegar and 5% clove (*Syzygium aromaticum* (L.) Merrill & Perry) oil caused mortality rates between 80% – 95%. In contrast, the formulation comprising coconuts shell wood vinegar and 5% citronella (*Cymbopogon nardus* (L.) Rendle) oil achieved mortality rates between 73.34% – 88.33% after five days (Indriati & Puspitasari, 2021).

Other considerations

Our analysis underscores these botanical agents' pivotal role as both efficacious and environmentally friendly alternatives to conventional pest control methods. Notably, many potent plants identified are distributed worldwide, emphasizing biodiversity's untapped potential in reshaping sustainable agriculture's future. Given the increasing global demand for sustainable agricultural practices, there is a burgeoning need for interdisciplinary research integrating botany, biochemistry, and agronomy findings. The highlighted studies also underscore the importance of extending research beyond mere identification to understanding the mechanisms of action and probable active substances, optimizing extraction processes, and developing efficient delivery systems for these botanical agents. Furthermore, recognizing the economic implications, there's an avenue for researchers to delve into the cost-effectiveness and scalability of these natural solutions. With a growing global emphasis on reducing the carbon footprint, the cultivation, production, and application of these indigenous plant-derived products could also bolster local economies, supporting farmers and creating job opportunities in the producer regions.

Numerous botanicals, such as *S. molle*, have demonstrated significant insecticidal activity against *Premnotrypes*, as highlighted in this review. Their abundance and ease of cultivation offer a potential substitute alternative for pest management. While the aqueous extract of *S. molle* may not have demonstrated high adult mortality rates (50% in 24 hours, using a concentration of 5%), it could still be an effective tool in pest management, potentially reducing the pest population by half and somewhat impacting egg hatching. Conversely, the plant's essential oil was more effective in deterring feeding and inhibiting egg hatching, with studies conducted by Cortez Villarroel (2018) further validating its economic effectiveness in potato crops. The more polar extracts of *S. molle* contain high concentrations of tannins, phenolics, and catechins (Cortez Florentino, 2018). These compounds are known to act as digestive enzyme inhibitors in insects due to their protein-binding ability (Hanhineva et al., 2010). Moreover, catechin may disrupt ion transport in larvae because of harm to the anal papillae and outer cuticle layer (Elumalai et al., 2016).

The EO of *S. molle*, abundant in Latin American countries, primarily consists of compounds like α-phellandrene, β-phellandrene, α-pinene, β-pinene, limonene, p-cymene, and myrcene (López de La Cruz & Caso Orihuela, 2015). Monoterpenes, such as α-pinene and p-cymene, are known to disrupt cellular membrane function due to their low polarity, thus breaking down the lipid bilayer (Salakhutdinov et al., 2017). These compounds can also easily penetrate target organisms' respiratory systems (Langsi et al., 2020). Limonene has also been found to inhibit oviposition and egg-hatching of insects due to its repellency and toxicity (Karr & Coats, 1988). Similarly, α-phellandrene harms vital insect organs, including the cuticle, brain, midgut, and fat body (Chaaban et al., 2019) while exhibiting larvicidal activity (Evergetis et al., 2013). Apart from *S. molle*'s essential oil, oils from *C. thymifolia*, *C. tomentosum*, and *E. helioscopia* also exhibited significant efficacy against *Premnotrypes* larvae. Recurring major substances in other active species, such as menthone, isomenthone, and 1,8-cineole, along with pulegone and carvacrol, have displayed high insecticidal or larvicidal properties in their isolated forms (Kumar et al., 2011). Compounds like 1,8-cineole can inhibit acetylcholinesterase activity in insects (Abdelgaleil et al., 2009; Kumar et al., 2011). In their turn, the EO's ketone compounds menthone and pulegone are $GABA_A$-R (Gama amino butyric acid A receptor) negative allosteric modulators, so that this receptor is one of the mains insecticide targets and is related to inhibition of nervous central system (Sánchez-Borzone et al., 2017). Furthermore, it was demonstrated that carvacrol is a potent inhibitor of the housefly [14C]-nicotine acetylcholine receptors binding in a non-competitive pattern, which is probably related to the toxic effect on the insect's nervous system (Tong et al., 2013).

When considering activity against *Hypothenemus hampei*, plants such as *T. vulgaris*, *R. chalepensis*, *C. ambrosioides*, and *C. nardus* displayed high mortality rates within 24 hours. Similar outcomes were observed with the EO from *S. terebinthifolia*, a species with the same genus as *S. molle*, also presenting wide distribution throughout the American continent (Clemente, 2006; Santos et al., 2009). As with *Premnotrypes*, oils rich in 1,8-cineole, pulegone, and menthone exhibited considerable activity against *Hypothenemus hampei*. In addition, other well-known insecticidal extracts and products, including citronella oil, neem (*A. indica*), and limonene, demonstrated activity against *Hypothenemus hampei*. Citronella oil interferes with haemocytes viability and inhibits acetylcholinesterase activity due to its principal monoterpenes (Aftab & Hakeem, 2021; Johnson et al., 2021). Neem affects insect development and reproduction, such as feeding, hormone function in juvenile stages, and molting processes, primarily attributed to its terpenoids (Brahmachari, 2004).

The synergism between specific components of EOs and between EOs from different species is well known (Mossa, 2016). Therefore, studies are suggested to verify the association of two or more EOs that present major compounds with different mechanisms of action. For *Premnotrypes* genus, the interaction between oils such as *S. molle* and *M. spicata* could be evaluated, since both plants are widely found in American countries. In the case of *H. hampei*, associations between *E. resinifera*, *M. mollis* and between them and neem could be interesting if evaluated in assays similar to those presented in this review.

The recurrent losses in key crops like potatoes and coffee due to pests underscore the need for sustainable and effective pest management solutions. Recognizing the dual challenges of economic and environmental sustainability, there is a pronounced move towards natural pesticides. Plants like *S. molle* and *S. terebinthifolia*, distributed in several countries worldwide, have shown potential as sources of organic pesticides (Vicenço et al., 2020). On the other side, synthetic pesticides have been linked to health issues in farm workers and local communities (Ayilara et al., 2023) and despite are effective in the short term, come with recurring costs. Also, the adverse environmental effects of synthetic pesticides, such as soil degradation and water contamination, can be mitigated by natural alternatives reducing the associated long-term environmental remediation costs (Pathak et al., 2022). Furthermore, increased resistance necessitates higher dosages and frequent applications, so adopting botanical alternatives can reduce these health risks, leading to a healthier, more productive workforce, and potentially lower public health expenses. As of 2020, the global pesticide market size was valued at over USD 55 billion (Fridonia Group, 2021). Transitioning to abundant local plants like *S. molle* can be more cost-effective in the long run, reducing dependence on imported synthetic chemicals (Bañuelas, 2018). Moreover, natural pesticides often work through diverse modes of action, reducing the likelihood of pests developing resistance and ensure long-term applicability and economic sustainability (Procópio et al., 2015; Tang et al., 2020). Since biological controllers from plants are obtained from organic producers who cultivate species in a standardized way, the risk of deforestation and environmental contamination is considered inexistent (Gamage et al., 2023). Beside this, the organic food market has been witnessing robust growth. By 2021, organic sales reached nearly USD 57.5 billion globally. Producers can tap into this burgeoning market by utilizing natural insecticides, positioning their products as sustainably grown and attracting premium prices (Waltover, 2023).

Finally, the studies described in this review can serve as a basis for the production of pest biocontrollers that are in accordance with sustainability policy, which includes not only large agricultural exporters but also the market of local agricultural producers, so that they can increase the reliability of its products using low toxicity and efficient resources at an affordable cost to their realities.

CONCLUSION AND PROSPECTS

Different extracts and EOs have displayed insecticidal, larvicidal, or ovicidal activity against *Premnotrypes vorax*, *P. latithorax* and *H. hampei*. The promising outcomes from these studies indicate that these natural substances could potentially be used commercially and comprehensively in the future. The review discussion points to the need for further research into formulation studies for enhancing the efficiency of the active principles. Additionally, this review also serves as a springboard, highlighting the immense promise of botanical insecticides and urging stakeholders to harness the full potential of certain plants, such as *S. molle* and *S. terebinthifolia*. As we move towards a future where sustainability is paramount, the findings presented here act as a beacon, guiding researchers, policymakers, and agriculturalists to work collaboratively in realizing the vision of a greener, pest-free agricultural landscape. Furthermore, commitment is necessary between researchers from different areas involved in the topic, such as chemists, biologists, agronomists, and others, so that studies such as those presented in this review are directed towards a viable application of their respective products.

REFERENCES

Abdelgaleil, S.A., Mohamed, M.I., Badawy, M.E. & El-Arami, S.A. 2009. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. *Journal of Chemical Ecology* **35**(5), 518–525. doi:10.1007/s10886-009-9635-3

Abewoy, D. 2022. Impact of coffee berry borer on global coffee industry: review. *International Journal of Novel Research in Engineering and Science* **9**(1), 1–8.

Aftab, T. & Hakeem, K.R. 2021. *Medicinal and aromatic plants: Healthcare and industrial applications*. Springer, Germany, 783 pp. doi:10.1007/978-3-030-58975-2

Ayilara, M.S., Adeleke, B.S., Akinola, S.A., Fayose, C.A., Adeyemi, U.T., Gbadegesin, L.A., Omole, R.K., Johnson, R.M., Uthman, Q.O. & Babalola, O.O. 2023. Biopesticides as a promising alternative to synthetic pesticides: a case for microbial pesticides, phytopesticides, and nanobiopesticides. *Frontiers in Microbiology* **14**, 1040901. doi:10.3389/fmicb.2023.1040901

Baldeón Ordóñez, X.D.R. 2012. *Actividad insecticida de los aceites esenciales de Tagetes minuta, Tagetes terniflora y Tagetes zypaquirensis sobre Premnotrypes vorax.* Bachelor Thesis, Escuela Superior Politécnica de Chimborazo, Chimborazo, Peru, 112 pp. (in Spanish).

Bañuelas, D.C. 2018. *Allelopathic impacts of Schinus molle (Peruvian pepper tree) on invasive and native plant communities of Southern California*. Master Thesis, California State Polytechnic University, Pomona, USA, 40 pp.

Benites, J., Guerrero-Castilla, A., Salas, F., Martinez, J.L., Jara-Aguilar, R., Venegas-Casanova, E.A., Suarez-Rebaza, L., Guerrero-Hurtado, J. & Buc Calderon, P. 2018. Chemical composition, *in vitro* cytotoxic and antioxidant activities of the essential oil of Peruvian *Minthostachys mollis* Griseb. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **17**(6), 566–574.

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.A. & MacLeod, A. 2020. Pest categorisation of the Andean potato weevil (APW) complex (Coleoptera: Curculionidae). *EFSA Journal* **18**(7), e06176. doi:10.2903/j.efsa.2020.6176

Brahmachari, G. 2004. Neem-an omnipotent plant: a retrospection. *ChemBioChem* **5**(4), 408–421.

Brito, W.A.D., Siquieroli, A.C.S., Andaló, V., Duarte, J.G., Sousa, R.M.F.D., Felisbino, J.K.R.P. & Silva, G.C.D. 2021. Botanical insecticide formulation with neem oil and D-limonene for coffee borer control. *Pesquisa Agropecuária Brasileira* **56**, e02000. doi:10.1590/S1678-3921.pab2021.v56.02000

Calle-Álvarez, J., Espinosa, A.M., Núñez, C.P., Bautista, E. & Pinzón, R. 2004. Actividad insecticida del aceite esencial de *Mintostachys mollis* (HBK) Griseb y sus componentes. *Revista Colombiana de Ciencias Químico Farmacéuticas* **33**(2), 137–144. (in Spanish).

Chaaban, A., Richardi, V.S., Carrer, A.R., Brum, J.S., Cipriano, R.R., Martins, C.E.N., Silva, M.A.N., Deschamps, C. & Molento, M.B. 2019. Insecticide activity of *Curcuma longa* (leaves) essential oil and its major compound α-phellandrene against *Lucilia cuprina* larvae (Diptera: Calliphoridae): histological and ultrastructural biomarkers assessment. *Pesticide Biochemistry and Physiology* **153**, 17–27. doi:10.1016/j.pestbp.2018.10.002

Clemente, A.D. 2006. *Chemical composition and biological activity of pink pepper (Schinus terebinthifolius Raddi) essential oil.* Master Thesis, Universidade Federal de Viçosa, Viçosa, Brazil, 63 pp. (in Portuguese).

Cortez Florentino, A. 2018. *Identification of secondary metabolites in leaves of Schinus molle (molle) from the Huañimba-Cajabamba Caserío*. Bachelor Thesis, Universidad Los Ángeles de Chimbote, Chimbote, Peru, 32 pp. (in Spanish).

Cortez Villarroel, E.M. 2018. *Evaluation of the field phase insecticidal activity of the essential oil from molle (Schinus molle L.), against the white worm (Premnotrypes vorax Hustache) of the potato in the Santa Rosa variety*. Bachelor Thesis, Universidad Técnica de Ambato, Ambato, Peru, 59 pp. (in Spanish).

Damon, A. 2000. A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomology Research* **90**, 453–465. doi:10.1017/S0007485300000584

Devaux, A., Goffart, J.P., Kromann, P., Andrade-Piedra, J., Polar, V. & Hareau, G. 2021. The potato of the future: opportunities and challenges in sustainable agri-food systems. *Potato Research* **64**(4), 681–720. doi:10.1007/s11540-021-09501-4

Echeverria, J. & Albuquerque, R.D.D.G. 2019. Nanoemulsions of essential oils: New tool for control of vector-borne diseases and *in vitro* effects on some parasitic agents. *Medicines* **6**(2), 42. doi:10.3390/medicines6020042

Elumalai, D., Hemavathi, M., Hemalatha, P., Deepaa, C.V. & Kaleena, P.K. 2016. Larvicidal activity of catechin isolated from *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research* **115**(3), 1203–1212. doi:10.1007/s00436-015-4856-6

Evergetis, E., Michaelaki, A. & Haroutounian, S.A. 2013. Exploitation of Apiaceae family essential oils as potent biopesticides and rich source of phellandrenes. *Industrial Crops and Products* **41**, 365–370. doi: 10.1016/j.indcrop.2012.04.058

Food and Agriculture Organization of the United Nations (FAO). 2022. *Crops and Livestock Products*. https://www.fao.org/faostat/en/#data/QCL (Accessed 27 November 2023).

Food and Agriculture Organization of the United Nations (FAO). 2023. *Food Outlook: Biannual report on global food markets.* https://www.fao.org/3/cc3020en/cc3020en.pdf (Accessed 27 November 2023).

Fridonia Group. 2021. *Global pesticides: Industry reports, global.* https://www.freedoniagroup.com/industry-study/global-pesticides-4131.htm (Accessed 22 September 2023).

Gamage, A., Gangahagedara, R., Gamage, J., Jayasinghe, N., Kodikara, N., Suraweera, P. & Merah, O. 2023. Role of organic farming for achieving sustainability in agriculture. *Farming System* **1**(1), 100005. doi:10.1016/j.farsys.2023.100005

Gammon, D.W., Liu, Z. & Becker, J.M. 2012. Carbofuran occupational dermal toxicity, exposure and risk assessment. *Pest Management Science* **68**(3), 362–370. doi:10.1002/ps.2270

Gil-Rivero, A.E., López-Medina, E., Mostacero-León, J. & De La Cruz-Castillo, A.J. 2019. Native potatoes with antioxidant potential, grown in northern Peru. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **18**(3), 289–324.

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H. & Poutanen, K. 2010. Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences* **11**(4), 1365–1402. doi: 10.3390/ijms11041365

Indriati, G. & Puspitasari, M. 2021. The potency of coconut shell wood vinegar and essential oils as botanical insecticides to control *Hypothenemus hampei* (Coleoptera: Curculionidae). *IOP Conference Series: Earth and Environmental Science* **762**, 012057. doi:10.1088/1755-1315/762/1/012057

Jankevica, L., Polis, O., Korica, A., Samsone, I., Laugale, V. & Daugavietis, M. 2018. Environmental risk assessment studies on new plant protection products which have been elaborated from coniferous tree bark. *Agronomy Research* **16**(5), 2056–2067. doi:10.15159/AR.18.189

Johnson, T.O., Ojo, O.A., Ikiriko, S., Ogunkua, J., Akinyemi, G.O., Rotimi, D.E., Oche, J.-R. & Adegboyega, A.E. 2021. Biochemical evaluation and molecular docking assessment of *Cymbopogon citratus* as a natural source of acetylcholine esterase (AChE)- targeting insecticides. *Biochemistry and Biophysics Reports* **28**, 101175.

Karr, L.L. & Coats, J.R. 1988. Insecticidal properties of *d*-limonene. *Journal of Pesticide Science* **13**(2), 287–290.

Kumar, P., Mishra, S., Malik, A. & Satya, S. 2011. Insecticidal properties of *Mentha* species: a review. *Industrial Crops and Products* **34**(1), 802–817. doi:10.1016/j.indcrop.2011.02.019

Langsi, J.D., Nukenine, E.N., Oumarou, K.M., Moktar, H., Fokunang, C.N. & Mbata, G.N. 2020. Evaluation of the insecticidal activities of *α*-pinene and 3-carene on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Insects* **11(**8), 540. doi: 10.3390/insects11080540

Lepse, L. & Zeipiņa, S. 2023. Increasing the sustainability of vegetable crops production by using intercropping. *Agronomy Research* **21(**2), 494–503. doi: 10.15159/ar.23.026

Loayza-Huillca, A., Atauchi, P.J., Peterson, A.T. & Yabar-Landa, A. E. 2023. Potential upslope and latitudinal range shifts for Andean potato weevils *Premnotrypes* species, in the tropical Andes of South America. *Crop Protection* **173**, 106378. doi:10.1016/j.cropro.2023.106378

López De La Cruz, R.P. & Caso Orihuela, N.V. 2015. *Yield and chemical composition of essential oils from Eucalyptus archeri and Schinus molle - Valle del Mantaro.* Bachelor Thesis, Universidad Nacional del Centro de Perú, Huancayo, Peru, 91 pp. (in Spanish).

López, I.C., Rivera, V.E., Yánez, A.W., Artieda, J.R. & Villacres, G.E. 2017. Evaluation of insecticide activity of *Schinus molle* on *Premnotrypes vorax* in potato. *Agronomía Costarricense* **41**(2), 93–101. doi:10.15517/rac.v41i2.31302

Mahfudz, M., Saleh, S., Antara, M., Anshary, A., Bachri, S., Made, U., Hasanah, U. & Rauf, R. A. 2019. Adoption and advantages of eco-friendly technology application at the shallot farming system in Indonesia. *Agronomy Research* **17**(4), 1679–1687. doi: 10.15159/ar.19.188

Mawussi, G., Tounou, A.K., Ayisah, K.D., Vilarem, G., Raynaud, C., Merlina, G. & Sanda, K. 2012. Chemical composition and insecticidal activity of *Ocimum canum* essential oil against coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *Journal of Essential Oil Bearing Plants* **15**(6), 955–963. doi: 10.1080/0972060X.2012.10662599

Mawussi, G., Vilarem, G., Raynaud, C., Merlina, G., Gbongli, A.K., Wegbe, K. & Sanda, K. 2009. Chemical composition and insecticidal activity of Aeollanthus pubescens essential oil against coffee berry borer (*Hypothenemus hampei* Ferrari) (Coleoptera: Scolytidae). *Journal of Essential Oil Bearing Plants* **12**(3), 327–332. doi: 10.1080/0972060X.2009.10643727

Mendesil, E., Tadesse, M. & Negash, M. 2012. Efficacy of plant essential oils against two major insect pests of coffee (coffee berry borer, *Hypothenemus hampei*, and antestia bug, *Antestiopsis intricata*) and maize weevil, *Sitophilus zeamais*. *Archives of Phytopathology and Plant Protection* **45**(3), 366–372. doi: 10.1080/03235408.2011.587286

Montesdeoca, E.S. & Gallegos, A.R. 2009. Effect of insecticides on potato “white worm” (*Premnotrypes vorax* H.) trapping and evaluation of larvae control in potato crop (*Solanum tuberosum* L.). La Esperanza – Carchi. *Agris* 1–14.

Mossa, A.T.H. 2016. Green pesticides: essential oils as biopesticides in insect-pest management. *Journal of Environmental Science and Technology* **9**(5), 354–378. doi: 10.3923/jest.2016.354.378

Novikova, I.I., Minin, V.B., Titova, J.A., Krasnobaeva, I.L., Zaharov, A.M. & Perekopsky, A.N. 2021. Biological effectiveness of a new multifunctional biopesticide in the protection of organic potatoes from diseases. *Agronomy Research* **19**(3), 1617–1626. doi: 10.15159/AR.21.135

Pathak, V.M., Verma, V.K., Rawat, B.S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N. & Cunill, J.M. 2022. Current status of pesticide effects on environment, human health and it’s eco-friendly management as bioremediation: A comprehensive review. *Frontiers in Microbiology* **13**, 962619. doi:10.3389/fmicb.2022.962619.

Peña Caiza, J.M. 2018. *Evaluation of the antifeedant effect and insecticidal activity of the essential oil from molle (Schinus molle L.) against the white worm of the potato (Premnotrypes vorax Hustache).* Bachelor Thesis, Universidad Técnica de Ambato, Ambato, Peru, 59 pp. (in Spanish).

Pérez-Álvarez, R., Arguelles-Cárdenas, J. & Aguilera-Gardamuño, E. 2010. Spatial distribution of *Premnotrypes vorax* (Hustache) (Coleoptera: Curculionidae) in potato crops. *Ciencia y Tecnología Agropecuaria* **11**(1), 11–20 (in Spanish).

Procópio, T.F., Fernandes, K.M., Pontual, E.V., Ximenes, R.M., de Oliveira, A.R.C., Souza, C.D.S. & Napoleão, T.H. 2015. *Schinus terebinthifolius* leaf extract causes midgut damage, interfering with survival and development of *Aedes aegypti* larvae. *PloS One* **10**(5), e0126612. doi:10.1371/journal.pone.0126612

Reyes, E.I., Farias, E.S., Silva, E.M., Filomeno, C.A., Plata, M.A., Picanço, M.C. & Barbosa, L.C. 2019. *Eucalyptus resinifera* essential oils have fumigant and repellent action against *Hypothenemus hampei*. *Crop Protect* **116**, 49–55. doi. 10.1016/j.cropro.2018.09.018

Rizos, E., Liberopoulos, E., Kosta, P., Efremidis, S. & Elisaf, M. 2004. Carbofuran-induced acute pancreatitis. *Journal of the Pancreas* **5**(1), 44–7.

Rojas Roque, J.C. 2007. *Evaluation of natural essential oils and chemical insecticides in the control of Andean weevil (Premnotrypes spp.) in the cultivation of potato (Solanum tuberosum ssp andigena), Omasuyos province*. Bachelor Thesis, Universidad Mayor de San Andrés, Lima, Peru, 79 pp. (in Spanish).

Romero Alvino, Y.J. 2021. *Use of garlic-based biopreparations in the mortality of Andean weevil (Premnotrypes spp.) adults under controlled conditions*. Bachelor Thesis, Universidad Nacional del Centro de Perú, El Mantaro, Peru, 57 pp. (in Spanish).

Salakhutdinov, N.F., Volcho, K.P. & Yarovaya, O.I. 2017. Monoterpenes as a renewable source of biologically active compounds. *Pure and Applied Chemistry* **89(**8), 1105–1117. doi:10.1515/pac-2017-0109

Sánchez-Borzone, M.E., Marin, L.D., & García, D.A. 2017. Effects of insecticidal ketones present in mint plants on GABAA receptor from mammalian neurons. *Pharmacognosy Magazine* **13**(49), 114. doi: 10.4103/0973-1296.197638

Santos, A.A., Farder-Gomes, C.F., Ribeiro, A.V., Costa, T.L., França, J.C.O., Bacci, L., Demuner, A.J., Serrão, J.E. & Picanço, M.C. 2022. Lethal and sublethal effects of an emulsion based on *Pogostemon cablin* (Lamiaceae) essential oil on the coffee berry borer, *Hypothenemus hampei*. *Environmental Science and Pollution Research International* **29**(30), 45763–45773. doi:10.1007/s11356-022-19183-1

Santos, M.R.A., Lima, R.A., Silva, A.G., Lima, D.K.S., Sallet, L.A.P., Teixeira, C.A.D. & Facundo, V.A. 2013. Chemical composition and insecticidal activity of the essential oil of *Schinus terebinthifolius* Raddi (Anacardiaceae) on coffee berry borer (*Hypothenemus hampei*) Ferrari. *Revista Brasileira de Plantas Medicinais* **15**(4 Suppl. 1), 757–762. doi:10.1590/S1516-05722013000500017

Santos, A.C.A.D., Rossato, M., Agostini, F., Serafini, L.A., Santos, P.L.D., Molon, R. & Moyna, P. 2009. Chemical composition of the essential oils from leaves and fruits of *Schinus molle* L. and *Schinus terebinthifolius* Raddi from Southern Brazil. *Journal of Essential Oil Bearing Plants* **12**(1), 16–25. doi:10.1080/0972060X.2009.10643686

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N. & Nelson, A. 2019. The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution* **3**(3), 430–439. doi:10.1038/s41559-018-0793-y

Sebastián, R. & Raghavan, S.C. 2015. Exposure to Endosulfan can result in male infertility due to testicular atrophy and reduced sperm count. *Cell Death & Disease* **1**(1), 1–10. doi:10.1038/cddiscovery.2015.20

Sharma, R.K., Jaiswal, S.K., Siddiqi, N.J. & Sharma, B. 2012. Effect of carbofuran on some biochemical indices of human erythrocytes *in vitro*. *Cellular and Molecular Biology* **58**(1), 103–109.

Smith, C.J. & Perfetti, T.A. 2020. A comparison of the persistence, toxicity, and exposure to high-volume natural plant-derived and synthetic pesticides. *Toxicology Research and Application* **4**. doi:10.1177/2397847320940561

Solís-Quispe, L., Pino, J.A., Tomaylla-Cruz, C., Solís Quispe, J.A., Aragón-Alencastre, L.J. & Solís Quispe, A. 2018. Chemical composition and larvicidal activity of the essential oils from *Minthostachys spicata* (Benth) Epling and *Clinopodium bolivianum* (Benth) Kuntze against *Premnotrypes latithorax* Pierce. *American Journal of Essential Oils and Natural Products* **6**(2), 22–28.

Talgre, L., Eremeev, V., Mäeorg, E. & Luik, A. 2023. Diversified cropping systems for promoting the beneficial insects-ground beetles (Coleoptera: Carabidae). *Agronomy Research* **21**(2), 592–597. doi: 10.15159/ar.23.027

Tang, H., Porras, G., Brown, M.M., Chassagne, F., Lyles, J.T., Bacsa, J., Horswill, A.R. & Quave, C.L. 2020. Triterpenoid acids isolated from *Schinus terebinthifolia* fruits reduce *Staphylococcus aureus* virulence and abate dermonecrosis. *Scientific reports* **10**(1), 8046. doi:10.1038/s41598-020-65080-3

Tong, F., Gross, A.D., Dolan, M.C. & Coats, J.R. 2013. The phenolic monoterpenoid carvacrol inhibits the binding of nicotine to the housefly nicotinic acetylcholine receptor. *Pest Management Science* **69**(7), 775–780. doi:10.1002/ps.3443

Triolo, F.A., Figueiredo, B., Martin, D.M. & Farrelly, F. 2023. Coffee: a global marketplace icon. *Consumption Markets & Culture* 1–10. doi: 10.1080/10253866.2023.2206129

United Nations. 2022. *Reducing food loss and waste: Taking action to transform food systems*. https://www.un.org/en/observances/end-food-waste-day (Accessed 16 October 2023).

Urquizo Nachimba, W.F. 2017. *Effect of the essential oil from shanshi (Coriaria thymifolia), tiglan (Clinopodium tomentosum) and sinverguenza (Euphorbia heliescola L), on the white gusano of the potato (Premnotrypes vorax Hustache)*. Bachelor Thesis, Universidad Técnica de Ambato, Ambato, Peru, 42 pp. (in Spanish).

Vega, F.E., Infante, F. & Johnson, A.J. 2015. The genus Hypothenemus, with emphasis on *H. hampei*, the coffee berry borer. In Vega, F.E. & Hofstetter, R.W. (eds), *Bark Beetles: Biology and Ecology of Native and Invasive Species*. Academic Press, USA, 427–494.

Vicenço, C.B., Silvestre, W.P., Silva, V.T., Menegol, I.V., Hahn, R.C., Lima, T.S., Agostini, F. & Pauletti, G.F. 2020. Bioactivity of *Schinus molle* L. and *Schinus terebinthifolia* Raddi. essential oils on *Anticarsia gemmatalis* (Hübner 1818). *Brazilian Archives of Biology and Technology* **63**, e20200111. doi:10.1590/1678-4324-2020200111

Waltover, S. 2023. *Organic food sales growth outpaces rest of grocery industry*. https://www.businessnewsdaily.com/2404-organic-industry-healthy-growth.html (Accessed 22 September 2023).

World Population Review (WPR). 2023. Coffee Producing Countries 2023. https://worldpopulationreview.com/country-rankings/coffee-producing-countries (Accessed 27 November 2023).

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In vitro Assessment of the Food Preference and Toxicity of Five Insecticides against The Land Snail *Eobania vermiculata* (Gastropoda; Helicidae)

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Abstract. The land snail *Eobania vermiculata* is one of the most cosmopolitan and harmful agricultural pests, causing economic devastation to many crops. For this purpose, the choice and non-choice methods was used to determine the palatability of certain plants for *E. vermiculata*. Moreover, the vulnerability of the snail was assessed via its exposure to five common insecticides (spirotetramat, sulfoxaflor, chlorantraniliprole, spinetoram and fipronil) using leaf-dipping technique under laboratory conditions. The median lethal dose was determined for each compound while biomarkers, such as enzymatic activity levels of AST, ALT, total protein TP, and lipid TL were used to evaluate sublethal effects. The findings of the no-choice feeding trial revealed that *E. vermiculata* significantly consumed a higher amount of *Lactuca scariola var. sativa* leaves compared to other tested plants. *Cichorium cicorea* leaves were found to be the least preferred by *E. vermiculata*, with an average of 1.71 g after 5 days. On the other hand, the results of the free choice feeding trial revealed that *L. scariola var. sativa* and *Brassica oleracea* leaves were the most frequently consumed by *E. vermiculata*. Conversely, *E. vermiculata* exhibited the lowest preference towards *Brassica rapa* leaves. The results of the molluscicidal activity indicated that the mortality rate is dose-dependent. After one month of exposure to a concentration of 1,000 ppm per 100 mL, chlorantraniliprole caused 46.4% mortality, followed by sulfoxaflor and fipronil, which exhibited equal mortality values of 42.9%. The latter insecticides revealed LC_{50} of 1,010.5, 2,501.9, and 1,444.7 ppm per 100 mL against *E. vermiculata*, respectively. Nevertheless, spinetoram and spirotetramat caused a lower mortality rate for *E. vermiculata*. The biochemical analysis results showed that the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins (LP), and the lipid profile of *E. vermiculata* have increased by 50% in response to the insecticides. Compared to the control and other compounds, spirotetramat increased total cholesterol by 33 mg dL^{-1}. The activity of ALT, AST, and triglycerides decreased after the application of spinetoram and fipronil treatment, with values reaching 13 u L^{-1}, 32 u L^{-1}, and 4 mg dL^{-1} of TL, respectively. However, no substantial effects of insecticides were observed on TP, Total cholesterol, LDH, or LP levels after the exposure period. The study's findings indicate that chlorantraniliprole, a novel insecticide group, could be a promising approach for controlling the land snail *E. vermiculata*. Unlike other, more hazardous insecticides, chlorantraniliprole has not previously been used to control snails. Furthermore, it appears to be safe for non-target organisms and mammals, making it an excellent choice for snail management.

Key words: *Eobania vermiculata*, food preferences, insecticides, mortality, biochemical analysis.

INTRODUCTION

The land snail, *Eobania vermiculata* (Műller, 1774), is a significant representative of the Helicidae land snails and has successfully expanded its range worldwide thanks to human activities. Its adaptability and resilience make it a fascinating subject of study for those interested in the impact of human influence on the environment. The species is native to the Mediterranean area but has found its way to many other countries. Land mollusks are important members of gastropods, and they cause significant damage to vegetables, field crops, ornamental plants, fruit trees, and ecosystems. Due to their high reproductive potential, nocturnal activity, and feeding habits, several species of snails and slugs are considered pests in agroecosystems worldwide, resulting in crop damage and economic losses. *Eobania vermiculata* is a well-known circum-Mediterranean land snail with a cosmopolitan distribution, affecting various crops, vegetables, orchards, and ornamental plants (Carlsson et al., 2004; Ismail, 2004; Barbara & Schembri, 2008; Cilia, 2011; Desouky & Busais 2012; Puizina et al., 2013; Colonese et al., 2014; Mienis et al., 2016; Ronsmans & Van den Neucker, 2016; Routray & De, 2016; Ali, 2017; Chellat et al., 2018; Abd El-Atti et al., 2020; Camilleri et al., 2021; Cheriti et al., 2021; Das et al., 2020; Dedov et al., 2022; Bayoumi et al., 2023; Racevičiūtė-Stupelienė et al., 2023 and Bronne & Delcourt, 2024).

Despite the generalist nature of the majority of land snails, they are capable of exhibiting temporal and spatial variation in their dietary preferences (Ampuero et al., 2023). Several studies have been conducted on the dietary preferences and patterns of various land snails. For example, carrots, lettuce, and cucumber were the most preferred and consumed by land snails such as, *Thaba pisana*, and *E. vermiculata* (Keshta et al., 2006). In addition, Shoieb (2008) demonstrated that ornamental plants in public gardens in Port Said, Egypt, have been infested with land snails, specifically *E. vermiculata*. Moreover, Al-Akraa et al. (2010) stated that *Peganum* and *Hisbicus* leaves were the most consumed by *Eobania vermiculata* over the experimental course of five days. Furthermore, Mohamed (2016) demonstrated that cabbage and lettuce were more preferable to land snails such as *Monacha cartusiana* and *Helicella vestalis* in comparison to other plants. Furthermore, Valarmathi (2017) demonstrated that among the twelve food materials presented, land snails *Cryptozona bistrialis* exhibited a strong preference for carrots, cabbage, cucumber, and chow chow. Additionally, Soha et al. (2020) displayed that *E. vermiculata* and *Monacha obstructa* preferred untreated lettuce leaves compared to treated plant leaves. Also, Bashandy & Awwad (2022) indicated that in the non-choice method, the leaves of cabbage and lettuce had the most palatability.

However, in the free-choice methods, land snails, *E. vermiculata* showed a preference for berseem leaves as their preferred food.

The use of synthetic pesticides or specific molluscicides is the most common method for regulating terrestrial gastropods (Radwan et al., 1992; El-Wakil & Attia, 1999; Moran et al., 2004; El-Shahaat et al., 2005; El-Shahaat et al., 2009; Eshra, 2014). Several studies have also been conducted to detect the molluscicidal effects of common molluscicides such as metaldehyde and methiocarb, which are applied as baits (Miller et al., 1988; Radwan, 1993). Unfortunately, because these compounds are hazardous, they cause toxicity in non-target organisms as well as harmful long-term effects on the ecosystem (Kenko & Ngameni, 2022; Kenko et al., 2022; Kenko Nkontcheu et al., 2023; Kenko et al., 2024).

Safe pesticides and molluscicides with distinct modes of action are critical. For instance, spirotetramat (Movento 10% SC), a spirocyclic tetramic acid derivative, is a fully systemic insecticide for sucking pests (Bretschneider et al., 2007). Spirotetramat is a lipid biosynthesis inhibitor (Nauen et al., 2006). Its mode of action leads to a reduction in the fecundity, fertility, and insect populations at all stages of pests. Furthermore, since its approval as an insecticide in 2010, sulfoxaflor (Transform 50%WG) has been used to safeguard a range of crops from a number of insect pests (Rossaro et al., 2018). Babcock et al. (2011) assert that sulfoxaflor's high efficacy and minimal cross-resistance with other pesticides make it an excellent substitute for some neonicotinoids, if not an improvement over them. Many authors have reevaluated the threats connected with sulfoxaflor to people, the environment, and non-target animals. They have stated that sulfoxaflor poses a major threat to honeybees and bumblebees but is only mildly hazardous to humans, birds, and most aquatic organisms (Bishop, 2015; Pan et al., 2017; Centner et al., 2018; Chakrabarti et al., 2020; Al Naggar & Paxton, 2021; Li et al., 2021; Bellisai et al., 2022; El-Din et al., 2022; Mundy-Heisz et al., 2022). With a license to control insects in a range of crops, the active ingredient of Coragen 20% SC, a new insecticide, is chlorantraniliprole (Kar et al., 2013; Bacca et al., 2021). According to Noha & Meligi (2019), Coragen, an insecticide, causes biochemical and histological changes in some important organs in male albino rats.

Spinetoram (Radiant 12% SC) is an insecticide belonging to the spinosyn category, known for its extended residual activity. It acts swiftly on the nervous system of insects through both contact and ingestion (Thompson et al., 2000). Spinosyns, a novel class of insecticides, exhibit high efficacy with minimal environmental impact. Their mode of action involves the breakdown of nicotinic acetylcholine receptors (Kirst, 2010). Spinetoram, which is chemically related to spinosad, a safe pesticide used in organic farming, effectively targets insects at low application rates while sparing beneficial insects. Its mechanism of action involves consistent stimulation of insect nicotinic acetylcholine receptors (Anonymous, 2014).

Fipronil is one of the highly effective pesticides that fall under the category of a new type of pesticide called phenylpyrazole pesticides. It functions by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels in the target organism (Raymond et al., 2005). Under controlled laboratory conditions, the toxicity of fipronil against the land snail *E. vermiculata* was found to be greater than its toxicity against the land snails *Theba pisana* and *Helicella vestalis*, according to a study by Eshra et al. (2016). The treatment had the highest impact on the proportion of eggs hatched in adult

E. vermiculata snails when compared to snails that were not treated (Hussein & Sabry, 2019).

Considering the wide range of effects these chemicals have on pest control and the limited information available on their impact on sub-lethal biochemical markers in land snails, such as *E. vermiculata*, and the consumption of plants by these snails, we can conclude that there is a lack of research in these specific areas. Therefore, this study aimed to investigate the feeding behaviour of the chocolate-band snail, *E. vermiculata,* and its control under laboratory conditions. The study will first evaluate the palatability of certain plant leaves to the chocolate-band snail, *E. vermiculata*, using two methods: free-choice and no-choice. Second, evaluate the molluscicidal efficacy of five insecticides belonging to the Tetramic acid, Sulfoximine, Anthranilic diamide, Spinosad, and Phenylpyrazole classes against the land snail *E. vermiculata*. Furthermore, examine the impact of low concentrations of these pesticides, when sprayed on the leaves and dipped in, on the functioning of five crucial enzymes in these terrestrial snails.

MATERIALS AND METHODS

Chemicals

The insecticides used in this study (Table 1), were provided by the Central Agricultural Pesticide Laboratory (CAPL) in Dokii, Egypt.

Table 1. Displays the insecticides investigated in this study against land snails *E. vermiculata*

Trade name	Active ingredient	Chemical group	Rate of application	Manufacturer / Year
Movento 10% SC	Spirotetramat	Tetramic acid	40 mL per 100 L	Bayer AG, German, 2020
Transform 50% WG	Sulfoxaflor	Sulfoximine	125 g per 200 L	Dow AgroSciences, UK, 2021
Coragen 20% SC	Chlorantraniliprole	Anthranilic diamide	60 mL per 200 L	FMC Corporation, France, 2020
Radiant 12% SC	Spinetoram	Spinosad	10, 15 and 20 mL per feddan	Corteva Agriscience, USA, 2021
Fipronil 5%	Fipronil	Phenylpyrazole	500 mL per100 L	MAC-GmbH, Germany, 2021

Collection of Land Snails *E. vermiculata*

Adult snails of *E. vermiculata* (Müller, 1774) (20.4 ± 1.1 mm in shell diameter and 2.4 ± 0.1 g in body weight) were hand-collected from the field of Citrus lemon trees in March 2022 from Beheira Governorate (30º30´43.9″N, 30º17´38.9″E) (Bashandy, 2018; Hussein & Sabry, 2019). The snails were then placed in transparent sacks and transferred to the research center of the Zoology Agricultural and Nematology Department, Faculty of Agriculture, Al-Azhar University. After being washed with freshwater, the collected snails were kept in a glass cage (60×40×30 cm) with 100 individuals per cage. The cage was filled with a mixture of sterilized clay and sand in a 1:1 ratio. The snails were fed lettuce leaves for 15 days under laboratory conditions at RH 60% ± 5 and a temperature of 22 ± 2 °C (Shetaia, 2005; Bashandy & Raddy, 2021).

Assessment of Feed Preferences of Land Snails *E. vermiculata*

No-choice Feeding for Land Snail *E. vermiculata*

Three plastic cups (10×14 cm) filled with blended soil to a depth of 5cm and with 60% moisture were used for the animals. Three replicates were used for each treatment. Each cup contained 10 healthy adult snails. A Known weight of plant leaves (Table 2) was given daily to *E. vermiculata*. The consumption of foodstuff by *E. vermiculata* was accounted daily for five days following the method previously published (Al-Akraa et al., 2010; Mohamed, 2016; Bashandy, 2018).

Free Choice Feeding for Land Snail *E. vermiculata*

Cabbage, Cos lettuce, Komatsuna, Chicory, Milky tassel, and London rocket leaves were used (Table 1). Three wooden boxes (50×40×20 cm) each were filled with mixed soil to a depth of 10 cm and maintained at 60% moisture content. Each box included 30 animals, which were placed in the center, and known weights of fresh leaf samples from each plant were placed around the snails on the box's sides. To eliminate partiality for a certain place, the food ingredients and their sides were changed regularly (Mohamed, Ghade, 2016). After being weighed, the leaf samples were replaced every day. For five days, the adjusted weight losses caused by *E. vermiculata* feeding were estimated.

Table 2. The plants used for feed preferences of land snails *E. vermiculata*

Family	Common name	Scientific name
Asteraceae	Chicory	*Cichorium cicorea Dumort.*
	Milky tassel	*Sonchus ciliatus* Lam.
	Cos lettuce	*Lactuca scariola* var. *sativa*
Brassicaceae	London rocket	*Arabis charbonnelii*
	Komatsuna	*Brassica rapa*
	Cabbage	*Brassica oleracea*

Determination of molluscicidal activity of five insecticides against land snail *E. vermiculata*

Pesticides evaluation experiments

Five insecticides were evaluated for their toxicity against adults of land snails (*E. vermiculata*) under laboratory conditions at 22 ± 2 °C and RH 60 ± 5 (Table 1). A series of seven concentrations of each compound (5, 10, 20, 50, 100, 300, and 1,000 ppm per 100 mL) was prepared by mixing an appropriate amount of each compound with one drop of Tween 80 and one drop of DMSO until the compounds became completely soluble. This was followed by the addition of the appropriate volume of water to create a homogeneous suspension (Gad et al., 2023). An appropriate size of lettuce leaf was dipped in each concentration and left for a minute, then taken out and left to dry, then served to snails. Ten snails were placed in a plastic box (15×10 cm) and supplied with a disc of lettuce. For each treatment, three replicates of ten land snails each were used. The boxes were covered with a muslin cloth and secured with a rubber band to prevent the snails from escaping. The remaining three replicates were provided with lettuce leaves immersed in water as a control). The plastic boxes were checked daily for a month. When needed, treated lettuce leaves were changed, and untreated animals were sprayed with only water to provide appropriate humidity. A lack of contraction indicated death, and dead snails were recorded and removed immediately.

Biochemical Analyses

The process of collecting and preparing tissues for sub-lethal biochemical tests

To investigate the effects of the five mentioned insecticides on *E. vermiculata* snails, their biochemical changes were examined by exposing them to a sub-lethal level of LC_{50}, as listed in Table 5, for seven days in a separate experiment. Each treatment involved triplicates, with each replicate consisting of ten snails. After seven days of treatment, the soft tissues of *E. vermiculata* snails were extracted from their shell and homogenized for one minute in 10 volumes (W/V) of 0.1 M phosphate buffer at PH 7.4 using a glass homogenizer. The homogenates were then centrifuged for 20 minutes at 1,000 xg in a cooling centrifuge (5417R) set to 4 °C. The supernatants were kept in a -20 °C freezer until they were used to determine the activities of alanine aminotransaminase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH), total protein (TP), and total lipid (TL). The supernatant was employed as an enzyme substrate (Laila & Genena, 2011; Bislimi et al., 2013; Banaee et al., 2019).

Enzymatic and biochemical measurements

Enzymatic measurements

The activities of AST and ALT were determined according to Reitman and Frankel's method (1957). The enzyme activities were reported in Units L^{-1}. Lactate dehydrogenase (LDH) was determined using the colorimetric method described by Cabaud & Wroblewski (1958).

Biochemical measurements

The protein content (TP) was determined using Bradford's technique (1976). Total lipids (TL) were estimated according to Knight et al. (1972). All the biochemical measurements used in this study were based on the methodology established by Radwan et al. (2008), specifically for snails.

Statistical analysis

The data were subjected to the One-Sample Kolmogorov-Smirnov Test using the SPSS program (version 20). The corrected mortality for land snails due to lethal toxicity was computed using the Abbott formula (1925), using Ldp line computer software (Bakr, 2005). The sub-lethal of the tested compound's LC_{50} values (LC_{25}), 95% confidence limits, and slope for the interval were calculated using Probit analysis as described by Finney (1971). Data were expressed as the mean ± standard error (SE). The data on food palatability and biochemical were analyzed by one-way ANOVA (Duncan´s test) at a significance level of $p \leq 0.05$ using CoStat computer software, version 2.6 CoStat program (2002).

RESULTS

Feed preferences of land snails *E. vermiculata*

No-choice feeding for land snail *E. vermiculata*

Data in Table 3 indicated that leaves of Cos lettuce were the most preferred by *E. vermiculata*, with an average consumption of 4.8 ± 0.1 g. Furthermore, cabbage, komatsuna, and milky tassel were also preferred by *E. vermiculata* with consumption averages of 4.0 ± 0.1, 3.3 ± 0.1, and 3.5 ± 0.1 g after 5 days, respectively. On the contrary, London rocket was moderately palatable with an average consumption of 2.5 ± 0.1 g, and chicory had the lowest palatable with an average consumption of 1.7 ± 0.0 g.

Table 3. Shows the *in vitro* no-choice consuming rate of the land snail *E. vermiculata* to fresh foliage of six plant species

Plants	Consuming rate/day					Mean ± SE
	1^{st}	2^{nd}	3^{rd}	4^{Th}	5^{Th}	
Cabbage (*Brassica oleracea*)	3.9	3.9	4.5	3.8	3.9	4.0 ± 0.1^{b}
Cos lettuce (*Lactuca sativa*)	4.7	4.9	4.6	4.9	4.9	4.8 ± 0.1^{a}
Komatsuna (*Brassica rapa*)	3.1	3.3	3.4	3.3	3.2	3.3 ± 0.1^{c}
Chicory (*Cichorium cicorea*)	1.7	1.8	1.7	1.7	1.7	1.7 ± 0.0^{e}
Milky tassel (*Sonchus ciliates*)	4.0	3.6	3.3	3.3	3.3	3.5 ± 0.1^{c}
London rocket (*Arabis charbonnelii*)	2.7	2.8	2.4	2.4	2.4	2.5 ± 0.1^{d}

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test at 0.05 = 0.26, ± SE = standard error ($n = 5$), and (30 land snails in three replicates).

Free choice feeding for land snail *E. vermiculata*

According to the statistically analyzed results of the data in Table 4, there was a significant difference ($p < 0.05$) in the average weight consumption of land snails *E. vermiculata*. Cos lettuce and cabbage were the most prevalently consumed, with values reaching 3.8 ± 0.2 and 3.4 ± 0.2 g, respectively. Chicory also exhibited a similar consumption rate, with an average consumption of 3.6 ± 0.1 g. Furthermore, there were no significant differences ($p < 0.05$) in the average intake of weight leaves between milky tassel and London rocket for land snails. On the other hand, komatsuna was the lowest consumed by snails during the 5-day experimental course, with an average amount of 1.6 ± 0.1 g.

Table 4. Shows the *in vitro* free-choice consuming rate of the land snail *E. vermiculata* on fresh foliage from six plant species

Plants	Consuming rate/day					Mean ± SE
	1^{st}	2^{nd}	3^{rd}	4^{Th}	5^{Th}	
Cabbage (*Brassica oleracea*)	3.3	3.3	3.4	4.0	3.0	3.4 ± 0.2^{b}
Cos lettuce (*Lactuca sativa*)	4.7	3.5	3.8	3.7	3.5	3.8 ± 0.2^{a}
Komatsuna (*Brassica rapa*)	1.7	1.5	1.5	1.9	1.5	1.6 ± 0.1^{d}
Chicory (*Cichorium cicorea*)	3.5	3.5	3.7	3.8	3.7	3.6 ± 0.1^{ab}
Milky tassel (*Sonchus ciliates*)	2.4	2.4	2.8	2.3	2.4	2.5 ± 0.1^{c}
London rocket (*Arabis charbonnelii*)	2.1	2.9	2.3	2.34	2.6	2.5 ± 0.1^{c}

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test at 0.05 = 0.39, ± SE = standard error ($n = 5$), and (30 land snails in three replicates).

Determination of molluscicidal activity of the five insecticides

The molluscicidal activity of five insecticides, when applied as lettuce leaf poison bait against *E. vermiculata*, is shown in Table 5. The data indicates that the mortality percentage increases with concentration and exposure period. From Table 5 and Figs 1 and 2, it was evident that the tested pesticides spirotetramat, sulfoxaflor, chlorantraniliprole, and spinetoram showed no lethal effect against the land snail *E. vermiculata* in the first six days of the trial. However, after three days, sulfoxaflor and fipronil at a concentration of 5 ppm exhibited mortality percentages of 7.1% and 10.7%, respectively, against *E. vermiculata*. Meanwhile, other insecticides displayed a low mortality rate at the same concentration. One week later, the mortality percentage increased gradually for the tested insecticides. After one month of exposure to 1,000 ppm, chlorantraniliprole significantly overwhelmed other pesticides and showed a mortality of 46.4% with an LC_{50} 1,010.5 ppm per 100 L. However, as the time elapsed to thirty days, sulfoxaflor and fipronil showed a gradual increase in the cumulative mortality percentage and exhibited a mortality percentage of 42.9% against *E. vermiculata* with lethal concentrations of 2,501.9 and 1,444.7 ppm per 100 mL, respectively.

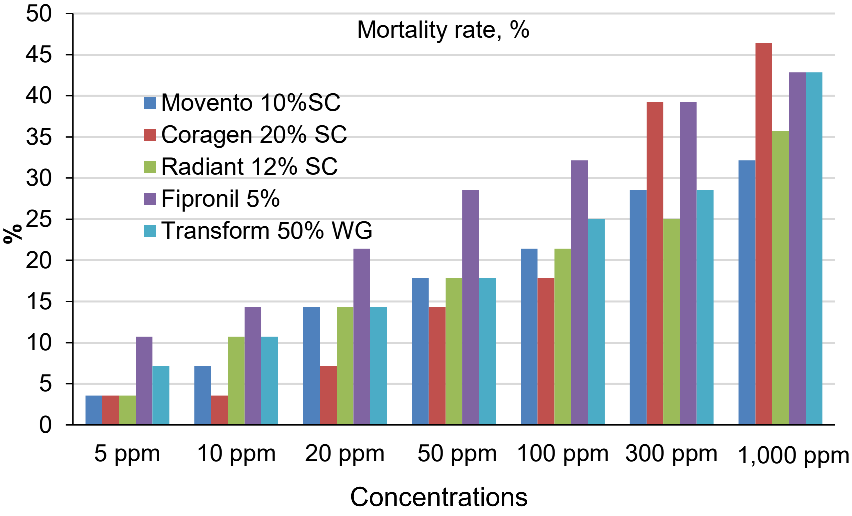

Figure 1. Mortality percentage of land snail, *E. vermiculata* by five insecticides after one month under laboratory conditions.

Table 5. Molluscicidal activity (LC_{50} and LC_{25}) of five insecticides against *E. vermiculata* under laboratory conditions

Insecticides	LC_{50}	Con. L.		Index	R	Slope ± SE	LC_{25}	Con. L.	
		1	2					1	2
Chlorantraniliprole	1,010	465	4,211	100	1	0.83 ± 0.15	156.7	85.9	313.3
Fipronil	1,444	389	65,185	69.9	1.4	0.46 ± 0.13	47.9	12.7	129.3
Sulfoxaflor	2,502	649	96,843	40.4	2.5	0.53 ± 0.14	130.3	53.0	432.6
Spirotetramat	4,857	979	654,834.47	20.8	4.8	0.51 ± 0.14	228.0	89.7	127.2
Spinetoram	5,244	985	1,147,465	19.3	5.2	0.49 ± 0.19	214.1	81.5	1,292.8

The index compared with Chlorantraniliprole; Resistance Ratio (RR) compared with Chlorantraniliprole; Con. L. (Confidence limit), (1) Lower limit, (2) Upper limit, SE (standard error).

Furthermore, at the end of the trial, spinetoram and spirotetramat exhibited lower mortality of 35.71% and 32.14%, respectively, with LC_{50} values of 5,244.45 and 4,857.20 ppm per 100 mL. No mortality was recorded during the experiment for snails fed on untreated control lettuce leaves. Therefore, the investigated insecticides can be arranged in descending order according to their mortality percentages as follows: chlorantraniliprole > sulfoxaflor > fipronil > spinetoram > spirotetramat.

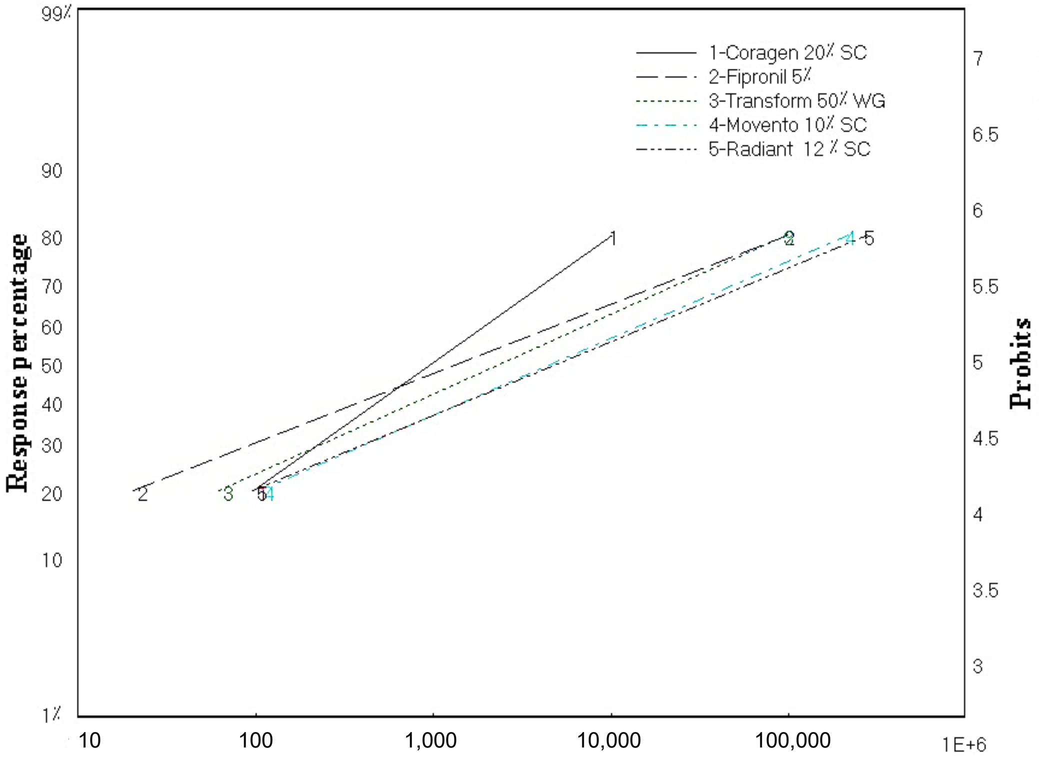

Figure 2. Probit regression lines representing the effect of insecticides leaf dipping against terrestrial snail, *E. vermiculata*.

Biochemical evaluation

The data in Table 6 show the enzymatic activity levels of AST, ALT, total protein TP, and lipid TL in the land snail *E. vermiculata* in response to five insecticides after seven days of exposure. When compared to other pesticides and controls, data showed that fipronil and sulfoxaflor increased ALT activity, with values reaching 120 u L^{-1} and 108 u L^{-1}, respectively. The *E. vermiculata* treated with spinetoram exhibited the lowest ALT value of 13 u L^{-1}. On the other hand, Spirotetramat and chlorantraniliprole revealed similar results, with control levels of 43 and 38 u L^{-1}, respectively. Radiant and spirotetramat significantly decreased AST activity on exposure days, with values 32 u L^{-1} and 73 u L^{-1}, respectively. On the other hand, Sulfoxaflor, fipronil, and chlorantraniliprole showed an increase in enzyme levels after 7 days of treatment, with values of 407 u L^{-1}, 333 u L^{-1}, and 164 u L^{-1}, respectively. When compared to untreated snails over the same period, these results indicated the least effectiveness on the activity of the AST enzyme. The data demonstrated that there was no significant difference ($p < 0.05$) among snails treated with insecticides and the control in terms of total protein levels. Furthermore, *E. vermiculata* treated with spinetoram, sulfoxaflor, spirotetramat, and chlorantraniliprole exhibited higher amounts of triglycerides: 4,728, 2,064, 798, and 271 mg dL^{-1}, respectively. On the contrary, fipronil reduced the numver of triglycerides to 4 mg dL^{-1} in total lipids. Seven days of spirotetramat exposure resulted in the largest rise in total cholesterol levels of the lipid profile, with an increase of 33 mg dL^{-1} compared to the control (3 mg dL^{-1}) and other pesticides (1 mg dL^{-1}). There is no significant difference among chlorantraniliprole, spinetoram, fipronil, and sulfoxaflor in terms of total cholesterol. The data demonstrated that there was no significant difference between the pesticides and the control group in terms of LDH levels in the lipid profile after the exposure period.

Table 6. Shows the effect of LC_{25} of five different pesticides on enzymatic levels in *E. vermiculata* tissues exposed to 5 ppm for seven days

Insecticides	ALT (u L^{-1})	AST (u L^{-1})	Total protein (g dL^{-1})	Lipid profile		LDH (u L^{-1})
				Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	
Spirotetramat	43^{c}	73^{e}	0.2^{a}	33^{a}	798^{c}	2^{a}
Chlorantraniliprole	38^{d}	164^{d}	0.3^{a}	1^{c}	271^{d}	1^{a}
Spinetoram	13^{e}	32^{f}	0.3^{a}	1^{c}	4,728^{a}	1^{a}
Fipronil	120^{a}	333^{c}	0.3^{a}	1^{c}	4^{f}	1^{a}
Sulfoxaflor	108^{b}	407^{b}	0.3^{a}	1^{c}	2,064^{b}	2^{a}
Control	37^{d}	448^{a}	0.2^{a}	3^{b}	6^{e}	2^{a}
LSD 0.05	1.97	1.78	0.178	1.03	1.78	1.26

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test.

Accordingly, fipronil and sulfoxaflor increased the activity of ALT, while sulfoxaflor, fipronil, and chlorantraniliprole were found to increase the levels of the enzyme AST after 7 days of treatment. Spinetoram resulted in the lowest values of ALT and AST. Additionally, fipronil caused a reduction in triglycerides in the TL. However, there is no significant difference observed between the pesticides and the control group in terms of the levels of total protein, Total cholesterol, and LDH in the lipid profile after the exposure period.

DISCUSSION

Snails are polyphagous and feed on a variety of plant materials, such as leaves and fruits, as well as on decaying organic matter (Albuquerque et al., 2008; Ademolu et al., 2011). Feed preference studies (Iglesias & Castillejo, 1999; Chevalier et al., 2000; Chevalier et al., 2003; Ebenso & Adeyemo, 2011; Mohamed, 2016; Bashandy & Awwad, 2022) have demonstrated the capacity of snails to choose their food when given free choice feeding and to retain memories of preferred feeds. Furthermore, Ogbu et al. (2014) showed that the different species of land snails have preferences for different feedstuffs and exhibit differences in feeding behavior.

In our study, the results showed significant ($p < 0.05$) differences in the preference of different feedstuff by land snails *E. vermiculata*. The most appetent feedstuffs consumed were cos lettuce, followed by cabbage. Similarly, Arafa (1997) reported the mean daily consumption (mg/snail) of *Eobania* sp. over 7 days, with lettuce, sweet peas, cabbage, and nursery rocket being consumed at rates of 1.1, 1.2, 1.4, 1.3, 1.5, 1.4, and 1.9 mg/snail, respectively. Similar to our findings, Abd El-Hak (1997) discovered that *Eabania* sp. favored new lettuce leaves, followed by peas and cabbage. Furthermore, the detailed study of Eshra (1997) supported our results, with lettuce leaves being the most preferred, followed by cabbage leaves. On the other hand, the fruits of carrot and squash were found to be the least favored. Additionally, Giant African land snails, such as *Archachatina marginata*, consume various vegetable plants including cabbage, pawpaw, pineapples, nuts, cherry, flowers, and potatoes (Okafor, 2001). On the other hand, Mahrous et al. (2002) found that *Monacha cartusiana* snails preferred cabbage and lettuce in larger quantities, while pepper, pea, and tomatoes were the least preferred. Additionally, giant African land snails, such as *A. marginata*, have been observed to favour approximately 500 different types of plants, including peanuts, beans, peas, cucumbers, and melons (Akintomide, 2004). Furthermore, Okonta, (2012) observed that *A. marginata* snails consumed a higher amount of palm fruits (*Elaeis guineensis*) compared to *Ipomea babatas* leaves. Additionally, the results of Mohamed-Ghada (2004, 2016) exhibited that cabbage and lettuce were the most palatable options for land snails, specifically *Monacha cartusiana,* and *Helicella vestalis*. with rates of 63.3% and 57.9%, respectively, for *Monacha cartusiana*, and 67.3% and 42.9%, respectively, for *Helicella vestalis*. Furthermore, the high consumption of two plants was (34.8, 41.5) and (30.9, 33.3) for two snails, respectively. Additionally, the study of Asran et al. (2016) revealed that *E. vermiculata* preferred lettuce, followed by squash, carrots, and potatoes. In contrast, Shoeib (1997) observed that *E. vermiculata* consumed more Dahlia leaves compared to cabbage and lettuce. Also, in the feed preference test by Nakhla & Tadros (1995), *E. vermiculata* showed a strong preference for banana plants. Moreover, Bashandy (2018) observed that cabbage was the most palatable, with an average consumption rate of 0.552 g, while London rocket and Snow thistle had the lowest consumption rates for the march slug, *Deroceras leave*, at 0.244 g and 0.215 g, respectively. Furthermore, according to Bashandy & Awwad (2022), in the non-choice method, cabbage and lettuce leaves were found to have the highest palatability, with consumption rates of 5.17 g and 3.76 g, respectively. However, in the free-choice method, berseem leaves had the highest food preference among land snails, *E. vermiculata*, with a consumption rate of 4.27 g over a period of five days.

The chemical control of *E. vermiculata* land snails through the application of pesticides is still the most effective approach, particularly over large areas (Asif, 2018). This study elucidated the efficacy of six insecticides at series concentrations in controlling *E. vermiculata* under laboratory conditions**.** Our findings revealed that chlorantraniliprole caused higher mortality compared to other chemicals used to control *E. vermiculata*. The results are similar to Liu et al. (2017), as they exhibited high toxicity levels of chlorantraniliprole to *Helicoverpa armigera* moths, resulting in a mortality of 86.67% during 24 h period at the concentration of 1 mg a.i. L^{-1}. Chlorantraniliprole, a key anthranilic diamide, is a novel chemical insecticide that has been reported as the most effective compound for controlling lepidopteran pests (Carscallen et al., 2019). It can induce feeding cessation and muscle paralysis, resulting in death by binding with ryanodine and promoting calcium release (Plata-Rueda et al., 2019). Moreover, sulfoxaflor and fipronil caused the death of less than 50 percent of land snails for one month. But Eshra et al. (2016) reported that fipronil had the highest toxicity against *E. vermiculata*, and mortality was 82.99–91.20% after 96 hrs. Also, Hussein & Sabry (2019) showed that the recommended field rate of Fipronil was very effective against the eggs of *E. vermiculata* at 22.7% compared with 96.3% in the control group. In this trial, spinetoram and spirotetramat had the lowest efficacy in terms of mortality for *E. vermiculata* snails. However, Sabry & Hussein (2022) demonstrated that spirotetramat in both conventional and nano formulations,revealed 100% and 53%

mortality with LC_{50} values of 7.7 and 35% against *E. vermiculata*. Furthermore, Al Naggar & Paxton (2021) and Chakrabarti et al. (2020) reported that sulfoxaflor is moderately toxic to mammals and birds and slightly toxic to most aquatic species, but it poses a high risk to honeybees and bumblebees when they come into contact with spray droplets shortly after application. Some studies have reported the high toxicity of sulfoxaflor to bees. Chlorantraniliprole is an anthranilic diamide insecticide that exhibits a high degree of specificity towards insect ryanodine receptors (RyRs), which play a crucial role in insect muscle contraction (Lahm et al., 2019). According to Brugger et al. (2010), chlorantraniliprole exhibited selectivity towards several beneficial parasitoid wasp species, including *Aphidius rhopalosiphi*, *Trichogramma dendrolimi*, *Trichogramma chilonis*, *Trichogramma pretiosum*, *Aphelinus mali*, *Dolichogenidea tasmanica*, and *Diadegma semiclausum* (Brugger et al., 2010). Furthermore, this pesticide exhibited little toxicity towards both the larvae and adults of the predators *Harmonia axyridis* and *Chrysoperla sinica* (Liu et al., 2016).

Pesticides cause biochemical impairment and lesions of tissues and cellular processes, resulting in hundreds-fold increases in the activity of enzymes (AST), (ALT), (ALP), and (LDH) in *Monacha cantiana* and *Theba pisana*, two land snail species, as a consequence of organ cell injury (Ali, 2004; Mahmoud, 2006; Celik et al., 2009; Ghouri et al., 2010; El-Gohary & Genena, 2011; Khalil, 2016). Furthermore, Bakry et al. (2013) discovered that lipid peroxidase (LP) activity increased in *Bulinus truncatus* after two weeks of exposure to sublethal concentrations of glyphosate. Additionally, Bislimi et al. (2013) showed that a high rate of cholesterol and total protein in the hemolymph of garden snails, *Helix pomatia* L. in the contaminated regions. Moreover, several biological targets on *E. vermiculata* were altered by the chemical compounds that were investigated, potentially resulting in severe detrimental impacts on the metabolism and cells of snails (Mahal et al., 2015). According to Abdelmonem (2016), the LD_{50} (102.32 µg/snail) of methomyl lannate inhibited AChE more in the brain ganglia than in the foot muscle of *E. vermiculata*. Except for ALP, there was a considerable elevation of hemolymph enzymes in snails exposed to 21.32 and 53.30 µg/snail for 48 hours via contact. Moreover, Esam (2023) showed that Bis-(1,2-diphenyl-2-(p-tolylimino)-ethanone decreased the activities of ALT, AST, and TP in *E. vermiculata* with mean values lower than the control, while treatment with a mixture of chemicals increased ALT, AST activities, and TP with mean values higher than the control. El-Bassouiny et al. (2022) demonstrated that spiroetramat (Movento) achieved a 24 h-LC_{50} of 12.05 ppm against the cotton bollworm, *Earias insulana*, and caused a significant change in the activities of transaminase enzymes (AST and ALT), phenol oxidase, and acetylcholinesterase. It also caused a significant decrease in total protein and lipids.

According to Das et al. (2019), sulfoxaflor was toxic to adult bees and caused significant changes in antioxidative (SOD, CAT), lipid peroxidation (POD, LPO, MDA), detoxification (GST, GR, GSH), and signal transduction-related (AChE, ACh) enzymes or products in both larvae and adult honeybees in the laboratory over 96 hours. Therefore, chlorantraniliprole takes into account the well-being of parasitoids and natural enemies, chlorantraniliprole proves to be a good candidate for controlling land snail *E. vermiculata*.

CONCLUSIONS

In conclusion, the results showed the most food preference for land snails, specifically *E. vermiculata* was Cos lettuce and cabbage leaves in both the ‘no-choice feeding’ and ‘free-choice feeding’ methods. Additionally, based on the previous findings, it was possible to arrange the pesticides used based on the extent of their effect on land snails and their vital enzymes as follows: chlorantraniliprole > sulfoxaflor > fipronil > spinetoram > spirotetramat. Therefore, these substances could be useful for managing land snails. Incorporating these insecticides into a comprehensive management strategy to mitigate any negative effects of land snails while ensuring the overall well-being of the environment. The most likely route of action of these chemicals on land snails still needs more research.

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REFERENCES

Abbott, W.S. 1925. A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology* **18**, 265–267.

Abd El-Atti, M.S., El-Sayed, A.S. & Said, R.M. 2020. Usage of pharmaceutical contraceptive drug for controlling *Eobania vermiculata* snails by baits technique. *Heliyon* **6**(12).

Abd El-Hak, A.I.A. 1997. *Studies on some land molluscs at Sharkia Governorate*. M.Sc. Thesis, Fac. Agric., Al-Azhar Univ., 137 pp.

Abdelmonem, M.K. 2016. Impact of methomyl lannate on physiological parameters of the land snail Eobania vermiculata. *The Journal of Basic & Applied Zoology* **74***,* 1–7.

Ademolu, K.O., Jayeola, O.A., Idowu, A.B. & Elemide, I.O. 2011. Circadian variation in locomotor and feeding periods of two land snail species. Arch Zootec **60**, 1323–1326.

Akintomide, I.A. 2004. Tropical snail farming Oak Ventures *publishers Lagos*, pp. 5–6.

Al Naggar, Y. & Paxton, R.J. 2021. The novel insecticides flupyradifurone and sulfoxaflor do not act synergistically with viral pathogens in reducing honeybee (*Apis mellifera*) survival but sulfoxaflor modulates host immunocompetence. *Microb. Biotechnol.* **14**(1), 227–240.

Al-Akraa, T.M.M., El-Danasory, M.A. & Mohafez, M.A. 2010. Food preference and food consumption of some land snails under laboratory conditions. *J. of Plant Protection and Pathology* **1**(4), 189–193.

Albuquerque, F.S., Peso-Aguiar, M.C. & Assuncao-Albuquerque, M.J.T. 2008. Distribution, feeding behaviour and control strategies of the exotic land snail Achatina fulica (Gastropoda: Pulmonata) in the northeast of *Brazil. Braz. J. Biol.* **68**, 837–842.

Ali, A.E. 2004. Toxicity and biochemical response of *Eobania vermiculata* land snail to niclosamide molluscicide under laboratory and field conditions. *Journal of Plant Protection and Pathology* **29**(8), 4751–4756.

Ali, M.A. 2017. Comparison among the Toxicity of Thymol and Certain Pesticides on Adults Survival and Egg Hatchability of the Glassy Clover Snail Monacha cartusiana (MÜLLER). *Journal of Plant Protection and Pathology* **8**(4), 189–194.

Ampuero, A., Rivera, F., Olórtegui, S. & Martel, C. 2023. Feeding behaviour and food preferences of native and introduced snail species from the Lomas formations, threatened seasonal fog oases. *Pedobiologia* **99**, 150893.

Anonymous, 2014. *Dow Product Safety Assessment: Spinetoram*. The Dow Chemical Company, pp. 6

Arafa, A.A. 1997. *Studies on some land mollusca at Sharkia Governorate*. M.Sc. Thesis Fac. Agric., Al-Azhar Univ., 137 pp.

Asif, M. 2018. Diverse Biologically Active Pyridazine Analogs: A Scaffold for the Highly Functionalized Heterocyclic Compounds. *Rev. J. Chem*. **8**, 280–300.

Asran, A.A., Keshta, T.M.S., Mohamed, Gh.R., Mona, A.A. & Abou-Hashem, A.A. 2016. Preference of brown garden snails Eobania vermiculata to some vegetables under laboratory conditions. *Egypt. J. of Appl. Sci* **31**(1), 1–8.

Babcock, J.M., Gerwick, C.B., Huang, J.X., Loso, M.R., Nakamura, G., Nolting, S.P., Rogers, R.B., Sparks, T.C., Thomas, J., Watson, G.B. & Zhu, Y. 2011. Biological characterization of sulfoxaflor, a novel insecticide. *Pest Manag. Sci*. **67**(3), 328–334.

Bacca, T., Cabrera, N.J. & Gutiérrez, Y 2021. Toxic effect of chlorantraniliprole on new- born larvae of the potato tuber moth *Tecia solanivora* (Lepidoptera: Gelechiidae). *Ann. Appl. Biol.* **179**(2), 169–175.

Bakr, E.M. 2005. A new software for measuring leaf area, and area damaged by Tetranychus urticae Koch. *Journal of Applied Entomology* **129**(3), 173–175.

Bakry, F.A., El-homossany, K., Abd El-Atti, M.S. & Ismaiel, S.M. 2013. Alterations in the fatty acid profile, antioxidant enzymes and protein pattern of *Biomophalaria alexandarina* snails exposed to the pesticides diazinon and profenofos. *Global J. Pharm* **1**(1), 27–36.

Banaee, M., Sureda, A., Taheri, S. & Hedayatzadeh, F. 2019. Sub-lethal effects of dimethoate alone and in combination with cadmium on biochemical parameters in freshwater snail, *Galba truncatula*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **220**, 62–70.

Barbara, N. & Schembri, P.J. 2008. The status of Otala punctata (Müller, 1774), a recently established terrestrial gastropod in Malta. *Bollettino Malacologico* **44**(5–8), 101–107.

Bashandy, A.S. & Awwad, M.H. 2022. An examination of palatableness of some leaf plants utilizing two terrestrial snails in Egypt. *Egypt. J. Plant Prot. Res. Inst.* **5**(2), 207–214.

Bashandy, A.S. 2018. *Studies on Some Terrestrial Gastropods Injurious to Some Important Crops at Giza Governorate*. Ph.D. thesis, Agric. Zoology and Nematology, Fac. of Agric., Al-Azhar Univ., 266 pp.

Bashandy, A.S. & Raddy, H.M. 2021. In Vitro Assessment of The Efficacy of Metaldehyde, Methomyl and Copper Sulfate on some Terrestrial Gastropods. *Journal of Plant Protection & Pathology* **12**(7).

Bayoumi, S.M., Omar, N.A., Mohanna, A.H., Ismail, S.A.A., Abed, M., El Sayed, A.M.A, El-Akhrasy, F.I. & Issa, M.A. 2023. Survey and population dynamics of land snails at Sharkia Governorate, Egypt. *Brazilian Journal of Biology* **84**, p.e271247.

Bellisai, G., Bernasconi, G., Brancato, A., Cabrera, L.C., Castellan, I., Ferreira, L., Giner, G., Greco, L., Jarrah, S., Leuschner, R., Magrans, J.O., Miron, I., Nave, S., Pedersen, R., Reich, H., Robinson, T., Ruocco, S., Santos, M., Pia Scarlato, A., Theobald, A. & Alessia Verani. 2022. Modification of the existing maximum residue levels for sulfoxaflor in various crops. *EFSA Journal* **20**(4), e07283.

Bishop, H. 2015. Pollinator Stewardship Council v. United States Environmental Protection Agency, 800 F. 3d 1176 (9th Cir. 2015). *Public Land & Resources Law Review* **6**, 24.

Bislimi, K., Behluli, A., Halili, J., Mazreku, I., Osmani, F. & Halili, F. 2013. Comparative analysis of some biochemical parameters in hemolymph of garden snail (*Helix pomatia* L.) of the Kastriot and Ferizaj regions, Kosovo. *International journal of engineering* **4**(6), 8269.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem* **72**, 248–254.

Bretschneider, T., Fischer, R. & Nauen, R. 2007. *Inhibitors of lipid synthesis (acetyl-CoA-carboxylase inhibitors) in: Modern Crop Protection Compounds*, Volume **1**, eds: Krämer, W., Schirmer, U., Wiley-VCH, Weinheim, Germany, Chapter 28, pp. 909–925.

Bronne, L. & Delcourt, J. 2024. The find of six species new to Belgium highlights the role of the stone trade as a pathway for non-native land snails (Gastropoda: Stylommatophora). *Belgian Journal of Zoology* **154**, 11–30.

Brugger, K.E., Cole, P.G., Newman, I.C., Parker, N., Scholz, B., Suvagia, P., Walker, G. & Hammond, T.G. 2010. Selectivity of chlorantraniliprole to parasitoid wasps. *Pest Manag Sci.* **66**(10), 1075–1081.

Cabaud, P.G. & Wroblewski, F. 1958. Colorimetric measurements of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol.* **30**, 234–236.

Camilleri, J., Cassar, L.F. & Schembri, P. 2021. Distribution and abundance of the alien helicid *Otala punctata* (Müller, 1774) (Mollusca Helicidae) at Baħrija, Malta, a site of agricultural and conservation importance. *Naturalista Siciliano* **45**(1–2), 55–70.

Carlsson, N.O.L., Bronmark, C. & Hansson, L.A. 2004. Invading herbivory: The golden apple snailalters ecosystem functioning in Asian wetlands. *Ecology* **85**, 1575–1580.

Carscallen, G.E., Kher, S.V. & Evenden, M.L. 2019. Efficacy of chlorantraniliprole seed treatments against armyworm (*Mythimna unipuncta* [Lepidoptera: Noctuidae]) larvae on corn (*Zea mays*). *Journal of Economic Entomology* **112**(1), 188–195.

Celik, I., Yilmaz, Z. & Turkoglu, V. 2009. Hematotoxic and hepatotoxic effects of dichlorvos at sublethal dosages in rats. *Environmental Toxicology: An International Journal* **24**(2), 128–132.

Centner, T.J., Brewer, B. & Leal, I. 2018. Reducing damages from sulfoxaflor use through mitigation measures to increase the protection of pollinator species. *Land use policy*, **75**, 70–76.

Chakrabarti, P., Carlson, E.A., Lucas, H.M., Melathopoulos, A.P. & Sagili, R.R. 2020. Field rates of Sivanto TM (flupyradifurone) and Transform® (sulfoxaflor) increase oxidative stress and induce apoptosis in honeybees (*Apis mellifera* L.). *PLoS One* **15**(5), 0233033.

Chellat, S., Toubal, L., Djerrab, A., Bourefis, A., Hamdi-Aissa, B. & Salmi-Laouar, S. 2018. Molluscan and sedimentological sequences of the late Quaternary deposits of Morsott region (NE Algeria) and their paleoenvironmental implication. *BSGF-Earth Sciences Bulletin* **189**(4–6), 17.

Cheriti, O., Belhiouani, H., El-Hadef-El-Okki, M., Neubert, E. & Sahli, L. 2021. Inventory of land snails from the Kebir Rhumel basin, northeast of Algeria. *Biodiversity* **22**(3–4), 110–130.

Chevalier, F.D., Diaz, R., McDew-White, M., Anderson, T.J. & Le Clec'h, W., 2020. The hemolymph of Biomphalaria snail vectors of schistosomiasis supports a diverse microbiome. *Environmental microbiology* **22**(12), 5450–5466.

Chevalier, L., Le Coz-Bouhnik, M. & Charrier, M., 2003. Influence of inorganic compounds on food selection by the brown garden snail Cornu aspersum (Muller)(Gastropoda: Pulmonata). *MALACOLOGIA-PHILADELPHIA*, **45**(1),125–132.

Cilia, D.P. 2011. Contributions to the malacology of Malta, 2: on the second record of *Otala punctata* (Müller, 1774) (Gastropoda: Helicidae) from Malta. *The Central Mediterranean Naturalist* **5**(3–4), 4–5.

CoHort Software, 2004. CoStat. California, USA.

Colonese, A.C., Zanchetta, G., Fallick, A. E., Manganelli, G., Cascio, P.L., Hausmann, N., Hausmann, A.I., Baneschi, C. & Regattieri, E. 2014. Oxygen and carbon isotopic composition of modern terrestrial gastropod shells from Lipari Island, Aeolian Archipelago (Sicily). *Palaeogeography, Palaeoclimatology, Palaeoecology* **394**, 119–127.

Costat program, 2002. Microcomputer Program Analysis. CoHort Software, Version 2.6, Monterey, CA, USA.

Das, P.P.G., Bhattacharyya, B., Bhagawnti, S., Dev, E.B., Manpoong, N.S. & Bhairavi, K.S. 2020. Slug: An emerging menace in agriculture: A review. *Journal of Entomology and Zoology Studies* **8**(4), 1–6.

Das, P., Bhuyan, R.P., Shandilya, U., Borah, B. & Gharphalia, B.J. 2019. Bioefficacy and phytotoxicity of Sulfoxalor 50% WG against tea mosquito bug (Helopeltis theivora) and green fly (Empoasca flavescens) and its effect against natural enemies in tea. *Journal of Entomology and Zoology Studies* **7**, 224–229.

Dedov, I.K., Mitov, P.G., Zapryanov, L., Georgiev, D. & Gashtarov, V. 2022. Distribution of the Invasive Land Snail *Eobania vermiculata* (OF Müller, 1774) (Gastropoda: Helicidae) in Bulgaria. *Acta Zoologica Bulgarica* **74**(4), 611–622.

Desouky, M.M. & Busais, S. 2012. Phylogenetic relationships of the land snail; Eobania vermiculata (Müller, 1774) from Egypt and Saudi Arabia. A combined morphological and molecular analysis. *The Journal of Basic & Applied Zoology* **65**(2), 144–151.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics* **11**(1), 1–42.

Ebenso, I.E. & Adeyemo, G.O., 2011. Foraging behaviour responses in the African giant land snail Achatina achatina. *Ethiopian Journal of Environmental Studies and Management* **4**(4). http://dx.doi.org/10.4314/ejesm.v4i4.6.

El-Bassouiny, H.M., Ahmed, A.F., Madany, W.A. & Selim, S. 2022. Impact of tetramic acid derivatives compounds against cotton Lepidoptera pests' neonate larvae Earias insulanaare (Boisd.). *International Journal of Entomology Research* **7**(4), 170–176.

El-Din, H.S., Helmy, W.S., Al Naggar, Y. & Ahmed, F.S. 2022. Chronic exposure to a field-realistic concentration of Closer® SC (24% sulfoxaflor) insecticide impacted the growth and foraging activity of honey bee colonies. *Apidologie* **53**(2), 22.

El-Gohary, Laila, R.A. & Genena. Marwa, A.M. 2011. Biochemical effect of three molluscicide baits against the two land snails, *Monacha cantiana* and *Eobania vermiculata* (Gastropoda: Helicidae): *International Journal of Agricultural Research* **6**(9), 682–690.

El-Shahaat, M.S., Eshra, E.H. & Abobakr, Y. 2005. Impact of Basamide® and methomyl bait on non-target pests and microbiological processes in soil. *Egypt. J. Agric. Res* **83**, 1007–1016.

El-Shahaat, M.S., Nagda, A.A., Eshra, E.H., Mesbah, H.A. & Emtiaz Ghoneim, I. 2009. Toxicity of certain copper fungicides and other pesticides to terrestrial snails. *J. Agric. Sci. Mansoura Univ* **34**, 5501–5507.

El-Wakil, H.B. & Attia, A.M. 1999. Effect of selected insecticides on the terrestrial snails *Eobania vermiculata* (Müller) and *Theba pisana* (Müller) with respect to some morphological changes in Egypt. *J. Environ. Sci. Health* **34**, 47–60.

Esam, M.E. 2023. Biochemical impacts and toxicity of Bis-(1,2-diphenyl-2-(p-tolylimino)-ethanone and its metal complexes against *Monacha obstructa* and *Eobania vermiculata* (Gastropoda: Hygromiidae: Helicidae). *Egypt. J. Plant Prot. Res. Inst* **6**(2), 143–149.

Eshra, E.H. 2014. Toxicity of methomyl, copper hydroxide and urea fertilizer on some land snails. *Annals of Agric. Sci., Ain Shams Univ.* **59**, 281–284.

Eshra, E.H., El-Shahaat, M.S. & Dewer, Y. 2016. Molluscicidal Potential of Two Neonicotinoids and Fipronil Against Three Terrestrial Snail Species. *International Journal of Zoological Investigations* **2**(1), 01–08.

Eshra, E.H.H. 1997. *Ecological and biological studies on land snails associated with some important economic crops*. M.Sc. Thesis Fac. Agric., Al-Azhar Univ., Egypt, 153 pp.

Finney, D.J. 1971. *Propit analysis*. Cambridge Univ. press, London 3rd. Ed., 333 pp.

Gabr, W.M., Youssef, A.S. & Khidr, F.K. 2006. Molluscicidal effect of certain compounds against two land snail species *Monacha obstructa* and *Eobania vermiculata* under laboratory and field conditions. *Egypt. J. Agric. Res.* **84**(1), 43–50.

Gad, A.F., Abdelgalil, G.M. & Radwan, M.A. 2023. Bio-molluscicidal potential and biochemical mechanisms of clove oil and its main component eugenol against the land snail, *Theba pisana*. *Pesticide Biochemistry and Physiology* **192**, 105407.

Ghouri, N., Preiss, D. & Sattar, N. 2010. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology* **52**(3), 1156–61.

Hussein, M.A. & Sabry, A.K.H. 2019. Assessment of some new pesticides as molluscicides against the adult and eggs of chocolate banded snail, *Eobania vermiculata*. *Bulletin of the National Research Centre* **43**, 1–5.

Iglesias, J. & Castillejo, J.J.M.S. 1999. Field observations on feeding of the land snail Helix aspersa Muller. *Journal of Molluscan Studies* **65**(4), 411–423.

Ismail, S.A. 2004. Eco biological studies on the brown garden snail, *Eobania vermiculata* Muller under laboratory and field conditions in Sharkia Governorate [Egypt]. *Zagazig Journal of Agricultural Research* **31**(1), 293–305.

Kar, A., Mandal, K. & Singh, B. 2013. Environmental fate of chlorantraniliprole residues on cauliflower using QuEChERS technique. *Environ Monit Assess* **185**(2), 1255–1263.

Kenko, D.B.N. & Ngameni, N.T. 2022. Assessment of ecotoxicological effects of agrochemicals on bees using the PRIMET model, in the Tiko plain (South-West Cameroon). *Heliyon* **8**(3).

Kenko, D.B.N., Ngameni, N.T. & Egbe, A.M. 2024. Evaluation of the implications of pesticide usage in agriculture on earthworms in the mono-modal equatorial agro-ecological zone of Cameroon. *Environment*, *Development and Sustainability* **26**(1), 2271–2290.

Kenko, D.B.N., Ngameni, N.T. & Kamta, P.N. 2022. Environmental assessment of the influence of pesticides on non-target arthropods using PRIMET, a pesticide hazard model, in the Tiko municipality, Southwest Cameroon. *Chemosphere* **308**, 136578.

Keshta, T., Abou Hashem, A.A. & Abd El Gaul, Y.M.A. 2006. Food preference estimation for some land snails species and their sensitivity to vertimec biocide under laboratory conditions comparing with stander molluscide cekumeta 5%. *Journal of Plant Protection and Pathology* **31**(12), 7997–8002.

Khalil, A.M. 2016. Impact of methomyl lannate on physiological parameters of the land snail *Eobania vermiculata*. *J. Basic Appl. Zool.* **74**, 1–7. https://doi.org/10.1016/j.jobaz.2015.12.005

Kirst, H.A. 2010. The spinosyn family of insecticides: realizing the potential of natural products research. *J. Antibiotic*s **63**, 101–111.

Knight, J.A., Anderson, S. & Rawle, J.M. 1972. Chemical basis of the sulfo-phospho- vanillin reaction for estimating total serum lipids. *Clin. Chem* **18**, 199–202.

Lahm, G.P., Cordova, D. & Barry, J.D. 2009. New and selective ryanodine receptor activators for insect control. *Bioorg Med Chem* **17**, 4127–4133. doi: 10.1016/j.bmc.2009.01.018

Laila, R.A. & Genena, M.A.M. 2011. Biochemical effect of three molluscicide baits against the two land snails, *Monacha cantiana* and *Eobania vermiculata* (Gastropoda: Helicidae). *International Journal of Agricultural Research* **6**(9), 682–690.

Li, J., Zhao, L., Qi, S., Zhao, W., Xue, X., Wu, L. & Huang, S. 2021. Sublethal effects of Isoclast™ Active (50% sulfoxaflor water dispersible granules) on larval and adult worker honey bees (Apis mellifera L.). *Ecotoxicology and Environmental Safety* **220**, 112379.

Liu, Y., Gao, Y., Liang, G. & Lu, Y. 2017. Chlorantraniliprole as a candidate pesticide used in combination with the attracticides for lepidopteran moths. *PLoS ONE* **12**(6), e0180255. https://doi.org/10.1371/journal.pone.0180255

Liu, Y.Q., Li, X.Y., Zhou, C., Liu, F. & Mu, W. 2016. Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. *Sci Rep*. **6**, 39060. doi: 10.1038/srep39060

Mahal, A., Abu-El-Halawa, R., Zabin, S.A., Ibrahim, M., Al-Refai, M. & Kaimari, T. 2015. Synthesis, characterization and antifungal activity of some metal complexes Derived from Quinoxaloylhydrazone. *World J. Org. Chem* **3**, 1–8.

Mahmoud, M.B. 2006. Biological and histological impact of the insecticides reagent and mimic on Biomphalaria alexandrina snails. *Egypt. J. Zool* **46**, 11–21.

Mahrous, M.H., Ibrahim, M.H. & Abd El-Aal, E.M. 2002. Occurrence, population density and importance value of land snails infesting different crops at Sharkia Governorate. *Zagazig J. Agric. Res*. **29**(2), 613–629.

Mienis, H.K., Rittner, O. & Vaisman, S. 2016. Information concerning *Eobania vermiculata*, I. On the presence of this exotic species in Israel (Mollusca, Gastropoda, Helicidae). *Triton* **35**, 15–22.

Miller, E., Swails, D., Olson, F. & Staten, R.T. 1988. White garden snail (Theba pisana Müller): Efficacy of selected bait and sprayable molluscicides. *J. Agric. Entomol*. **5**, 189–197.

Mohamed, Gh.R. 2004. *Ecological and biological studies on some species of snails*. M.Sc. Thesis Fac. Agric. Moshthor, Zagazig Univ. (Banha branch), 215 pp.

Mohamed, G.R. 2016. Susceptibility and consumption rates of *Monacha cartusiana* and *Helicella vestalis* land snails to certain plants. *Middle East Journal of Applied Sciences* **6**(4), 896–899.

Moran, S., Gotlib, Y. & Yaakov, B. 2004. Management of land snails in cut green ornamentals by copper hydroxide formulations. *Crop Protection* **23**, 647–650.

Mundy-Heisz, K.A., Prosser, R.S. & Raine, N.E. 2022. Acute oral toxicity and risks of four classes of systemic insecticide to the Common Eastern Bumblebee (Bombus impatiens). *Chemosphere* **295**, 133771.

Nakhla, J.M. & Tadros, A.W. 1995. Studies on the seasonal abundance of land snails on date palm shoots in Sharkia Governorate. *Egypt. J. Res.* **73**(2), 347–355.

Nauen, R., Bretschneider, T., Elbert, A., Fischer, R., Reckmann, U. & Van Waetermeulen, X. 2006. Biological and mechanistic considerations on the mode of action of spirotetramat. In: *11th IUPAC Int. Congress of Pesticide Chemistry*, pp. 6–10.

Nkontcheu, D.B.K., Fotio, A.L., Kenfack, A.D., Taboue, G.C.T., Acha, D.A. & Fokam, E.B. 2023. Ecological risk assessment of pesticides based on earthworms in soils on the southeast slopes of Mount Cameroon. *Soil & Environmental Health* **1**(4), 100047.

Noha, M. & Meligi Hfhah, S.M. 2019. Coragen induced toxicity and the ameliorative effect of an Origanum majorana L. in male albino Rats. *J. Am. Sci* **15**(9), 33–44.

Ogbu, C.C., Ani, A.O. & Emeh, M. 2014. Feed preferences and feeding behaviour of two species of African giant land snails. *Archivos de zootecnia* **63**(242), 337–347.

Okafor, F.U. 2001. Edible land snails: a manual of biological management and farming of snails. *Splendid publishers*, Lagos. *Splendid Publishers*, pp. 1–54.

Okonta, B.O. 2012. Performance of giant African land snail *Archachatina marginata* (Swainson) fed with selected diets. *Global journal of Bioscience and Biotechnology* **1**(2), 182–185.

Pan, F., Lu, Y. & Wang, L. 2017. Toxicity and sublethal effects of sulfoxaflor on the red imported fire ant, *Solenopsis invicta*. *Ecotoxicology and Environmental Safety* **139**, 377–383.

Pesticides, A. & Authority, V.M. 2009. Evaluation of the new active SPIROTETRAMAT in the product MOVENTO 240 SC INSECTICIDE. APVMA, April, pp. 9–10.

Plata-Rueda, A., Martínez, L.C., Costa, N.C.R., Zanuncio, J.C., de Sena Fernandes, M.E., Serrão, J.E., ... & Fernandes, F.L. 2019. Chlorantraniliprole–mediated effects on survival, walking abilities, and respiration in the coffee berry borer, Hypothenemus hampei. *Ecotoxicology and Environmental Safety* **172**, 53–58. https://doi.org/10.3390/toxics11070618

Puizina, J., Puljas, S., Fredotović, Ž., Šamanić, I. & Pleslić, G. 2013. Phylogenetic relationships among populations of the Vineyard Snail *Cernuella virgata* (Da Costa, 1778). *International Scholarly Research Notices,* 2013, 638325, 9.

Racevičiūtė-Stupelienė, A., Vilienė, V., Vilma, E.Ž. & Nutautaitė, Š.M. 2023. Selenomethionine Utilisation in Fattening Pig Diets and Impact on Productivity and Final Production Quality. *ICOFAAS 2023*, 210.

Radwan, M.A., Essawy, A.E., Abdelmeguied, N.E., Hamed, S.S. & Ahmed, A.E. 2008. Biochemical and histochemical studies on the digestive gland of *Eobania vermiculata* snails treated with carbamate pesticides. *Pestic. Biochem. Physiol.* **90**, 154–167.

Radwan, M.A. 1993. A technique for testing the efficacy of molluscicidal baits against land snails. *Com. Sci. Dev. Res* **651**, 17–26.

Radwan, M.A., El-Wakil, H.B. & Osman, K.A. 1992. Toxicity and biochemical impact of certain oxime carbamate pesticide against terrestrial snail, Theba pisana (Müller). *J. Environ. Sci. Health* B **27**, 759–773.

Raymond, D.V., Matsuda, K., Sattelle, B.M., Rauh, J.J. & Sattelle, D.B. 2005. Ion channels: molecular targets of neuroactive insecticides. *Invertebr Neurosci* **5**(3–4), 119–133.

Ronsmans, J. & Van den Neucker, T. 2016. A persistent population of the chocolate-band snail *Eobania vermiculata* (Gastropoda: Helicidae) in Belgium. *Belgian Journal of Zoology*, **146**(1).

Rossaro, B., Convertini, S. & Bacci, L. 2018. A review of sulfoxaflor, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. *J. Èntomol. Acarol. Res*. **50** (3).

Routray, S. & Dey, D., 2016. Snails and slugs as crop pests. *Rashtriya Krishi*, 40–41.

Sabry, A.K.H. & Hussein, M.A., 2022. Evaluation of conventional and nanoformulations of some pesticides against the adults of chocolate banded snail, Eobania vermiculata (OF Müller, 1774). *Egyptian Journal of Chemistry* **65**(10), 625–629.

Shetaia, Z.S. 2005. *Integrated control of land snail's pests in the fields of Sharkia Governorate*. Ph.D. Thesis in Agric. Zoology and Nematology, Al-Azhar University, 147 pp.

Shoeib, Maha A. 1997. *Ecological studies on some common snail species and their control in Ismailia Governorate*. M.Sc. Thesis, Fac. Agric. Suez Canal Univ., Egypt, 115–120 pp.

Shoieb, M.H. 2008. Occurrence and distribution of terrestrial molluscs in Suez Canal Governorates and South of Sinai. *Egypt. J. Agric. Res*. **86**(3), 989–994.

Soha, A.M., Fatma, M.E.G. & Abu El-Kheer, R.K. 2020. Repulsive effect of potassium tartrate against *Eobania vermiculata* and *Monacha obstructa* (Gastropoda: Helicidae: Hygromiidae) land snails under laboratory and field conditions. *Egyptian Journal of Plant Protection Research Institute* **3**(1), 389–397.

Thompson, G.D., Dutton, R. & Sparks, T.C. 2000. Spinosad - a case study: an example from a natural products discovery programme. *Pest. Mgt. Sci*. **56**, 696–702.

Valarmathi, V. 2017. Food preference and feeding behaviour of the land snail *Cryptozona bistrialis* in Nagapattinam, Tamil Nadu India. *Int. J. zool. Appl. Biosci.* **2**(2), 90–94.

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Bioactivity of a methanolic extract of *Peganum harmala* L. seeds on the inflorescence rot agent (*Mauginiella scaettae)* and the fusarium rot agent (*Fusarium oxysporum* fsp *albedinis*) of date palm

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Abstract. The antifungal activity of the methanolic extract of *Peganum harmala* L. seeds harvested in Algerian Sahara was assessed on the mycelial growth of *Mauginiella scaettae* the causal agent of inflorescence rot and *Fusarium oxysporum f.sp albedinis* the causal agent of vascular fusariosis of date palm (Bayoud). The phytochemical analyses revealed the absence of tannins, flavonoids, steroids and coumarins, and among others, alkaloids, saponosides and terpenoids. The antifungal tests of different concentrations prove a strong inhibitory activity of the seed extract towards *Mauginiella scaettae* with an average inhibition rate of 100% after 72 h from 20% concentration to 100% (v/v). For *Fusarium oxysporum* f. *sp albedinis*, the inhibition rate increased with the extract concentration and the mycelial growth in the treated plates showed a low growth rate compared to the growth of this fungus in the control. The ANOVA test reveals that the extract of *Peganum harmala* seeds is very effective against *Mauginiella scaettae* and *Fusarium oxysporum* f.sp *albedinis*, it appears to have antifungal and mycelial growth inhibitory activity.

Key words: *Peganum harmala* L, *Mauginiella scaettae*, *Fusarium oxysporum* f. sp *albedinis*.

INTRODUCTION

The date palm culture is a specificity of arid and semi-arid regions in Algeria, totaling 20 million date palms on an area of 170,500 hectares and a production of

1,151,909 tons of dates (FAO, 2020). In the desert, this heritage plays essential ecological, economic and social roles. Nevertheless, this heritage witnessed significant production losses caused by a variety of pests. Among these, *Mauginiella scaettae* and *Fusarium oxysporum* f.sp *albedinis* (*Foa*) are responsible for inflorescence rot and fatal vascular fusariosis of date palms, with a direct impact on date production and the longevity of the tree (Djerbi, 1983) in addition, many plant species synthesize substances (phenols, sesquiterpenes and glucosides) that have antifungal properties and may be of interest for crop protection, (De Corato et al., 2007). Among these species *Peganum harmala* L. has been used for a long time in traditional medicine (Chehma, 2006; Zougagh et al., 2019), and is known for its antibacterial, antifungal, antiviral and insecticidal properties (Jinous & Fereshteh, 2012) *Peganum harmala* L. (Zygophyllaceae), a spontaneous perennial plant from the Algerian northern Sahara (Ozenda, 1977).

The aim of this research is to assess the anti-fungal activity of the extract of the seeds of *Peganum harmala* L against *M. scaettae* and *F. oxysporum*.

MATERIALS AND METHODS

Plant material: the extracts were obtained from *Peganum harmala* L. seeds collected in the region of Ouedlabyad [32°32'49.4 "N; 3°36'44.2 "E], province of Daya Ben Dahoua, state of Ghardaia. The seeds were successively rinsed with water, dried in the shade (20 days) at room temperature (20 to 25 °C) and finally crushed before extraction.

Fungal material: a strain of *M. scaettae* was isolated from small pieces of contaminated spathe, soaked in bleach (60%) for 5 minutes and rinsed with sterile distilled water. Once dried, they were cultured in Potato Dextrose Agar (PDA) medium and incubated for 7 days at 21 °C. To obtain pure isolates, successive transplants were made by transferring mycelial fragments into a new PDA medium. The pure strain of *M. scaettae* was obtained after isolation and purification in the laboratory of the university of Ghardaia and identified with Mr. Bensaci Messaoud Bachagha. The *Foa* strain was isolated, transplanted and identified at the laboratory of the regional plant protection station in Ghardaia.

Preparation of the methanolic extracts: they were obtained by solubilization in a mixture of distilled water and methanol (1/3–2/3) (v/v), through extraction by reflux. The extraction device consists of a flask (1,000 mL) heated with a heating mantle and topped with a refrigerating mechanism. A quantity of 100 g of seeds powder of *Peganum harmala* is deposited in the flask containing 600 mL of water-methanol solution, the mixture was boiled at 45 °C for six hours; then the mixture was filtered. To remove the methanol, the filtrate was evaporated under vacuum in a rotary evaporator set at 50 °C and a rotation speed of 80 rpm (Tonk et al., 2006).

Antifungal trials: they were performed according to the technique of Grover & Moore. (1962): dilutions were prepared to obtain final extract concentrations of 5%, 10%, 20%, 30%, 40%, 50% and 100%. In sterile Petri plates of 9 cm diameter, 15 mL of PDA and 1 mL of extract were placed with circular shaking to distribute the extract uniformly. The plates with and without extract (controls) were inoculated as follows: a mycelial disc of the phytopathogenic fungus with a diameter of 5 mm taken from the periphery of a 7-day-old culture is aseptically placed in the centre. The procedure is repeated three times for each concentration of extract. Petri plates were incubated at 21 ± 2 °C for seven days. The diameters of the fungal colonies were measured daily (Pandey et al., 1982).

Phytochemical analyses: the phytochemical screening was performed according to the methods described by several authors (Harborne, 1973; Trease & Evans, 1989; Diallo, 2000; Dohou, 2003; Mibindzou, 2004 and Koffi, 2015).

HPLC Analysis:HPLC analyses were performed on a Waters liquid chromatograph equipped with a model pumpLC-20ADXR and PDA detector using C18 reversed-phase packing column (GraceSmart RP18, 4.6×150 mm, 5 µm; Grace, Deerfield, IL, USA) for the separation, the column was thermostated at 30 °C using a CTO-20A column oven and Empower v.2 Software (Waters Spa, Milford, MA, USA) was used for the acquisition of data. Isocratic elution was performed using the mobile phase, which consisted of ultrapure water methanol (40: 60, v/v) and injected directly on-line degassed by using Degassex, mod. DG-4400 (Phenomenex, Torrance, CA, USA) at a flow rate of 1 mL min^{-1}. The standard solutions (100 µ mL^{-1}) were diluted in the mobile phase, then all the prepared sample solutions of extract and the standard were centrifuged and 10 µL of the supernatant was injected into HPLC and achieved at wavelength of 435 nm with a bandwidth of 4 nm, the time of HPLC experience is 75 mn.

Statistical analysis: A two way ANOVA was performed to verify whether the observed effect is growth dependent and/or concentration dependent/time dependent. The data obtained was subjected to a randomized repeated measures analysis of variance using *Statistica* 10 software. The results were also subjected to a multiple comparison of means using the Tukey HSD (Honest Significance Test).

RESULTS AND DISCUSSION

Extracts composition: the phytochemical tests confirmed the presence of secondary metabolites in the extracts of *P. harmala* L. seed, with an extraction yield of 20.01%. Phytochemical analyses were carried out following classical technics (observation of reactions) to highlight the presence of chemical groups known for their antifungal effects. In Table 1 of phytochemical screening of *P. harmala* L seed extracts revealed the absence of tannins, flavonoids, and reducing compounds, as well as coumarins and steroids. Terpenoids are moderately present, while free quinones and alkaloids are abundant. These results are similar to those of Bouabedelli et al. (2016) and Babaousmail et al. (2014). Based on those results reported in the literature, the presence of alkaloids in our *P. harmala* L. seeds extract was elucidated using HPLC chromatography method. The quantitative analysis was carried out to detect the presence of five known alkaloids, namely Harmol, harmine, harmaline, and peganine (vasicine), in methanol seeds extracts

Table 1. Phytochemical screening results of *Peganum harmala* L. seeds extracts

Chemical compounds		Seeds
Tannins	Catechism	-
	Gallic	-
Flavonoids	Catechism	-
	Gallic	-
	Anthocyanins	-
	Catechols	-
	Leucoanthocyanins	-
Coumarins		-
Free quinones		+++
Alkaloids		+++
Terpenoids		++
Saponosides		+
Steroids		-

(-) – Absence; (+) – low concentration; (++) – medium concentration; (+++) – high concentration.

of *P. harmala* L. which were confirmed by comparing the retention times on HPLC chromatogram spectra of the extract with the reference samples as shown in Fig. 2 by comparison with the same time as the external standard substance, we concluded that the first peak in HPLC chromatogram eluting at 18.435 min with 10.983% of the average contents was in accordance with Harmol, the second peak eluting at 30.831 min was tentatively identified as Harmaline with an average contents of 8.414%. The retention times for harmine were observed to be at 43.604 minutes, with average contents of 54.815. We observed that the *P. harmala* seeds produced peaks in HPLC chromatogram and the peak corresponding to vasicine was observed which revealed that this bioactive molecule was also identified and quantified in the dry seeds (Herraiz et al., 2017; Abbas et al., 2021). According to these results, harmol, harmine, and harmaline were the main alkaloids in extracts, with a total average content of 74.212% and harmine was noted as the major β-carboline alkaloids compounds in this plant as shown in Table 2.

Table 2. HPLC chromatogram data for alkaloids detected in *P. harmala* L methanol seeds extracts

2	Ret. time	Substance	Area, %
1	18.435	Harmol	10.983
2	30.831	Harmaline	8.414
3	43.604	Harmine	54.815

Inhibition rate

According to Doumbouya et al. (2012), the inhibition rate (IR) of mycelial growth compared to the control is calculated according to the formula:

$$IR (\%) = 100 \times (dC - dE) / dC$$

dC – Diameter of the control colony; dE – Diameter of the colony treated with the extract.

Table 3. Growth inhibition of *Mauginiella scaettae* in the presence of *P. harmala* seed extracts

Inhibition rate	72 h	96 h	120 h	144 h	168 h	Average IR
5%	21.96 ± 0.06	31.04 ± 0.06	11.21 ± 0.13	10.93 ± 0.12	4.52 ± 0.19	15.93
10%	86.68 ± 0.16	77.98 ± 0.21	66.32 ± 0.28	65.21 ± 0.30	61.69 ± 0.44	71.57
20%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
30%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
40%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
50%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100
100%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100

The antifungal tests according to the method of Grover & Moore (1962) showed that in the presence of the *Peganum harmala* extract we noted that there is great effectiveness in inhibiting of mycelial discs. The concentrations of extracts of *Peganum harmala* seeds have an inhibitory action on the mycelial growth of the strains of *Foa*, the inhibition also appeared at the lowest extract concentration (5%) with 15.93% inhibition rate and increased to a maximum (nearly 84%) with the pure extract (Tables 3 and 4). On *M. scaettae*, inhibition is significant (71.57%) from 10% extract, becoming total from 20% extract concentration. There is no mycelial growth in Petri plates treated with 20%, 30%, 40%, 50%, 100% extract concentration.

Table 4. Growth inhibitions of *Fusarium oxysporum* f.sp *albedinis in the* presence of *P. harmala* L. seed extract

Inhibition rate	72 h	96 h	12 0h	144 h	168 h	Average IR
5%	24.33 ± 1.15	20.59 ± 0.71	17.96 ± 0.07	27.84 ± 0.14	24.99 ± 0.28	23.14
10%	35.12 ± 1.73	36.29 ± 0.76	35.16 ± 0.08	40.52 ± 0.21	32.39 ± 0.13	35.90
20%	54.10 ± 1.53	39.24 ± 0.58	40.65 ± 0.12	45.58 ± 0.15	40.91 ± 0.23	44.09
30%	66.26 ± 0.29	56.88 ± 0.58	56.26 ± 0.06	58.22 ± 0.10	54.55 ± 0.06	58.44
40%	62.21 ± 0.29	60.82 ± 0.58	59.40 ± 0.06	62.67 ± 0.03	61.37 ± 0.06	61.29
50%	81.10 ± 0.76	74.53 ± 1.53	70.32 ± 0.06	70.91 ± 0.12	67.61 ± 0.07	72.89
100%	83.78 ± 0.06	85.29 ± 0.58	82.84 ± 0.12	84.81 ± 0.17	82.95 ± 0.20	83.94

Determination of the minimum inhibitory concentration (MIC)

The MIC corresponds to the lowest concentration of extract for which no growth of the treated fungus colony is observed, visible to the naked eye; therefore, it has a fungistatic effect and does not provide any information on the situation of the fungus population; particularly it does not allow to specify whether it has been partially or totally killed or whether it has simply stopped the growth (Berezin & Dellamonica, 1999). The fungi treated with concentrations that showed a total absence of mycelial growth were transferred to plates containing PDA medium to confirm the minimum inhibitory concentrations (MIC). We noted that a dose of 20% is the MIC for *Peganum harmala* L seed extract against *M. scaettae* with zero mycelial growth and even after a second mycelial disc subculture.

Mycelial growth rate (MG)

According to Cahagnier & Richard-Molard. (1998), the rate of mycelial growth at each concentration is determined by the formula:

$$VC = [D1/Te1] + [(D2-D1)/Te2] + [(D3-D2)/Te3] + ... + [(Dn-Dn-1)/Ten]$$

D – Diameter of the growth area of each day (mm); Te – Incubation time (day).

P. harmala L seed extract affects the mycelial growth rate of both *M. scaettae* and *Foa*, the average daily growth rate of which is low compared to the controls in all treated boxes (Fig. 1).

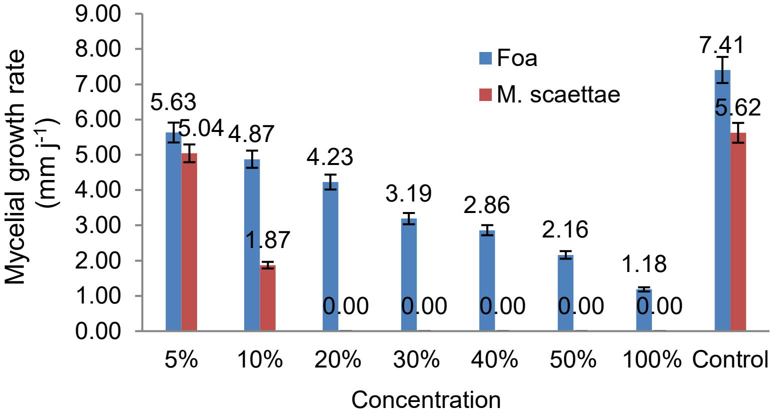

Figure 1. Mycelial growth rate of *M. scaettae* in *the* presence of different concentrations of *P. harmala* L. seed extract.

Statistical analysis

ANOVA reveals that all the concentrations of *P. harmala* L seed extract show a significant difference as an inhibitory extract of both phythopathogenic fungi (Table 5), *Fusarium oxysporum* f.sp *albedinis*, *Mauginiella scaettae* (*F* = 102.173; *P* = 0) (*F* = 129.9257; *P* = 0). The same was true for the 'time' factor (*F* = 302.490; *P* = 0) (*F* = 334.0321; *P* = 0). The interaction between 'factor', 'concentration' and 'time' (both factors) is significant. The antifungal action of *P. harmala* L seed extracts is highly significant on *M. scaettae* and significant on *Foa* (Fig. 1).

Table 5. Analysis of variance (ANOVA)

Effect	Pests	SS	Degree freedom	MS	*F*	*P*
Concentration	*Foa*	8,286.15	7	469.45	102.173	0.00000
	M. scaettae	4,804.140	7	686.306	129.9257	0.00000
Time	*Foa*	1,230.84	4	307.71	302.490	0.00000
	M. scaettae	444,158	4	111.04	334.0321	0.00000
Concentration x Time	*Foa*	245.38	28	8.76	8.76	0.00000
	M. scaettae	851.017	28	30.393	30.393	0.00000

The mycelial growth in the presence of the methanolic extract was evaluated during seven days of incubation, at the optimal growth temperature of *M. scaettae* (21 °C) and the optimal growth temperature of *Foa* (25 °C). The antifungal activity was estimated by comparing the mycelial growth of the boxes treated with the extracts at different concentrations with that of the control.

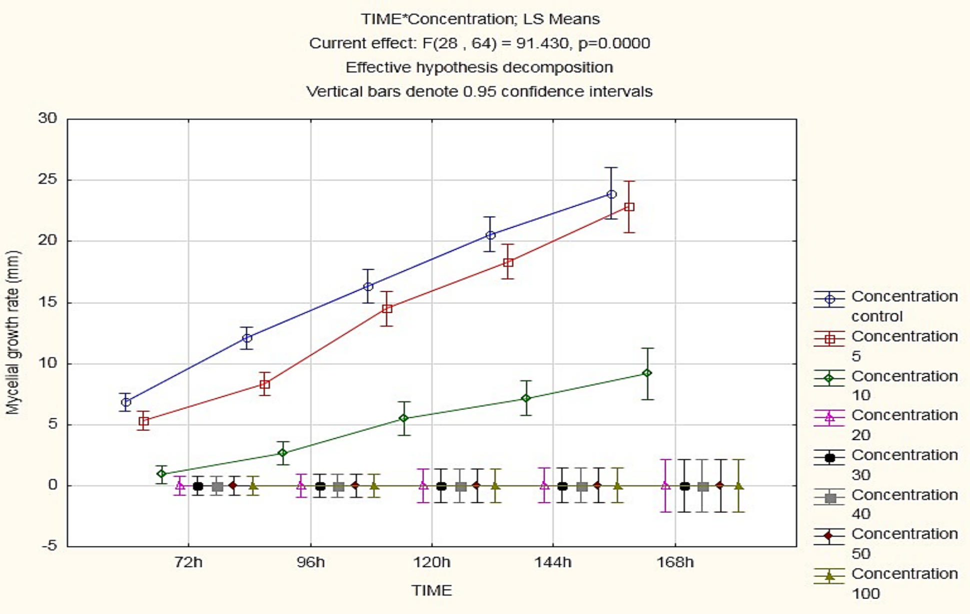

Figure 2. Effect of methanolic extract of *Peganum harmala* L. on the growth of *Mauginiella scaettae.*

The seed extract was more active on *M. scaettae* showing a fungistatic effect with slowed mycelial growth at 5% concentration and a fungicidal effect at 20% concentration at which mycelial growth becomes null (Fig. 2). The concentrations of extracts of *Peganum harmala* seeds have an inhibitory action on the mycelial growth of the strains of *Foa* (Fig. 3).

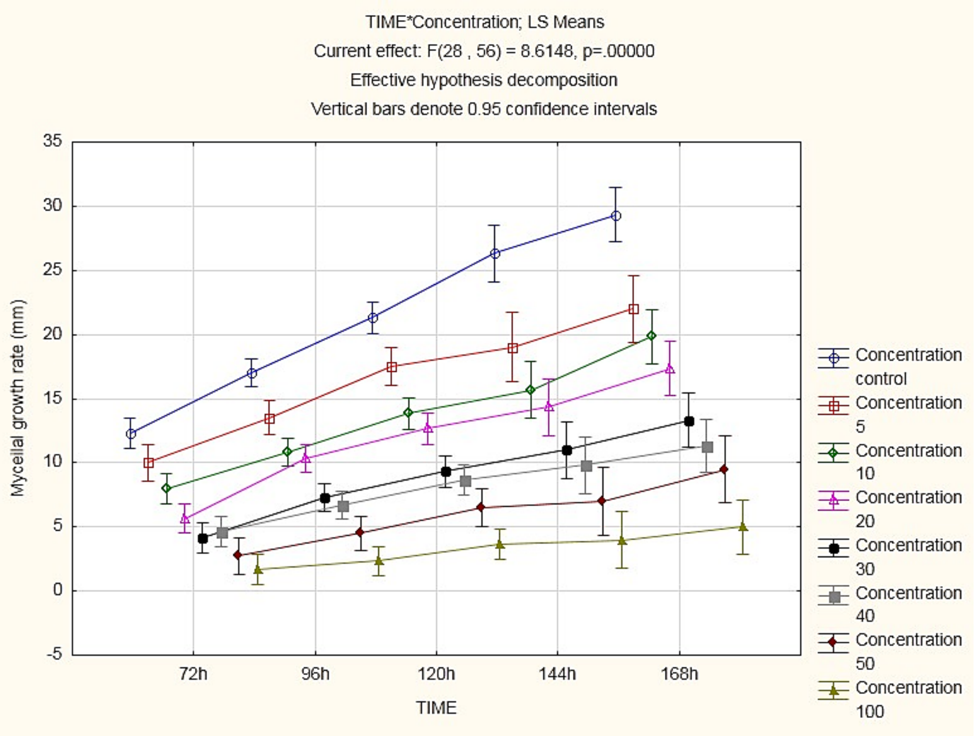

Figure 3. Effect of methanolic extract of *Peganum harmala* L. on the growth of *Fusarium oxysporum*f. sp. *albedinis*.

The antifungal activity of plant extracts is due to their biologically active compounds. The antifungal activity of this extract against *Mauginiella scaettae* is attributed mainly to the terpenoids found in high concentrations (+++) in the seed extract, wich have an antibacterial effect and were also reported by Shane et al. (1999).

Saadabi (2006) reports for several plant species and different solvents that compared to aqueous or chloroformic extraction, methanolic extracts have a more anti-fungal effect, an effect antimicrobien often associated among others with richness in alkaloids.

Many studies have noted that the alkaloids of *P. harmala* L. seeds have anti-microbial activity namely antifungal, antibacterial (Prashanth & John, 1999; Saadabi, 2006; Nenaah, 2010) and insecticidal (Rharrabe et al., 2007). This is demonstrated by the results related to the rates of inhibition of mycelial growth of *M. scaettae* and *Foa* (Tables 3 and 4).

The saponins are relatively abundant in the extracts of seeds. According to the work of Viollon & Chaumont (1994), saponins have an inhibitory effect on fungal growth. While terpenoids present in the seeds in good quantity are recognized for their antimicrobial activities and alkaloids have antibacterial activity (Kuc, 1985).

This result was accordance with these findings in previous reports by Herraiz et al., (2010); Aziz et al. (2017), who detected an amount of harmol, harmaline, and harmine in the *Peganum harmala* L seed methanol extract. On the other hand, Bukhari et al., 2008 and Iranshahy et al., 2019) indicated that the *P. harmala* methanol extract seeds contained harman alkaloids (harmine and harmaline) and the same substance was identified by the HPLC analysis of *P. harmala* collected from the mountain of Saint Katherine (Sinai, Egypt).

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According to the literature, the presence of alkaloids in our *P. harmala* L. seeds extract was elucidated using HPLC chromatography method. The quantitative analysis was carried out to detect the presence of four known alkaloids, namely harmol, harmine, harmaline, and peganine (Vasicine), in methanol seeds extracts of *P. harmala* L. which were confirmed by comparing the retention times on HPLC chromatogram spectra of the extract with the reference samples as shown in Fig. 2. By comparison with the same time as the external standard substance, we concluded that the first peak in HPLC chromatogram eluting at 18.435 min with 10.983% of average contents was in accordance with harmol, the second peak eluting at 30.831 min was tentatively identified as harmaline with an average content of 8.414%. The retention times for harmine were observed at 43.604 minutes with average contents of 54.815. We observed that the *P. harmala* seeds were produced four peaks in HPLC chromatogram and the peak corresponding to vasicine was observed which revealed that this bioactive molecule was also identified and quantified in the dry seeds (Herraiz et al., 2017; Abbas et al., 2021). According to these results, harmol, harmine, harmaline were the main alkaloids in extracts with a total average content of 74.212% and harmine was noted as the major β-carboline alkaloids compounds in this plant as shown in Fig. 3 and Table 1 this result was in accordance with the findings of Herraiz et al. (2010); Aziz et al. (2017)where they were detected amount of harmol, harmaline, and harmine in the *Peganum harmala* L seed methanol extract. They detected amount of harmol, harmaline, and harmine in the

Peganum harmala L. seeds methanol extract. On the other hand, (Bukhari et al., 2008; Iranshahy et al., 2019; Rofida et al., 2021) indicated that the *Peganum harmala* L. contains harman alkaloids (harmine and harmaline) and the same substance was identified by the HPLC analysis of *Peganum harmala* L. collected from the mountain of Saint Katherine (Sinai, Egypt). In addition, the three major alkaloids peganine, harmol, and harmine were detected by Sherif et al. (2021) in the plant extract isolated from dried mature seeds of *Peganum harmala* L.

CONCLUSIONS

The Saharian plants have different uses in traditional pharmacopoeia due to their active materials derived from secondary metabolites contained in their different organs, *Peganum harmala* L. has been reported as an insecticidal toxic plant in some tests. The different doses used were selected after several preliminary tests and meet the purpose of the work, which is the detection of the minimum inhibitory concentration of mycelial growth. The results of the tests showed that the seed extract of *Peganum harmala* L. has inhibitory activity on *Mauginiella scaettae* from 20% (v/v) concentration and therefore can be considered an effective antifungal agent to treat the inflorescence rot disease of date palm. This work can be deepened to determine the chemical compounds responsible for this antifungal bioactivity. Also, the results obtained on these two date palm pathogenic fungi are still preliminary and they have to be extended to other plants in the Algerian Sahara, as well as the execution of anti-microbial effect tests combined to their mixtures and the extension of these tests to other pathogens.

REFERENCES

Abbas, M.W., Hussain, M., Qamar, M., Ali, S., Shafiq, Z., Wilairatana, P. & Mubarak, M.S. 2021. Antioxidant and Anti-Inflammatory Effects of Peganum harmala Extracts: An In Vitro and In Vivo Study. *Molecules* **26**, 6084. https://doi.org/10.3390/molecules26196084

Aziz, M.A., Khan, A.H., Adnan, M. & Izatullah, I. 2017. Traditional uses of medicinal plants reported by the indigenous communities and local herbal practitioners of Bajaur Agency, Federally Administrated Tribal Areas. Pak. *J. Ethnopharmacol.* **198**, 268–281.

Babaousmail, M., Idder, M.A. & Kemassi, A. 2014. First Attempts to Repel Scale Insects Using Plant Extracts: Effect on The Date Palm Scale Parlatoria blanchardiTarg. (Hemiptera: Diaspididae). *World Journal of Environmental Biosciences* **7**(4), 59–63.

Berezin, B.E. & Dellamonica, P. 1999. *Antibiotic treatement in clinical practice*. ED Masson, pp. 51.

Bouabedelli, F., Missoun, F., Benhamimed, E. & Djebli, N. 2016. Phytochemical and antimicrobial study of the seeds and leaves of Peganum harmala L. against urinary tract infection pathogens. *Asian Pacific Journal of Tropical Disease* **6**, 822–826.

Bukhari, N., Choi, J., Jeon, C., Park, H., Kim, W., Khan, M. & Leet, S. 2008. Phytochemical studies of the alkaloids from Peganum harmala. *AppliedChem* **12**, 101–104.

Cahagnier, B. & Richard-Molard, D. 1998. Mycological analysis in molds of low hydratation foods. Ed. Tec & Doc, 1998, pp. 140–158.

Chehma, A. 2006. *Catalogue* of *Spontaneous Plants* in the *Northern* of *Algerian Sahara*. Dar Elhouda Ain M'lila, Algeria, ppp. 1–140 (in French).

De Corato, U., Trupo, M., Leone, G.P.G., Di Sanzo, G. Di., Zingarelli, G. & Adami, M. 2007. Antifungal activity of leaf extracts of laurel, sweet orange, and olive, obtained using the supercritical carbon dioxide technique. *Journal of Plant Pathology* **89**(3, Supplement), S27–S68.

Diallo, D. 2000. *Ethno pharmacological survey of medicinal plants in Mali and phytochemical study of four of them: Glinusoppositifolius (Azoceae), Diospyrosabyssinica (Ebenaceae), Entadaafricana (Minosaceae), Trichilia emetic (Meliaceae).* D. thesis, Faculty of Science, University of Lausanne, Switzerland, 42 pp.

Djerbi. 1983. Diseases of the Date Palm. FAO Regional Project for Palm and Dates Research Centre in the Near East and North Africa, 106 pp.

Dohou, R., Yamni, K., Tahrouch, S., Hassani, L. I., Badoc, A. & Gmira, N. 2003. Phytochemical screening of an endemic iberomarocaine, Thymelaealythroides. *Bulletin-Société de Pharmacie de Bordeaux* **142**(1/4), 61–78.

Doumbouya, M., Abo, K., Lepengue, A.N., Camara, B., Kankok, Aidara, D. & Kone, D. 2012. Comparative in vitro activities of two synthetic fungicides and two essential oils, on telluric fungi of vegetable cropsin Ivory Coast. *Journal of Applied Biosciences* **50**, 3520–3532. ISSN 1997-5902.

FAO, 2020.agricultural.statistics.https://www.fao.org/faostat/fr/#data/QCL.Consulté on 24 April 2022.

Grover, R.K. & Moorej, D. 1962. Toximetric studies of fungicides against brown rot organism Sclerotiniafructicola and S. laxa. *Phytopathology* **52**, 876–880.

Harborne. 1973. *Phytochemicalmethods. A guide to modern techniques of plants analysis*. Third Edition, 110 pp.

Herraiz, T., González, D., Ancín-Azpilicueta, C., Arán, H. & Guillén, V.J. 2010. Carboline alkaloids in Peganumharmala and inhibition of human monoamine oxidase (MAO). *Food Chem. Toxicol.* **48**, 839–845.

Herraiz, T., Guillén, H., Arán, V.J. & Salgado, A. 2017. Identification, occurrence and activity of quinazoline alkaloids in Peganumharmala. *Food Chem. Toxicol.* **103**, 261–269.

Iranshahy, M., Fazly-Bazzaz, S., Haririzadeh, G., Abootorabi, B.Z., Mohamadi, A.M. & Khashyarmanesh, Z. 2019. Chemical composition and antibacterial properties of Peganumharmala L. *Avicenna J Phytomed* **9**(6), 530–537. doi: 10.22038/AJP.2019.13382

Jinous, A. & Fereshteh, R. 2012. Chemistry, pharmacology and medicinal properties of Peganumharmala L. *African Journal of Pharmacy and Pharmacology* **6**(22), 1573–1580.

Koffi, A., Bla, K., Yapi, H., Bidie, A. & Djaman, A. 2015. Phytochemical Screening of Some Medicinal Plants in Côte D'ivoire and Evaluation of their Extraction Efficiency. *International Journal of Pharmacognosy and Phytochemical Research* **7**(3), 563–569.

Kuc, J. 1985. Increasing crop productivity and value by increasing disease resistance through nongenetic techniques. **In:** Forest potentials: productivity and value, Ballard R (ed), Weyerhaeuser Company Press, Centralia, 147–190.

Mibindzou, M.A. 2004. *Screening phytochimical of two plant species Crotaliaretusa L (papilionaceae) and HalleaciliataAubrev&Pelleger.* (*rubiaceae) collected in Gabon*, PhD thesis, Mali, 58 pp.

Naeini, A., Khosravi, A.R., Chitsaz, M., Shokri, H. & Kamelnejad, M. 2009. Anti Candidaalbicans activity of some Iranian plants used in traditional medicine. *Journal of Medical Mycology* **19**(3), 168–172.

Nenaah, G. 2010. Antibacterial and antifungal activities of (beta)-carboline alkaloids of Peganumharmala (L) seeds and their combination effects. *Fitoterapia* **81**, 779–782.

Ozenda, 1977. *Flore du Sahara*, 2eme ed paris 1977, 322 pp.

Pandey, D.K., Tripathin, N., Tripathir, D. & Dixit, S.N. 1982. Fungitoxic and phytotoxic properties of the essential oil of Hyptissuaveolens. *Journal of Plant Diseases and Protection* **89**(6), 344– 349.

Prashanth, D. & John, S. 1999. Antibacterial activity of Peganumharmala. *Fitoterapia* **70**(4), 438–439. ISSN 0367-326X

Rharrabe, K., Bakrim, A., Ghailani, N. & Sayah, F. 2007. Bioinsecticidal effect of harmalineonPlodiainterpunctelladevelopment (Lepidoptera Pyralidae). *Pestic. Biochem. Physiol*ogy **89**, 137–145.

Rofida, A.S., Tarek, F.E., Dalaal, M.A., Muhammed, A.S. & Hanan, S.E. 2021. *Peganum harmala* enhanced GLP-1 and restored insulin signaling to allivate ALCL3 induced Alzheimer like pathology model. *Scientifc. Reports* **11**, 12040 https://doi.org/10.1038/s41598-021-90545-4

Saadabi, A.M. 2006. Antifungal activity of some Saudi plants used in traditional medicine. *Asian J. Plant Sci.* **5**, 907–909.

Shane, G.G., Grant, S.W., Julie, L.M. & David, N.L. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour Fragr. Journal* **14**, 322–332.

Sherif, A.F, Marwa, Y.I., Basma, M.S., Meselhy, R.M. & Hassan, M.E.A. 2021. *Peganum harmala* Alkaloids Self-Assembled Supramolecular Nanocapsules with Enhanced Antioxidant and Cytotoxic Activities. *ACS Omega American chemical society* **6**(18), 11954–11963.

Tonk, S., Bartarya, R., Kumari, K.M., Bhatnagar, V.P. & Srivastava, S.S. 2006. Effective method for extraction of larvicidal component from leaves of Azadirachtaindicaand Artemisia annua Linn. *Journal of Environmental Biology* **27**(1), 103–105.

Trease, G.E. & Evans, W.C. 1989. *Pharmacognosy*. 11th Edition, Bailliere Tindall, London, 45–50.

Viollon, C. & Chaumont, J.P. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. *Mycopathologia* **128**(suppl 3), 151–153.

Zougagh, S., Belghiti, A., Rochd, T., Zerdani, I. & Mouslim, J. 2019. Medicinal and Aromatic Plants Used in Traditional Treatment of the Oral Pathology: The Ethnobotanical Survey in the Economic Capital Casablanca, Morocco (North Africa). *Natural Products and Bioprospecting* **9**, 35–48.

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Boron and magnesium foliar application increase grain yield of durum wheat under drought by improving some physiological parameters

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Abstract. Grain yield of wheat is primarily limited by water stress. Therefore, to increase productivity under drought conditions, a pot experiment was carried out at Maru Agricultural Research Station (MARS), Jordan, during the year 2021 to investigate the effect of foliar fertilizer by boron and magnesium under drought at either tillering or anthesis stages on some physiological parameters and yield components of two varieties of durum wheat. Foliar application by combined boron and magnesium had significantly improved the transpiration rate and relative water content (RWC) of wheat varieties at both tillering (4.39 µg cm^{-2}s^{-1} and 82.55%, respectively) and anthesis (7.43 µg cm^{-2}s^{-1} and 77.28%, respectively) growth stages, when compared with controls at tillering (3.56 µg cm^{-2}s^{-1} and 76.63%, respectively) and anthesis (5.79 µg cm^{-2}s^{-1} and 66.21%, respectively). RWC was significantly the highest by foliar boron (79.4%) at tillering stage under drought. Meanwhile, total chlorophyll content by SPAD was significantly the highest by combined boron and magnesium (46.8) during anthesis stage under drought. In general, the results indicated that var. Maru 1 had significantly higher grain yield (20.1 g/ plant) than var. Hourani (12.1 g/plant) may be due to differences in genetic makeup. Foliar application by combined boron and magnesium significantly increased wheat varieties' grain weight at tillering (18.2 g/plant) and anthesis (8.7 g/ plant) drought when compared with controls at either tillering (13.7 g/plant) or anthesis (5.5 g/plant) drought. However, foliar application did not significantly improve the grain weight under well-watered conditions. Our findings showed that the foliar application is more important at antheis drought than at tillering for increasing grain yield of wheat by improving of some physiological parameters.

Keywords: anthesis, micronutrient, transpiration rate, *Triticum durum*, water stress, yield component.

INTRODUCTION

Wheat (*Triticum durum*) is among the most crucial field crops grown under rainfed conditions in Jordan and considered essential for food security at the national and global level. Extreme climate changes and increased water scarcity challenge global food security, further impaired due to the need to feed a growing global population (Lesk et al., 2016; Munaweera et al., 2022). Drought is a major environmental (abiotic) stress and the most unpredictable constraint, adversely affecting crop production. Drought harms plants by disturbing many plant activities, including the carbon assimilation rate, decreased turgor, and changes in leaf gas exchange, thus causing a reduction in yield (Hussain et al., 2018). Reduced chlorophyll due to water stress causes chlorosis and reduces photosynthesis (Yang et al., 2001; Tyagi & Pandey, 2022). In addition, drought also reduces leaf relative water content (RWC) and stomatal conductance, ultimately leading to reduced growth and biomass production (Bayat et al., 2016; Caser et al., 2018). Moreover, drought stress can occur at any growth stage. However, the drought stress most commonly occurs after anthesis in wheat (Rebetzke et al., 2009; Ru et al., 2022). The yield losses of wheat during the reproductive phase due to drought might be due to its deleterious effects on morphological and physiological traits (Wasaya et al., 2021). Drought leads to tissue dehydration which may cause metabolic injury at later growth stages (Yang et al., 2003; Bandurska, 2022).

Under water stress, plant growth is inhibited due to the disruption of mineral nutrient transportation from the soil solution to the roots (Kohli et al., 2022). Low soil moistures restrict root growth and thus lowers the uptake of nutrients through the roots (Ge et al., 2012). Therefore, foliar fertilizer's efficiency is higher than soil application's under stress conditions (Hu et al., 2008). The supply of nutrients via the roots is restricted under drought because of the negative effect of drought on nutrient availability. The foliar application of different nutrients on different crops and at different growth stages can increase crops' tolerance mechanism and therefore enhance crop yield (Lavon et al., 1999; Tuiwong et al., 2022).

Boron (B) is required by plants in micro quantities and had stimulating resistance responses against drought stress (Awasthi et al., 2022). The nutritional supply of B resulted in improved stomatal conductance and carbon assimilation through full-sized leaf expansion (Waraich et al., 2011). B application improves growth, enhances plant stress tolerance, and improves grain production and water use efficiency (Hussain et al., 2012; Karim et al., 2012). In addition, magnesium (Mg) is a macronutrient required for chlorophyll synthesis and, thus is essential for the photosynthesis process by plants. Also, Mg enhanced drought tolerance and played a vital role in all the biochemical and physiological processes of plants through different pathways, such as the metabolism of carbohydrates and synthesis of proteins (Cakmak & Yazici, 2010).

Several studies have shown that foliar application of boron (Sarkar et al., 2007; Kutman et al., 2010; Naeem et al., 2018) and magnesium (Rodrigues et al., 2021), can increase the yield of crops. Precisely, we hypothesize that the combined application of foliar boron and magnesium could efficiently alleviate drought stress impacts on wheat than applying each solely; thus, the growth and yield of treated wheat increased significantly under such adverse conditions. However, limited or no information is available regarding the effect of the combined foliar application of boron and magnesium on the growth and yield of durum wheat under water stress. Therefore, this study was designed to evaluate the effect of B and Mg application alone and in combination in improving some physiological traits and yield components of two varieties of durum wheat grown under tillering or anthesis drought stress.

MATERIALS AND METHODS

Plant materials

This experiment used two durum wheat (*Triticum durum* L.) varieties (Hourani and Maru1). Maru1 is an improved variety released and registered in 2019–2020, while Hourani is an old variety (released in 1976) known as a drought-tolerant variety (Almeselmani et al., 2013).

Soil preparation and seed sowing

A pot experiment was conducted in a glasshouse at Maru Agricultural Research Station (MARS), Jordan. Seventy two pots (27 cm diameter × 27 cm height) were used for this experiment, and each pot was filled with 4 kg of clay soil mixed with peat (1:1) (v/v). About 2 liters of water were added to each pot until field capacity. Then, diammonium phosphate (18% N and 46% P_2O_5) fertilizer was applied to each pot at 10 g m^{-2}. Three sterilized wheat seeds were placed 2–3 cm below the soil surface. Wheat varieties were sown on 10th January, 2021. Plants were thinned to two seedlings per pot at the two-leaf stage one week after emergence.

Growth conditions and treatments

The temperature in the greenhouse was controlled at 25/15 °C (day/night). The greenhouse's relative humidity (RH) was maintained at approximately 60%. Pots were supplied with NPK fertilizer every week from the beginning of tillering. Foliar spraying was applied twice during the experiment; once at tillering drought and another at anthesis drought and compared with those sprayed at well-watered conditions. In more details, fertilizer treatments (Boron, Magnesium, and Boron+ Magnesium) were sprayed one day before beginning of drought either at tillering or anthesis growth stages, while controls were sprayed with distilled water. Boron was sprayed in the form of boric acid (H_3BO_3) at concentration of 0.3 g L^{-1} (% B in boric acid=17.48%), whereas magnesium in the form of magnesium sulphate ($MgSO_4.7H_2O$) at a concentration of 5 g L^{-1} (% Mg in magnesium sulphate=9.86%, Abou El-Nour & Shaaban, 2012). Each pot was received about 50 mL of foliar solution/ treatment until the leaf surface was wet. Drought was imposed by withholding watering for 7 days at at tillering (GS 22; D1) or anthesis (GS 65; D2) according to the Zadoks scale (Zadoks et al., 1974) on separate sets of plants, and compared with well watered (WW) plants which were regularly watered to field capacity. Pots were watered a twice per week during the vegetative stage, and three times per a week from anthesis stage until maturity. Drought at tillering stage was imposed from 15th February to 22th February, 2021 while those at anthesis stage from 20th March to 27th March, 2021.

Relative water content (RWC)

Relative water content (RWC) was determined according to the method of Slatyer (1967). Samples of about 1 cm^2 from the fully expanded leaves and flag leaves after one week of drought at tillering and anthesis stages, respectively were excised to determine RWC and placed in a cooler containing ice bricks. The samples were placed into small tubes and transferred within three hours after excision to the laboratory to determine the fresh weights (Wf). Leaf samples were placed in deionized water in small tubes kept overnight in a refrigerator at 4 °C. The following morning, leaf samples were carefully blotted with tissue paper, to remove excess water from the leaf surface, and re-weighed to determine turgid weight (Wt). Dry weight (Wd) was determined after oven drying the leaf samples for 24 hours at 80 °C. RWC was calculated as a percentage from the equation:

$$\text{RWC}\ \% = \frac{\text{Wf} - \text{Wd}}{\text{Wt} - \text{Wd}}\ \text{x}100 \qquad (1)$$

Physiological measurements

Transpiration rate (µg cm^{-2} s^{-1}) was measured with a portable steady state porometer (LICOR model LI-1600), while total chlorophyll content (TCC) was determined non-destructively using a portable chlorophyll meter; SPAD 502 Chlorophyll Meter (Spectrum Technologies Inc., Plainfield, IL, USA) on the same leaf as RWC prior to excision at the beginning and one week after plant stresses at either tillering or anthesis stages. Physiological measurements were made on mid-day between 12–2 pm.

Growth and yield components

At full maturity stage, the number of tillers and heads, and proportion of fertile tillers per plant were counted. The plants were harvested on 15th May, 2021 when plants had reached final maturity. The above-ground plant parts were harvested and separated into vegetative and head parts. Threshing separated the grains from heads, and grain weight was determined for each pot. Total dry weight of shoots was determined after drying in an oven at 80 °C for two days. The one thousand-grain weight was determined from the weight of 200- seeds per sample. Harvest index (HI) was calculated by dividing grain weight by total (grain plus shoot) weight.

Statistical analysis

The experiment was performed in a factorial completely randomized design with three factors: two wheat varieties (Hourani and Maru 1), three drought conditions: well-watered (WW), drought at tillering stage (D1) and drought at anthesis stage (D2), and four foliar fertilizer treatments (B, Mg, B+Mg and controls). There were three replicates for each treatment. Pots were placed randomly on the greenhouse bench. Data were analyzed by factorial ANOVA using Statistix 8.1 (Analytical Software 2005). When there were significant interactions, one-way ANOVA was used and means were separated by least significant differences (LSD).

RESULTS AND DISCUSSION

Effect of foliar fertilizer on some physiological measurements under tillering drought

Analysis of variance for variety, foliar fertilizer and drought treatments at tillering stage (D1) and their interaction effects on some physiological parameters is shown in Table 1. All treatments had a significant main effect on transpiration rate. However, only drought treatment significantly affected total chlorophyll content by SPAD, whereas relative water content (RWC) was significantly affected by foliar fertilizer and drought treatments.

Table 1. Analysis of variance (Mean squares values) showing the effect of foliar fertilizer and wheat varieties under tillering (D1) and anthesis (D2) drought conditions on transpiration rate (T), total chlorophyll content by SPAD, and relative water content (RWC)

		Mean Squares at D1			Mean Squares at D2		
Source of variation	DF	T	SPAD	RWC	T	SPAD	RWC
Variety (V)	1	2.25^{*}	0.20^{ns}	2.85^{ns}	0.29^{ns}	5.60^{ns}	34.1^{ns}
Foliar fertilizer (F)	3	1.63^{**}	12.09^{ns}	176.64^{**}	5.91^{**}	33.61^{**}	251.3^{**}
Drought (D)	1	27.99^{**}	406.58^{**}	$4{,}978.43^{**}$	742.53^{**}	$1{,}136.85^{**}$	$18{,}963.5^{**}$
V x F	3	0.084^{ns}	7.62^{ns}	2.02^{ns}	0.77^{ns}	3.87^{ns}	18.8^{ns}
V x D	1	4.01^{**}	0.630^{ns}	0.06^{ns}	0.11^{ns}	3.63^{ns}	42.2^{ns}
F x D	3	2.19^{**}	14.1^{ns}	157.96^{**}	1.465^{ns}	17.18^{**}	278.9^{**}
V x F x D	3	0.075^{ns}	11.66^{ns}	13.69^{ns}	0.89^{ns}	20.08^{**}	17.2^{ns}
Error	30	0.3175	12.23	23.32	0.82	3.56	19.5
CV (%)		13.79	7.11	5.89	13.37	3.88	6.11

*, ** and ^{ns}, denote significant at 5%, 1%, and not significant, respectively.

Effect of foliar fertilizer treatments for wheat varieties on transpiration rate, total chlorophyll content by SPAD and relative water content (RWC) at the beginning (day 0) and end (day 7) of tillering drought (D1) are presented in Table 2. Drought at day 7 significantly reduced transpiration rate, SPAD and RWC compared to day 0. There was no significant effect of wheat varieties on SPAD and RWC. However, transpiration rate was significantly ($P < 0.05$) higher in var. Maru 1 than those in var. Hourani. Moreover, controls had significantly lower transpiration rate and RWC than foliar fertilizer treatments by B, Mg, and B + Mg (Table 2).

Table 2. Mean values of transpiration rate, total chlorophyll content by SPAD, and relative water content (RWC) for two durum wheat varieties (Hourani and Maru 1) and four foliar fertilizer treatments (B, Mg, B+ Mg and controls) under beginning of tillering drought (Day 0) and end of tillering drought (Day 7). Values are the mean of three replications. According to least significant difference (*LSD*) test, different letters within the same columns indicate significant differences ($p < 0.05$). B: Boron; Mg: Magnesium; B+ Mg: combined boron and magnesium

Variety	Drought	Transpiration rate (μg cm^{-2} s^{-1})	SPAD	RWC (%)
Hourani	Day 0	4.92	52.06	91.93
	Day 7	2.82	46.47	71.49
Variety mean		3.87B	49.26A	81.71A
Maru 1	Day 0	4.78	52.16	92.34
	Day 7	3.83	46.11	72.05
Variety mean		4.30A	49.13A	82.19A
Foliar treatment				
B	Day 0	4.82	50.95	92.18
	Day 7	3.39	48.10	79.39
Treatment mean		4.11A	49.53A	85.78A
Mg	Day 0	4.86	52.85	92.79
	Day 7	3.73	45.32	72.89
Treatment mean		4.29A	49.08A	82.84A
B + Mg	Day 0	4.79	53.07	91.84
	Day 7	3.99	47.52	73.26
Treatment mean		4.39A	50.29A	82.55A

Table 2 (continued)

Control	Day 0	4.94	51.57	91.74
	Day 7	2.18	44.22	61.52
Treatment mean		3.56B	47.89A	76.63B
Drought mean				
	Day 0	4.85A	52.11A	92.13A
	Day 7	3.32B	46.29B	71.77B
LSD (0.05)				
Variety		0.33	2.06	2.85
Foliar treatment		0.47	2.92	4.03
Drought		0.33	2.06	2.85

There were significant ($P < 0.01$) foliar fertilizer × drought interaction effect for RWC (Fig. 1, A) and also variety x drought interaction for transpiration rate (Fig. 1, B). Foliar fertilizer treatments had no significant effect on RWC at day 0. However, Plant treated with B had significantly higher RWC than Mg or B+ Mg at day 7 (Fig. 1, A).

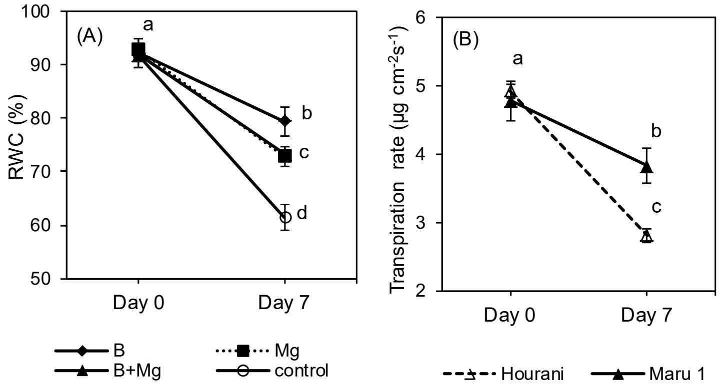

Figure 1. (A) Foliar fertilizer x drought interaction effect on RWC %, and (B) variety x drought interaction effect on transpiration rate during tillering stage. Lines with the same letter are not significantly different at $P < 0.05$ using least significant difference. Error bars show standard errors, $n = 3$.

There was no significant difference between wheat varieties on transpiration rate at day 0. However, transpiration rate was higher by 36% in var. Maru 1 at day 7 of drought (Fig. 1, B). Moreover, there were no significant differences between foliar treatments at day 0 of drought, while controls had significantly less transpiration rate than other foliar treatments at day 7 of drought (data not shown).

Effect of foliar fertilizer on some physiological measurements under anthesis drought

Table analysis of variance showed that foliar fertilizer and drought treatments had a high significant ($P < 0.01$) main effect on all physiological parameters of the study (Table 1). Foliar fertilizer x drought interaction was only high significant ($P < 0.01$) for SPAD and RWC measurements. Also, there was a high significant Variety x Foliar x Drought interaction effect on SPAD parameter.

Table 3 shows the main effect of foliar fertilizer treatments for wheat varieties on transpiration rate, SPAD and RWC at the beginning (day 0) and end (day 7) of anthesis drought (D2). Day 7 drought had significantly reduced transpiration rate, SPAD and RWC by 73%, 18% and 43%, respectively, compared with those at day 0. Additionally, measured physiological parameters revealed a significant reduction in controls compared to other foliar fertilizer treatments. However, B+ Mg treatment had significantly higher RWC than either B or Mg- treatments.

Table 3. Mean values of transpiration rate, total chlorophyll content by SPAD, and relative water content (RWC) for two durum wheat varieties (Hourani and Maru 1) and four foliar fertilizer treatments (B, Mg, B+ Mg and controls) under beginning of anthesis drought (Day 0) and end of anthesis drought (Day 7). Values are the mean of three replications. According to least significant difference (*LSD*) test, different letters within the same columns indicate significant differences ($p < 0.05$). B: Boron; Mg: Magnesium; B+ Mg: combined boron and magnesium

Variety	Drought	Transpiration rate ($\mu g\ cm^{-2}\ s^{-1}$)	SPAD	RWC (%)
Hourani	Day 0	10.84	53.39	92.28
	Day 7	2.88	43.11	50.65
Variety mean		6.8 A	48.25A	71.47A
Maru 1	Day 0	10.59	53.53	92.09
	Day 7	2.82	44.34	54.21
Variety mean		6.70A	48.93A	73.15A
Foliar treatment				
B	Day 0	10.78	54.33	92.55
	Day 7	2.92	43.45	52.48
Treatment mean		6.85A	48.89A	72.52B
Mg	Day 0	10.87	54.03	90.97
	Day 7	3.25	44.42	55.50
Treatment mean		7.06A	49.23A	73.23B
B + Mg	Day 0	11.01	53.28	92.53
	Day 7	3.85	46.83	62.03
Treatment mean		7.43A	50.06A	77.28A
Control	Day 0	10.21	52.18	92.69
	Day 7	1.38	40.20	39.72
Treatment mean		5.79B	46.19B	66.21C
Drought mean				
	Day 0	10.72A	53.46A	92.19A
	Day 7	2.85B	43.73B	52.43B
LSD (0.05)				
Variety		0.53	1.11	2.60
Foliar treatment		0.76	1.57	3.68
Drought		0.53	1.11	2.60

Foliar fertilizer x drought interaction significantly affected total chlorophyll content by SPAD at D2. Foliar fertilizer treatments did not significantly affect SPAD at day 0 of drought. However, B+ Mg treatment had significantly higher SPAD value than either B or Mg treatments at day 7 of drought (Fig. 2). This interaction effect was similar for RWC (data not shown) where B+ Mg treatment had significantly higher RWC (62%) than either B (52.48%) or Mg (55.5%) treatments at day 7 of drought but without significant effect at day 0 of drought.

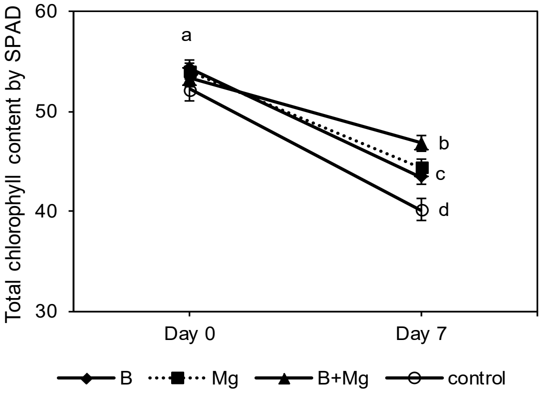

Figure 2. Foliar fertilizer x drought interaction effect on total chlorophyll content by SPAD during anthesis stage. Lines with the same letter are not significantly different at $P < 0.05$ using least significant difference. Error bars show standard errors, $n = 3$.

Fig. 3 shows Variety x Foliar fertilizer x Drought interaction effect on total chlorophyll content by SPAD during anthesis stage. Only B-treatment in var. Hourani had significantly higher SPAD values than controls of var. Maru 1 at day 0 of drought. However, there were no significant differences between controls of var. Maru 1 and B-treatment of var. Hourani at day 7 of drought.

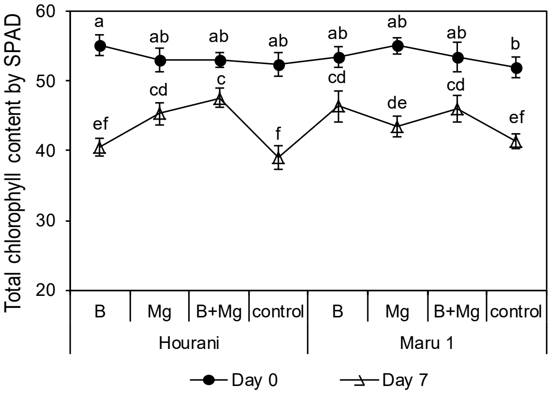

Figure 3. Variety x Foliar fertilizer x Drought interaction effect on total chlorophyll content by SPAD during anthesis stage. Lines with the same letter are not significantly different at $P < 0.05$ using least significant difference. Error bars show standard errors, $n = 3$.

Foliar application effectively improved wheat transpiration rate only under drought conditions. Similarly, Karim et al. (2012) found that the foliar application of boron (B) significantly increased transpiration of winter wheat under drought stress. It has been suggested that plant mineral nutrient status plays a vital role in improving plant resistance to stress conditions (Nadim et al., 2013). The response of micronutrient application to various abiotic stresses depends on the crop, growth stage and concentration of the nutrient solution (Siddiqui et al., 2022). The key mechanisms affecting the ability of micro-and macro-nutrients to alleviate the effects of drought stress include enhancing water uptake and transport, regulating stomatal behavior and transpirational water loss (Waraich et al., 2011; Wang et al., 2021). Our study showed that the efficiency of foliar fertilizers was not significantly different for transpiration rate. Putra et al. (2012) found a similar finding on *Musa* sp. Moreover, transpiration rate of both wheat varieties significantly varied at tillering drought. It is well documented that wheat varieties grown under drought conditions demonstrate natural genetic difference in traits related to drought tolerance (Budak et al., 2013).

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This study showed that drought significantly reduced the transpiration rate of both wheat varieties under different growth stages. Similar results were obtained by Wasaya et al. (2021); Marček et al. (2019) and Karim et al. (2012). Our results showed that wheat transpiration at anthesis drought was lower approximately 14% than at tillering drought. This may due to a higher water uptake of larger root biomass at anthesis time. Similarly, Aldahadha et al. (2019) found that transpiration rates during the anthesis were higher than during the vegetative phase. Therefore, the soil water supply is more rapidly exhausted at anthesis drought (Morgan, 1977).

Foliar application by combined boron and magnesium had increased total chlorophyll content by SPAD at anthesis drought. These results are consistent with other studies (Saad & El-Kholy 2000; Thalooth et al., 2006), indicating that Mg had a main role in chlorophyll formation, activation of enzymes, and it may increase plant resistance to water stress. Similarly, foliar application by B increased total chlorophyll content at late growth stages of winter wheat (Karim et al., 2012). Thus, these findings indicated the prominence of foliar application by combined boron and magnesium to reduce the harmful effects of drought stress that often occur during anthesis. Our results also revealed that total chlorophyll content of wheat was decreased by drought with higher reduction in SPAD values was recorded at anthesis growth stage. Similarly, a decrease in photosynthetic pigments including chlorophyll content in wheat was observed previously with increasing of drought (Kalaji et al., 2016; Chowdhury et al., 2021). Nikolaeva et al. (2010) found that the chlorophyll content decreased by 13–15% only after a 7-day drought period. Abdelkader et al. (2007) documented that the decrease in chlorophyll content as one of the most important limiting factors for plant photosynthetic activity under drought conditions.

It is well known that relative water content (RWC) is an important characteristic that measures water status in plants reflecting the ongoing metabolic activities in tissues and that may be used as a reliable indicator of drought tolerance (Chowdhury et al., 2021). The present study revealed that drought reduced RWC in both wheat varieties. These results were in close agreement with the findings obtained by Chowdhury et al. (2021). A higher reduction in RWC during anthesis drought may be due to higher transpiration rates during anthesis, similar to a wheat study reported by Aldahadha et al. (2019). In the current study, foliar fertilizer significantly increased RWC of wheat under drought conditions. Similar results were obtained by Wasaya et al. (2017) and Ahmad et al. (2019) who found that the foliar application of macro and micro-nutrients improved RWC for some crops. Higher RWC under combined boron and magnesium might be due to higher chlorophyll formation during the drought, whereas the increase in RWC by application of foliar boron might be due to leaf membrane stability (Sayed, 1998) and higher resistance against abiotic stresses (Shehzad et al., 2018; Awasthi et al., 2022).

Effect of foliar fertilizer on growth and yield component of wheat under drought

Analysis of variance for variety, foliar fertilizer and drought treatments and their interaction effects on several wheat growth and yield parameters is presented in Table 4. Wheat variety and drought treatments had a high significant ($P < 0.01$) main effect on all measured wheat growth and yield components. However, foliar fertilizer treatments had a high significant ($P < 0.01$) effect on grain number/plant (GN), grain weight/plant (GW), 1,000-grain weight/plant (1000-GW) and dry matter weight/ plant (DMW) only. There was a high significant variety x drought interaction for all measured growth and yield components except for 1000-GW, DMW, and harvest index (HI). However, foliar fertilizer x drought interaction was only significant ($P < 0.05$) for 1000-GW and GW (Table 4).

Table 4. Analysis of variance (Mean Squares values) showing the effect of foliar fertilizer and wheat varieties under different drought conditions on tiller number/plant (TN), head number/plant (HN), grain number/plant(GN/plt), 1,000-grain weight/ plant (1000-GW), dry matter weight/plant (DMW/plt) and harvest index (HI)

Mean Squares								
Source of variation	DF	TN	HN	GN/plt	1000-GW	GW/plt	DMW/plt	HI
Variety (V)	1	12.50**	10.12**	366,439**	501.92**	1,171.76**	188.82**	0.1303**
Foliar fertilizer (F)	3	0.84^{ns}	1.13^{ns}	14,615**	65.77**	40.75**	53.70**	0.0015^{ns}
Drought (D)	2	40.07**	60.51**	245,353**	2,537.6 **	1,385.97**	768.01**	0.1400**
V x F	3	0.51^{ns}	0.48^{ns}	223^{ns}	4.17^{ns}	1.93^{ns}	12.91^{ns}	0.0011^{ns}
V x D	2	2.69**	3.04**	18,205**	2.26^{ns}	88.80**	13.49^{ns}	0.0012^{ns}
F x D	6	0.08^{ns}	0.21^{ns}	2,723^{ns}	13.38*	6.08*	12.60^{ns}	0.0017^{ns}
V x F x D	6	0.34^{ns}	0.43^{ns}	2,431^{ns}	4.35^{ns}	2.20^{ns}	1.50^{ns}	0.0012^{ns}
Error	46	0.52	0.48	1,930	5.39	2.38	7.34	0.0015
CV (%)		7.78	7.83	11.52	5.81	9.59	13.1	9.55

*, ** and ^{ns}, denote significant at 5%, 1%, and not significant, respectively.

The main means of foliar fertilizer and drought treatments for growth and yield of the two wheat varieties are presented in Table 5. Number of heads (HN) and tillers (TN) per plant were significantly different ($P < 0.01$) between wheat varieties and drought treatments. The HN and TN for var. Maru 1 was significantly higher than those for var. Hourani. Moreover, well-watered (WW) plants had significantly higher HN and TN than those droughted at either D1 or D2. HN was reduced by 13% and 30% for D1 and D2 treatments, respectively, compared to WW treatment. However, foliar treatments did not significantly affect HN and TN (Table 5).

Number of grains (GN) per plant, grain weight (GW) per plant and 1,000-grain weight (TGW) were significantly affected by wheat varieties, drought and foliar treatments (Table 5). Variety Maru1 showed significantly a higher GN, GW and TGW per plant ($P < 0.01$) than var. Hourani. Both D1 and D2 significantly reduced GN, GW and TGW per plant compared with WW treatment, with higher reduction in D2 than in D1. The GW per plant was decreased by 26% and 65% at D1 and D2, respectively when compared with WW conditions. Yet, drought had a smaller effect on grain number than grain weight. In comparison with WW plants, D1 and D2 reduced the GN per plant by 19% and 42%, respectively. On the other hand, plants that were not sprayed by foliar fertilizer (controls) had significantly reduced GW, GN and TGW per plant by 18%, 16% and 6%, respectively when compared with those sprayed by boron and magnesium combination (B + Mg).

Table 5. Main effect of wheat varieties (Hourani and Maru 1), foliar fertilizer treatments and drought treatments on growth and yield components

Main effect	TN	HN	GN	TGW (g)	GW (g)	DMW (g)	HI
Variety							
Hourani	9.0b	8.5b	310.1b	37.3b	12.1b	19.1b	0.37b
Maru 1	9.8a	9.3a	452.8a	42.6a	20.1a	22.3a	0.46a
Foliar treatment							
B	9.5a	9.1a	396.4a	40.3b	16.8a	21.3a	0.42a
Mg	9.5a	9.0a	383.1a	42.0a	16.8a	21.4a	0.42a
B+ Mg	9.3a	9.0a	405.2a	40.0b	16.9a	21.9a	0.42a
Control	9.0a	8.5a	340.9b	37.4c	13.8b	18.1b	0.40a
Drought treatment							
WW	10.6a	10.4a	479.1a	48.0a	23.2a	26.9a	0.46a
D1	9.3b	9.0b	388.0b	44.0b	17.1b	19.3b	0.46a
D2	8.1c	7.3c	277.2c	28.3c	8.1c	15.9c	0.33b
LSD (0.05)							
Variety	0.34	0.33	20.84	1.10	0.73	1.29	0.019
Foliar treatment	0.49	0.47	29.48	1.56	1.04	1.82	0.027
Drought	0.42	0.40	25.53	1.35	0.89	1.58	0.023

B: Boron; Mg: Magnesium; B+ Mg: combined boron and magnesium; WW: well-watered; D1: drought at tillering; D2: drought at anthesis; TN: number of tillers per plant; HN: number of heads per plant; GN: number of grains per plant; TGW: 1000-grain weight; GW: grain weight per plant; DMW: shoot dry weight per plant excluding grain; HI: harvest index; *LSD*: least significant difference at $P < 0.05$. Figures labeled with the same letter in each column are not significantly different.

Dry matter weight (DMW) per plant was similarly affected by treatments as the GW and GN (Table 5). Variety and drought at anthesis (D2) significantly affected harvest index (HI). The overall mean HI of var. Maru1 (0.46) was significantly higher than HI of var. Hourani (0.37). There was no difference between HI of WW and D1 plants; however, HI of D2 was reduced to 0.33. The main effect of foliar fertilizer treatments was not significant for HI.

The interaction effect between variety and drought treatments on HN and GW per plant is presented in Fig. 4. The HN per plant was significantly higher in var. Maru1 than var. Hourani under both WW and D1 conditions, but there was no significant difference between the two varieties at D2 (Fig. 4, A). The reduction of GW per plant in var. Hourani was significantly higher at WW and D1 than D2 compared to var. Maru 1 (Fig. 4, B).

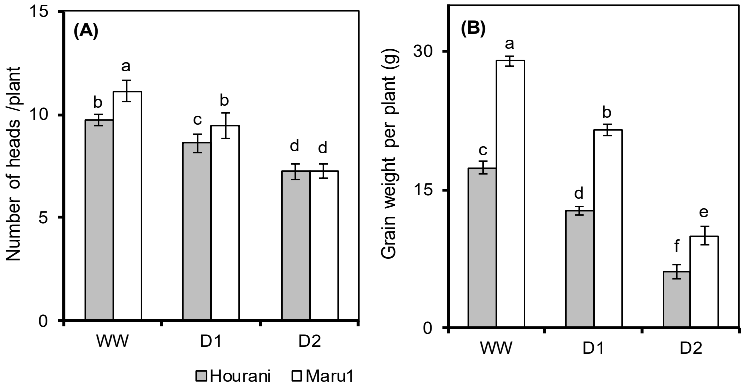

Figure 4. Variety x drought interaction effect on (A) number of heads per plant and (B) grain weight per plant. Columns with the same letter are not significantly different at $P < 0.05$ using least significant difference. Error bars show standard errors, $n = 3$.

There was significant ($P < 0.05$) foliar fertilizer × drought interaction effect for GW per plant (Fig. 5). Combined foliar fertilizer (B+ Mg) treatment did not improve GW under WW conditions compared to controls. However, when compared with controls, B+ Mg treatment had significantly increased the GW per plant by 33% and 57% at D1 and D2, respectively. Similarly, combined foliar boron and magnesium had improved 1000-GW by 6% and 15% at D1 and D2, respectively (data not shown).

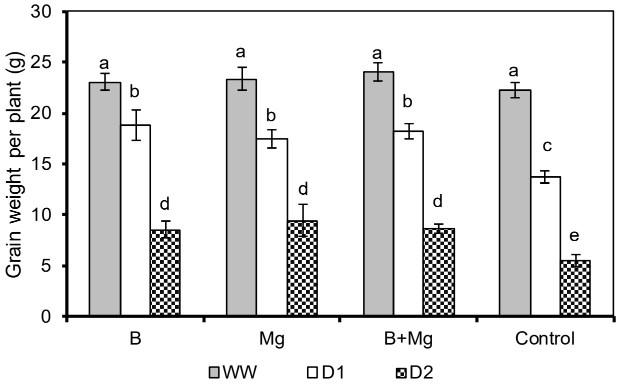

Figure 5. Foliar fertilizer x drought interaction effect on grain weight per plant. Columns with the same letter are not significantly different at $P < 0.05$ using least significant difference. Error bars show standard errors, $n = 3$.

In general, wheat yield and yield components were reduced when the drought was imposed, and its effect was more significant under anthesis drought than tillering drought. A reduction in the growth and metabolic activities leads to a reduction in agronomic and yield attributes under drought conditions (Hussain et al., 2018). Water stress occurring during reproductive phase caused a greater reduction in grain weight and number per plant and in the number of tillers and heads per plant (Aldahadha et al., 2019; Agrawal et al., 2021). A higher reduction in grain yield parameters under anthesis drought might be due to less retention of RWC, reflecting fewer metabolic activities (Clarke & McCaig, 1982). The reduced number of grains may be due to low spikelet per spike and spike length under drought. Thus, the flowering stage proved to be the most sensitive to water deficit. Drought stresses at vegetative or flowering stage considerably decreased total biomass of wheat. Similar findings were obtained by Blum (2005) and Bavita et al. (2015). Decreased 1,000-grain weight was reported by Plaut et al. (2004) under drought at flowering stage due to less efficient and disturbed nutrient uptake and limited photosynthetic translation within the plant which hastened maturity producing shriveled kernels. Reduced yield and yield-related traits under water stress might be due to a reduction in chlorophyll and photosynthetic parameters including stomatal conductance and transpiration rate (Wasaya et al., 2021). Our results also indicated significant differences between both wheat varieties in terms of yield due to a difference in genetic makeup of variety. Yield differences between varieties were greater under well-watered and tillering drought conditions, indicating higher drought tolerance at anthesis stage.

Our study demonstrated that the foliar application increased grain yield of durum wheat under different drought conditions may be due to the crucial role of fertilizers in enhancing photosynthesis, transpiration rate, pollen viability, number of grains per spike and higher concentrations of these nutrients in the grain. Similar results were obtained by Karim et al. (2012) and Abdel-Motagally & El-Zohri (2018). Our findings also agreed with the results of Karim et al. (2012) who found that winter wheat grain yield was not improved by foliar applications in the absence of drought. Furthermore, the foliar application of B and Mg was more effective at anthesis drought for improving the grain yield and 1000-grain weight. These results were similar to findings of Aown et al. (2012) who found that the foliar application of potassium was the most effective at anthesis stage. However, Abdel-Motagally & El-Zohri (2018) found that booting stage was the best time for boron application to get higher grains production.

CONCLUSIONS

Drought stress at either tillering or anthesis growth stage inhibits durum wheat varieties' physiological, growth and yield parameters. Yield reduction was greater during anthesis drought than during tillering. Therefore, exogenous application of B and Mg on wheat under drought alleviated the negative effects of water deficit. Results of this pot experiment revealed that foliar application of B and Mg had enhanced leaf transpiration rate and relative water contents, which improved wheat crop yield. However, the foliar application of B and Mg in combination performed similar as in single nutrient. The results from this study showed the significance of foliar application at anthesis stage for improvement wheat yield under drought conditions. It is a highly recommended to apply a combined foliar boron and magnesium or as a single form by either boron or magnesium especially during the late drought for achieving a higher wheat grain yield. Further study is required to examine the effect of foliar application and drought on wheat yield and growth under field conditions.

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REFERENCES

Abdelkader, A.F., Aronsson, H. & Sundqvist, C. 2007. High salt-stress in wheat leaves (*Triticum aestivum*) causes retardation of chlorophyll accumulation due to a limited rate of protochlorophyllide formation. *Physiologia Plantarum* **130**(1), 157–166. doi: 10.1111/j.1399-3054.2007.00885.x

Abdel-Motagally, F.M.F. & El-Zohri, M. 2018. Improvement of wheat yield grown under drought stress by boron foliar application at different growth stages. *Journal of the Saudi Society of Agricultural Sciences* **17**(2), 178–185. doi: 10.1016/j.jssas.2016.03.005

Abou El-Nour, E.A. & Shaaban, M.M. 2012. Response of wheat plants to magnesium sulphate fertilization. *American Journal of Plant Nutrition and Fertilization Technology* **2**, 56–63.

Agrawal, S., Vinaya, K.Y., Verma, A.K. & Mishra, R.C. 2021. Studies on foliar application of Thio-urea on morphophysiological and yield potential in different wheat [*Triticum aestivum* L.] under two conditions. *The Pharma Innovation Journal* **10**(10), 1049–1052.

Ahmad, S., Kamran, M., Ding, R., Meng, X., Wang, H., Ahmad, I., Fahad, S. & Han, Q. 2019. Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic capacity and antioxidant defense system of maize seedlings. *Peer. J.* **7**, e7793. doi: 10.7717/peerj.7793

Aldahadha, A., Warwick, N.W.M. & Backhouse, D. 2019. Water relations and yield of wheat (*Triticum aestivum* L.) exposed to interactions of drought and fungal root diseases (*Rhizoctonia* and *Pythium*). *Archives of Agronomy and Soil Science* **65**(4), 507–520. doi: 10.1080/03650340.2018.1511893

Almeselmani, M., Saud, A., Al Zubi, K., Hareri, F., Al-Nasan, M., Ammar, M.A., Kanbar, O.Z. & Al-Naseef, H. 2013. Physiological traits associated with yield improvement under rainfed condition in new wheat varieties. *Jordan Journal of Agricultural Sciences* **9**(1), 12–23.

Analytical Software. 2005. Statistix 8.1 User's Manual. Analytical Software, Tallahassee, Florida. USA.

Aown, M., Raza, S., Saleem, M.F., Anjum, S.A., Khaliq, T. & Wahid, M.A. 2012. Foliar application of potassium under water deficit conditions improved the growth and yield of wheat (*Triticum aestivum* L.). *The Journal of Animal and Plant Sciences* **22**(2), 431–437.

Awasthi, S., Chauhan, R. & Srivastava, S. 2022. The importance of beneficial and essential trace and ultratrace elements in plant nutrition, growth, and stress tolerance. In *Plant nutrition and food security in the era of climate change*, 27–46. Academic Press. Academic Press. doi: 10.1016/B978-0-12-822916-3.00001-9

Bandurska, H. 2022. Drought stress responses: coping strategy and resistance. *Plants* **11**(7), 922. doi: 10.3390/plants11070922

Bavita, A., Kaur, R. & Bains, N.S. 2015. Variation of invertase activities in four wheat cultivars as influenced by thiourea and high temperature. *Acta Physiologiae Plantarum* **37**, 1712–1730. doi: 10.1007/s11738-014-1712-8

Bayat, H., Nemati, H., Tehranifar, A. & Gazanchian, A. 2016. Screening different crested wheatgrass (*Agropyron cristatum* L. Gaertner) accessions for drought stress tolerance. *Archives of Agronomy and Soil Science* **62**(6), 769–780. doi: 10.1080/03650340.2015.1094182

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential–are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* **56**(11), 1159–1168. doi: 10.1071/AR05069

Budak, H., Kantar, M. & Kurtoglu, K.Y. 2013. Drought tolerance in modern and wild wheat. *Scientific World Journal* **2013**, 1–16. Article ID 548246. doi: 10.1155/2013/548246

Cakmak, I. & Yazici, A.M. 2010. Magnesium: a forgotten element in crop production. *Better Crops* **94**(2), 23–25.

Caser, M., Angiolillo, F.D., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, L., Pistelli, L. & Scariot, V. 2018. Ecophysiological and phytochemical responses of *Salvia sinaloensis* Fern. to drought stress. *Journal of Plant Growth Regulation* **84**, 383–394. doi: 10.1007/s10725-017-0349-1

Chowdhury, M.K., Hasan, M.A., Bahadur, M.M., Islam, M.R., Hakim, M.A.M., Iqbal, A., Javed, T., Raza, A., Shabbir, R., Sorour, S., Elsanafawy, N.E.M., Anwar, S., Alamri, S., Sabagh, A.E. & Islam, M.S. 2021. Evaluation of drought tolerance of some wheat (*Triticum aestivum* L.) genotypes through phenology, growth, and physiological indices. *Agronomy* **11**(9), 1792. doi:10.3390/agronomy11091792

Clarke, J.M. & McCaig, T.N. 1982. Evaluation of techniques for screening for drought resistance in wheat. *Crop Science* **22**(3), 503–506. doi: 10.2135/cropsci1982.0011183X002200030015x

Ge, T., Sui, F., Bai, L., Tong, C. & Sun, N. 2012. Effects of water stress on growth, biomass partitioning, and water-use efficiency in summer maize (*Zea mays* L.) throughout the growth cycle. *Acta Physiologiae Plantarum* **34**, 1043–1053. doi: 10.1007/s11738-011-0901-y

Hu, Y., Burucs, Z. & Schmidhalter, U. 2008. Effect of foliar fertilization application on the growth and mineral nutrient content of maize seedlings under drought and salinity. *Soil Science and Plant Nutrition* **54**(1), 133–141. doi: 10.1111/j.1747-0765.2007.00224.x

Hussain, M., Khan, M.A., Khan, M.B., Farooq, M. & Farooq, S. 2018. Drought stress in sunflower: physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management Journal* **201**, 152–166. doi: 10.1016/j.agwat.2018.01.028

Hussain, M., Farooq, S., Hasan, W., Ul-allah, S. & Tanveer, M. 2012. Boron application improves the growth, yield and net economic return of rice. *Rice Science* **19**(3), 259–262. doi: 10.1016/S1672-6308(12)60049-3

Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., Cetner, M.D., Łukasik, I., Goltsev, V. & Ladle, R.J. 2016. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum* **38**(102), 1–11. doi: 10.1007/s11738-016-2113-y

Karim, M.R., Zhang, Y.Q., Zhao, R.R., Chen, X.P., Zhang, F.S. & Zou, C.Q. 2012. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. *Journal of Plant Nutrition and Soil Science* **175**(1), 142–151. doi: 10.1002/jpln.201100141

Kohli, S.K., Kaur, H., Khanna, K., Handa, N., Bhardwaj, R., Rinklebe, J. & Ahmad, P. 2022. Boron in plants: uptake, deficiency and biological potential. *Plant Growth Regulation* **100**(2), 267–282. doi: 10.1007/s10725-022-00844-7

Kutman, U.B., Yildiz, B., Ozturk, L. & Cakmak, I. 2010. Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chemistry* **87**(1), 1–9. doi: 10.1094/CCHEM-87-1-0001

Lavon, R., Salomon, R. & Goldschmidt, E.E. 1999. Effect of potassium, magnesium and calcium deficiencies on nitrogen constituents and chloroplast components in citrus leaves. *Journal of the American Society for Horticultural Science* **124**(2), 158–162. doi: 10.21273/JASHS.124.2.158

Lesk, C., Rowhani, P. & Ramankutty, N. 2016. Influence of extreme weather disasters on global crop production. *Nature* **529**(7584), 84–87. doi: 10.1038/nature16467

Marček, T., Hamow, K.A., Végh, B., Janda, T. & Darko, E. 2019. Metabolic response to drought in six winter wheat genotypes. *PloS One* **14**(2), e0212411. doi: 10.1371/journal.pone.0212411

Morgan, J.M. 1977. Changes in diffusive conductance and water potential of wheat plants before and after anthesis. *Australian Journal of Plant Physiology* **4**(1), 75–86. doi: 10.1071/PP9770075

Munaweera, T.I., Jayawardana, N.U., Rajaratnam, R. & Dissanayake, N. 2022. Modern plant biotechnology as a strategy in addressing climate change and attaining food security. *Agriculture and Food Security* **11**(1), 1–28. doi: 10.1186/s40066-022-00369-2

Nadim, M.A., Awan, I.U., Baloch, M.S., Naeem, K. & Khalid, N. 2013. Micronutrient use efficiency in wheat as affected by different application methods. *Pakistan Journal of Botany* **45**(3), 887–892.

Naeem, M., Naeem, M.S., Ahmad, R., Ahmad, R. & Ashraf, M.Y. 2018. Improving drought tolerance in maize by foliar application of boron: water status, antioxidative defense and photosynthetic capacity. *Archives of Agronomy and Soil Science* **64**, 626–39. doi: 10.1080/03650340.2017.1370541

Nikolaeva, M.K., Maevskaya, S.N., Shugaev, A.G. & Bukhov, N.G. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russian Journal of Plant Physiology* **57**, 87–95. doi: 10.1134/S1021443710010127

Plaut, Z., Butow, B.J., Blumenthal, C.S. & Wrigley, W. 2004. Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. *Field Crops Research* **86**(2–3), 185–198. doi:10.1016/j.fcr.2003.08.005

Putra, E.T.S., Zakaria, W., Abdullah, N.A.P. & Saleh, G.B. 2012. Stomatal morphology, conductance and transpiration of Musa sp. cv. Rastali in relation to magnesium, boron and silicon availability. *American Journal of Plant Physiology* **7**(2), 84–96. doi: 10.3923/ajpp.2012.84.96

Rebetzke, G.J., Chapman, S.C., McIntyre, C.L., Richards, R.A., Condon, A.G., Watt, M. & Van-Heerwarden, A.F. 2009. Grain yield improvement in water–limited environments. In *Wheat science and trade*. Ed. B. Carver, 215–249. Ames (IA): Wiley-Blackwell. doi: 10.1002/9780813818832.ch11

Rodrigues, V.A., Crusciol, C.A.C., Bossolani, J.W., Moretti, L.G., Portugal, J.R., Mundt, T.T., de Oliveira, S.L., Garcia, A., Calonego, J.C. & Lollato, R.P. 2021. Magnesium foliar supplementation increases grain yield of soybean and maize by improving photosynthetic carbon metabolism and antioxidant metabolism. *Plants* **10**(4), 797. doi: 10.3390/plants10040797

Ru, C., Hu, X., Chen, D., Song, T., Wang, W., Lv, M. & Hansen, N.C. 2022. Nitrogen modulates the effects of short-term heat, drought and combined stresses after anthesis on photosynthesis, nitrogen metabolism, yield, and water and nitrogen use efficiency of wheat. *Water* **14**(9), 1407. doi: 10.3390/w14091407

Saad, A.O.M. & El-Kholy, M.A. 2000. Response of some faba bean to phosphorus and magnesium fertilization. *Egyptian Journal of Agronomy* **22**, 19–32.

Sarkar, D., Mandal, B. & Kundu, M.C. 2007. Increasing use efficiency of boron fertilizers by rescheduling the time and methods of application for crops in India. *Plant and Soil* **301**, 77–85. doi: 10.1007/s11104-007-9423-1

Sayed, S.A. 1998. Impacts of boron application on maize plants growing under flooded and unflooded conditions. *Biologia plantarum* **41**(4), 101–109. doi: 10.1023/A:1001720702268

Shehzad, M.A., Maqsood, M., Nawaz, F., Abbas, T. & Yasin, S. 2018. Boron-induced improvement in physiological, biochemical and growth attributes in sunflower (*Helianthus annuus* L.) exposed to terminal drought stress. *Journal of Plant Nutrition* **41**(8), 943–955. doi: 10.1080/01904167.2018.1431663

Siddiqui, H., Singh, P., Arif, Y., Sami, F., Naaz, R. & Hayat, S 2022. Role of micronutrients in providing abiotic stress tolerance. In *Microbial biofertilizers and micronutrient availability*. Eds. S. T. Khan and A. Malik. Springer, Cham. doi: 10.1007/978-3-030-76609-2_6

Slatyer, R.O. 1967. *Plant-water relationships*. Academic Press, New York, 366 pp.

Thalooth, A.T., Tawfik, M.M. & Magda, M.H. 2006. A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mungbean plants grown under water stress conditions. *World Journal of Agricultural Sciences* **2**(1), 37–46.

Tuiwong, P., Lordkaew, S., Veeradittakit, J., Jamjod, S. & Prom-u-thai, C. 2022. Efficacy of nitrogen and zinc application at different growth stages on yield, grain zinc, and nitrogen concentration in rice. *Agronomy* **12**(9), 2093. doi: 10.3390/agronomy12092093

Tyagi, M. & Pandey, G.C. 2022. Physiology of heat and drought tolerance in wheat: An overview. *Journal of Cereal Research* **14**(1), 13–25. doi: 10.25174/2582-2675/2022

Wang, M., Wang, R. & Mur, L.A.J. 2021. Functions of silicon in plant drought stress responses. *Horticulture Research* **8**(254), 1–13. doi: 10.1038/s41438-021-00681-1

Waraich, E.A., Ahmad, R., Saifullah, M.Y. & Ashraf, E. 2011. Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science* **5**(6), 764–77.

Wasaya, A., Manzoor, S., Yasir, T.A., Sarwar, N., Mubeen, K., Ismail, I.A., Raza, A., Rehman, A., Hossain, A. & Sabagh, A. 2021. Evaluation of fourteen bread wheat (*Triticum aestivum* L.) genotypes by observing gas exchange parameters, relative water and chlorophyll content, and yield attributes under drought stress. *Sustainability* **13**(9), 4799. doi: 10.3390/su13094799

Wasaya, A., Shabir, M.S., Hussain, M., Ansar, M., Aziz, A., Hassan, W. & Ahmad, I. 2017. Foliar application of zinc and boron improved the productivity and net returns of maize grown under rainfed conditions of pothwar plateau. *Journal of Soil Science and Plant Nutrition* **17**(1), 33–45. doi: 10.4067/S0718-95162017005000003

Yang, J., Zhang, J., Wang, Z., Zhu, Q. & Liu, L. 2001. Water deficit induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. *Agronomy Journal* **93**(1), 196–206. doi: 10.2134/agronj2001.931196x

Yang, J.C., Zhang, J.H., Wang, Z.Q., Zhu, Q.S. & Liu, L.J. 2003. Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. *Plant, Cell and Environment* **26**(10), 1621–1631. doi: 10.1046/j.1365-3040.2003.01081.x

Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**(6), 415–421. doi: 10.1111/j.1365-3180.1974.tb01084.x

Agronomy Research **22**(S2), 636–658, 2024
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Assessment of environmental impacts: a life cycle analysis of wheat and rice production in Madhya Pradesh

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Abstract. The production of cereals is one of the primary activities that is responsible for most of the environmental degradation that is caused by agricultural activities. In this study, an attempt was made to determine the ecosystem & resource emissions along with emissions affecting human health, causing due to agricultural activities. LCA is used to conduct an analysis of 17 types of emissions caused by rice and wheat production per hectare in Madhya Pradesh. Based on LCIA and Monte Carlo simulation, the study provides valuable insights into the regional environmental emissions associated with direct seeded rice (DSR), irrigated wheat (IW) and rainfed wheat (RW). Study shows that except for Marine eutrophication (MEUT) and Agricultural land use (ALU), rice production has relatively higher impact than wheat production. Irrigated wheat production found with higher potential of causing non-cancerous diseases caused by air pollution, whereas rice production has the potential to contribute to cancer disease. The production of rice and wheat in Madhya Pradesh state cumulatively contributes 0.008 Gt CO_2 eq. (0.10% of global total) to the global agrifood system GHG emission within farmgate. Since majority of the emissions are caused by soil & crop nutrients and fuel consumption, here it became important to adopt sustainable agricultural practices & biofuel to lessen the environmental impact of wheat & rice production and make sustainable agro-food system of Madhya Pradesh. Based on study results emission mitigation policies have been suggested taking the existing policies into consideration.

Key words: carbon footprint, environmental impact, life cycle assessment, rice, wheat.

INTRODUCTION

Food is one of the most essential items for human beings. However, there are enormous issues created by modern-day food production and consumption activities. The study conducted by Gleick et al. (2014) estimates that about 70 percent of the all-fresh water is used for growing the agriculture crops; Nesheim & Malden (2015) reveals that huge CO_2 are emitted when fossil fuels are used during various aspects of the food cycle. Also, the depletion of natural resources, such as cutting down forests, are some of few negative impacts of food production and consumption activities. Food production and consumption have a serious negative impact on the environment. Since food commodities may travel great distances from production to consumption, the effects of food are spread out and vary over the entire planet due to the nature of the global economy. Studies have revealed that the ecosystem is impacted by the increased CO_2 emission that transpires during different stages of the life cycle of food crops. In 2018, CO_2 emission from Crop and livestock activities within the farm gate increased from 4.6 Gegatonnes CO_2 equivalent to 5.3 Gegatonnes CO_2 equivalent as compared to 2000 (FAO, Analytical Brief 18, 2021). Even among crops environmental stress varies; for example, Pathak et al. (2010) have shown that the production of regular rice results in GHG emissions that are roughly 10.2 and 43.3 times higher than those of wheat and vegetables, respectively.

Agricultural production adds to the climate change by emitting GHG (Jimmy et al., 2017; Green et al., 2018; Taki et al., 2018; Tayefeh et al, 2018; Lynch et al., 2021; Nayak et al., 2022) whereas increased climate change leads to higher temperature and unfavorable weather conditions which ultimately cause a negative impact on production yields (Adams et al., 1998; Arora, 2019; Malhi et al., 2021; Saravanakumar et al., 2022; Sengupta & Mohanasundari, 2023a; Sengupta & Mohanasundari, 2023b). This cycle of cause and effect poses a grave threat to the food security of nations and poses challenges to environmental sustainability.

Wheat and rice production play significant roles in global agricultural systems, but they also contribute to emissions that have environmental implications. These emissions not only contribute to climate change but also contribute to air pollution and impact ecosystem, resources, and human health. At global level Sustainable production and consumption are the hot topic of discussion among the scholarly community. But still there are just a handful of studies on interrogating environmental stress caused by agricultural practices & production generally in India and particularly in Madhya Pradesh state. The studies that demonstrate the environmental impact of a variety of Indian Agri-products from cradle to farm gate will aid in re-evaluating our production and production techniques with an eye towards sustainability.

We need to understand the linkages between agricultural production practices and environmental degradation if we are to reduce agriculture's environmental impact. Several studies have been conducted to measure the regionalized environmental impacts of different agricultural produced across the globe. However, most of the studies are region-specific and using different methods, very few studies has been conducted using LCA for India. Keeping this context, the purpose of this study is to assess the Environmental impact, especially carbon footprint of Madhya Pradesh's agricultural production, with the intention of contributing further to the policy development for agri-food system. Wheat and Rice are two major agricultural products in Madhya Pradesh; hence the study has analyzed the environmental impact of both agricultural produce, which can help in shrinking the carbon emission by adopting sustainable production practices.

Why Madhya Pradesh?

Madhya Pradesh, located in central India, has a predominantly subtropical climate. The state experiences three major seasons: summer (March to June), monsoon (July to September), and winter (October to February). The climatic conditions vary across different regions of Madhya Pradesh, but in general, the state provides favorable conditions for wheat and rice production. Madhya Pradesh is the second largest state in India with a total area use of approximately 6,083’ thousand hectares for wheat production & 10th largest in area covered in rice production covering approximately 2,117’ thousand hectares (India Stat). The state is mainly agrarian, and agriculture plays a crucial role in the states economy, with varied quantity of production across district as shown in Fig. 1.

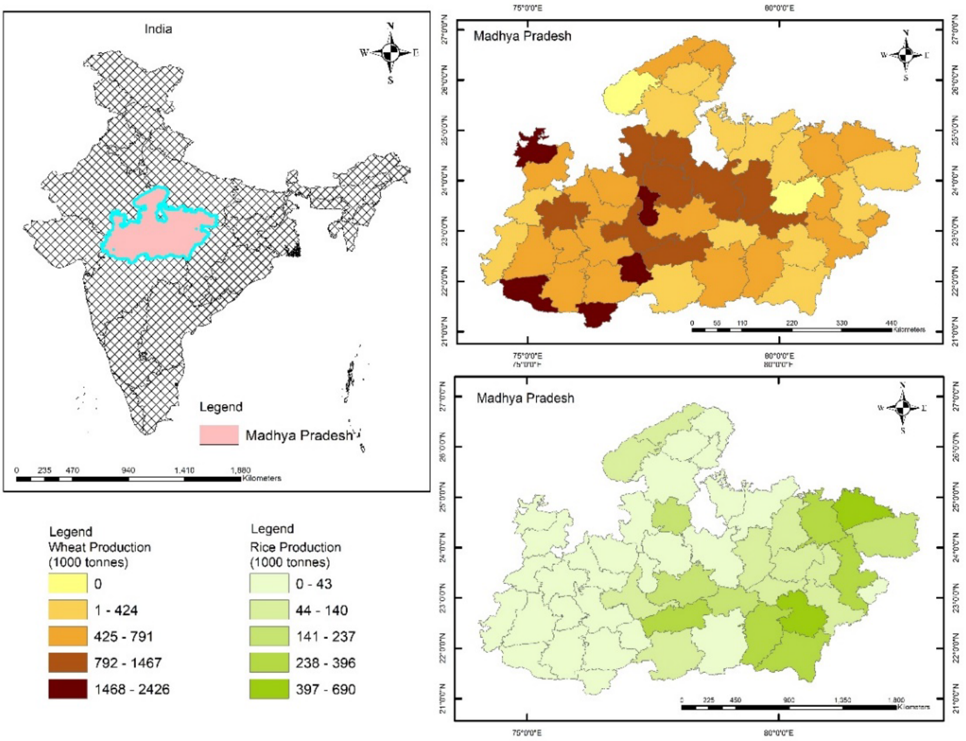

Figure 1. Study area map.

Source: Authors creation based on production data from - Production statistics, Ministry of Agriculture and Farmers welfare.

The state is one of the major producers of wheat & rice in India (Ranked 2nd and 12th for wheat & rice respectively), and the favorable temperature and moderate precipitation during winter contribute to its successful growth. Rice is predominantly cultivated during the monsoon season, while wheat is primarily grown during the winter season. Adequate irrigation and timely rainfall play crucial roles in achieving successful crop yields. It is widely recognized that there are regional variations in environmental impacts resulting from agricultural crop production. Thus, assessing the environmental impact contribution of these two crops of Madhya Pradesh is important for policymakers to intervene & mitigate emissions and bench marking the best practices to lean towards agricultural sustainability. This study will provide a localized comprehension of the environmental impact of agricultural crop production in Madhya Pradesh. Until now, no study has been conducted specifically on Madhya Pradesh; this study will fill this gap and add to the existing knowledge of study. This would aid in the identification of regional challenges and opportunities for sustainable agricultural practices.

LITERATURE REVIEW

Emissions from agriculture

Agriculture has a significant impact on the ecosystem, human health, and resources through various emissions it generates. These emissions go beyond anthropogenic sources and can have direct consequences on the environment and society. Different studies (Roer et al., 2012; Fusi et al., 2014; Achten et al., 2015; Falcone et al., 2019; Mancuso et al., 2019 and Selvaraj et al., 2021) has been conducted across globe on measuring the emission of different crops including wheat & rice, few major studies have been depicted in Table 1, which have covered many emissions in the study.

Table 1. Studies on environmental impacts of agricultural produce

Author, Year	Method & region	FU	Emission covered		
			Ecosystem	Resources	Human health
Roer et al., 2012	LCA, Central Southeast Norway	kg^{-1} production	GWP, FWET, FEUT, MET, MEUT, TEAF, TETO	FRS	HCT, HNCT, OD, PMF, POF
Fusi et al., 2014	LCA, Italy	Area harvested ha^{-1}	GWP, TEAF, FWET, MET	FRS	OD, HCT, HNCT
Achten et al., 2015	LCA, EU	kg^{-1} production	GWP, TEAF, FWET, MET, ALU	-	-
Falcone et al., 2019	LCA, Italy	Area harvested ha^{-1}	GWP, TEAF, TETO, ALU, ULO, NLT, MET, FWET, MEUT, FEUT	FRS, MRS, WD	OD, HCT, HNCT, IR, POF, PMF
Mancuso et al., 2019	LCA, EU	Ton^{-1} Production	GWP, TEAF, FWET, FEUT, TETO, MEUT, MET	-	-
Selvaraj et al., 2021	LCA, India	Area harvested season^{-1}	ALU, GWP, FWET, FEUT, MET, MEUT, TEAF, NLT, TETO	WD, FRS, MRS	IR, HCT, HNCT, OD, PMF
This Study	LCA, M.P.-Central India	Area harvested ha^{-1}	FWET, FEUT, GWP, ALU, MET, MEUT, OFHH, OFTE, TEAF, TETO	FRS, MRS	PMF, HCT, HNCT, IR, OD

Source: Authors' compilation.

Selvaraj et al. (2021) conducted a comprehensive assessment on the overall impact of various agricultural products, such as rice and wheat, in India. Emissions are broadly categorized in 3 categories based on ReCiPe 2016 endpoint indicators: emission to Ecosystem, emission to Resources and emission to Human Health. Represented as, Agricultural land use (ALU), Global warming potential (GWP), Freshwater ecotoxicity (FWET), Freshwater eutrophication (FEUT), Marine ecotoxicity (MET), Marine eutrophication (MEUT), Terrestrial acidification (TEAF), Terrestrial ecotoxicity (TETO), Natural land transformation (NLT), Urban land occupation (ULO), Ozone formation, Human health (OFHH), Ozone formation, Terrestrial ecosystems (OFTE), Water depletion (WD), Fossil resource scarcity (FRS), Mineral resource scarcity (MRS), Ionizing radiation (IR), Human carcinogenic toxicity (HCT), Human non-carcinogenic toxicity (HNCT), Ozone depletion (OD), Fine particulate matter formation (PMF) and Photochemical oxidant formulation as (POF). Numerous studies on a global scale have investigated a wide variety of impact categories associated with agricultural production. These studies have evaluated various emissions and their associated environmental impacts. The table below (Table 1) displays the various studies and emissions covered. In the discussion section, findings from these studies have been compared with those of this study.

GHG emission

Agricultural production is a significant contributor to the overall environmental burden, particularly in terms of global warming. The reduction of agricultural emissions, mainly methane (CH_4) and nitrous oxide (N_2O), could play a crucial role in combating climate change (Lynch et al., 2021). Studies have shown that agricultural practices and the use of nutrients and fertilizers have a substantial impact on emissions. Excessive use of nitrogen fertilizers in wheat and barley cultivation has been found to increase environmental impact (Fallahpour et al., 2012). Conventional tillage in wheat production has also been identified as a contributor to greenhouse gas (GHG) emissions, which can be reduced by implementing zero tillage methods (Aryal et al., 2014). Rice, being a staple for more than half of the global population, is an essential agricultural product, particularly in Asia, where it is widely consumed (FAO, 2011; Muthayya et al., 2014; Miranda et al., 2015). Rice emissions account for more than 30% of CO_2 equivalent emissions in Bangladesh (FAO, 2017). Nitrogen fertilizers used in rice fields have been found to contribute significantly to global warming, with LCA studies indicating that rice fields and nitrogen fertilizers are responsible for 29.29% of global warming (Jimmy et al., 2017). The global warming potential (GWP) of rice production increases linearly with inputs such as fertilizers (Tayefeh et al., 2018). The environmental impact of different crops varies based on geographical region, inputs, and cultivation techniques. Wheat production emits 1.27 t CO_2 equivalent per hectare, while rice production emits 2.44 t CO_2 equivalents per hectare (Nayak et al., 2022). The amount of nitrogen fertilizer used in wheat production significantly influences its carbon footprint, with values ranging between 292.3 and 765.3 kg CO_2 equivalent per hectare (Kumar et al., 2021). LCA studies on wheat production in various regions have estimated emissions at approximately 229.6 kg CO_2 equivalents per ton in New South Wales, 5,455 kg carbon emissions per hectare in China, and 680.36 kg CO_2 equivalents per ton for irrigated wheat production and 381.30 kg CO_2 equivalents per ton for rainfed wheat production (Brock et al., 2012; Zhang et al., 2017; Mondani et al., 2017). Wheat production in different countries also shows variations in carbon emissions, such as 154 kg CO_2 equivalents per ton in Spain and 600–1,400 kg CO_2 equivalents per hectare annually in Canada (Lechón et al., 2005; Gan et al., 2012).

It is important to note that carbon footprint estimates are specific to the geographic region and production systems studied and may not be directly applicable to other regions or systems. Different methodologies and assumptions can lead to variations in carbon footprint estimates. For example, a study in Finland found that wheat production caused 2,330 kg CO_2-eq. per hectare or 590 g CO_2-eq. per kilogram (Rajaniemi et al., 2011). Another study in Iran reported carbon emissions ranging from 805.46 to 1,164.12 kg CO_2 eq. per hectare for different levels of nitrogen fertilizer use (Fallahpour et al., 2012). Studies evaluating the environmental impact of winter wheat production using the life cycle assessment (LCA) method have shown that utilizing less than 150 kg ha^{-1} of nitrogen can significantly reduce the aggregated environmental indicator

(Eco-X) (Brentrup et al., 2004a; Brentrup et al., 2004b). Below Table 2 & 3 dipicted the GHG emission from wheat & rice production from different literatures.

Table 2. Carbon emission from Wheat production

GHG emission	Study area	Method	Author, Year
1.27 t CO_2 eq. h^{-1}	India	Emission coefficients	Nayak et al. (2022)
292.3–765.3 kg CO^2 eq. h^{-1}	Jharkhand	Cool Farm Tool	Kumar et al. (2021)
229.6 kg CO^2 eq. t^{-1}	NSW	LCA	Brock et al. (2012)
600–1,400 kg CO^2 eq. h^{-1} yr^{-1}.	Canada	Site-specific data & empirical modeling	Gan et al. (2012)
0.2–0.6 kg CO^2 eq. kg^{-1}	Sweden	LCA	Röös et al. (2011)
805.46–1,164.12 kg CO_2 eq. h^{-1}	Iran	LCA	Fallahpour et al. (2012)
154 kg CO^2 eq. t^{-1}	Spain	LCA	Lechón et al. (2005)
Wheat (2,330 kg CO_2-eq. ha^{-1}) (590 g CO_2-eq. kg^{-1})	Finland	LCA	Rajaniemi et al. (2011)
Irrigated (680.36 kg CO_2 eq. t^{-1}) Rainfed (381.30 kg CO_2 eq. t^{-1})	Iran	Emission coefficients	Mondani et al. (2017)
5,455 kg CO_2 eq. h^{-1} or 0.75 kg CO_2 eq. kg^{-1}	China	LCA	Zhang et al. (2017)

Table 3. Carbon emission from Rice production

GHG emission	Study area	Method	Author, Year
8.80 ± 5.71 t CO_2 eq. h^{-1}	Punjab	CROPWAT Model	Kashyap & Agarwal (2021)
2.44 t CO_2 eq. h^{-1}	India	Emission coefficients	Nayak et al. (2022)
2,000 kg CO_2 eq. ha^{-1} yr^{-1} (Upland rice)	India	CFT	Vetter et al. (2017)
20,000 kg CO_2 eq. ha^{-1} yr^{-1} (Paddy rice)	India	CFT	Vetter et al. (2017)
11,881 kg Carbon emission ha^{-1} or 1.60 kg Carbon emission kg^{-1}	China	LCA	Zhang et al. (2017)
0.37 kg CO_2 eq. ha^{-1}	China	Emission coefficients	Cheng et al. (2014)
0.333 for rice and 0.413 for basmati rice	India	Emission coefficients	Pathak et al. (2010)
1.34 kg CO_2 eq. kg^{-1} (monsoon season) 2.85 kg CO_2 eq. kg^{-1} (dry season)	Bangladesh	IPCC Tier 1 method	Shew et al. (2019)

Source: (Table 2 & 3) Authors compilation.

Agricultural activities not only contribute to climate change & ecosystem emissions, but they also attribute harm to resources & society. However, it is ’well-documented that regional variations in these impacts exist due to diverse climatic and geographic factors. Surprisingly, until now, no impact assessment has been reported for the state of Madhya Pradesh in India, despite it being situated in the central region and having an exceptionally suitable climate. This cutting-edge study is the first of its kind to thoroughly assess the impacts on region's ecosystem, resources, and environment. The objective of the study is to assess the environmental impact of the Wheat & Rice production in central region of India, specially focusing on GHG emission. As evidenced, the variations in emissions across geography, study questioned, what is the level of average environmental degradation caused from wheat and rice production per hectare in Madhya Pradesh?

DATA AND METHODS

Data collection & Conversion

The data of production, yield, and cultivated area of various foodgrains were collected from 'India stat' and all the sowing requirements (Seed rate, Fertilizers, FYM, Pesticides & Nutrients) of various crops is collected from Farmer Welfare and Agriculture Development Department, Madhya Pradesh, given as a blanket requirement of the foodgrains. Since the data on Electricity and Fuel consumption in agricultural production for India is not available on 'Agribalyse' and 'Ecoinvent' database of LCA. Lieu to that information related to other inputs such as fuel and electricity consumption is collected from various sources and considering them as proxy inputs. The energy use is converted for present scenario by multiplying the specific energy requirement (Singh et al., 2007) per kg to the study time yield of wheat, For Rice energy requirement proxy was taken from Ranguwal & Singh (2022) and converted by reducing the energy used in sowing/ seedling, diesel and fertilizers from total MJ h^{-1} used in rice production. The production yield data for the crops for Madhya Pradesh is collected from 'India Stat' for the year 2020–2021, along with the fuel & energy use from Singh et al. (2007) and Ranguwal & Singh (2022).

Methodology

Life cycle assessment (LCA) is a method for quantifying and evaluating the environmental consequences of a product, process, or activity over its life cycle. It is the accumulation and assessment of a product system's inputs, outputs, and potential environmental impacts throughout its life span. LCA can assist in identifying more sustainable options. To reach the objective of the study, and measure environmental stress caused by crop production system- Life Cycle Assessment (LCA) approach has been used utilizing 'OpenLCA' software and 'Ecoinvent' and 'Agribalyse' data sources and some secondary information collected from various sources like government reports and research to assess the environmental impact. ReCiPe 2016 Midpoint method has been used to assess the detailed environmental harm. The uncertainty in the inputs is reduced using sensitivity analysis using Monte Carlo simulation of 1,000 iterations. ReCiPe 2016 Midpoint has used below mentioned impact calculation equation to estimate the midpoint impact scores for each category:

$$Ii = \sum_{i=1}^{n} (C_i * P\,i * Di)$$

where: I_i is the midpoint impact score for a specific impact category (e.g., climate change, human toxicity, freshwater eutrophication, etc.). n is the number of elementary flows within the impact category. C_i represents the characterization factor of the i^{th} elementary flow, representing the relative contribution of the flow to the impact category, typically in a unit of kg CO_2-eq, kg PM2.5-eq, or similar impact units (Emission conversion factor). P_i shows the amount of the i^{th} input consumed in the production process. And lastly, D_i shows the regional (or country-specific) damage potential of the i^{th} elementary flow, characterizing the damage to the region or country because of the flow's emissions or consumption per unit of the elementary flow. Characterization factor & region specific damage potential in ReCiPe 2016 has been mentioned by Huijbregts et al. (2017a, 2017b).

Since the data exerted from Practice of Package is for whole Madhya Pradesh, it is assumed to be normally distributed. 1,000 random value of inputs (P_i) are generated for Monte carlo simulation. LCA was perfromed with each random input. At last the mean of all emission from 1,000 runs of calculations is considered as most possible emission potential. 'OpenLCA' software has been used for these analysis.

Goal & Scope: This study's objective is to (1) to assess and compare the environmental burdens (on Ecosystem, Resources and Human Health) associated with the production of two of the most prevalent crops in Madhya Pradesh (India), namely Rice and Wheat. A cradle-to-farm gate method (within system) was utilized to carry out the research for this study.

System boundaries: System boundaries play an important role in starting life cycle assessment for any produced. The system investigation includes all the inputs in the form of fertilizer, pesticides & nutrients, fuel, along with other basic and suggested (blanket) sowing requirements. System analyzed conventional tillage scenario in crop production and measured the output in form of crop grain meanwhile excluded subsequent processes like the transformation of grain into feed, consumption, and crop residue management as shown in Fig. 2. ReCiPe (2016) Midpoint method was used to assess the environmental impact of the wheat and rice production at farm gate. System also undertakes the backward linkages associated with the inputs, where 'Agribalyse' databased is used for India or global level with utmost priority.

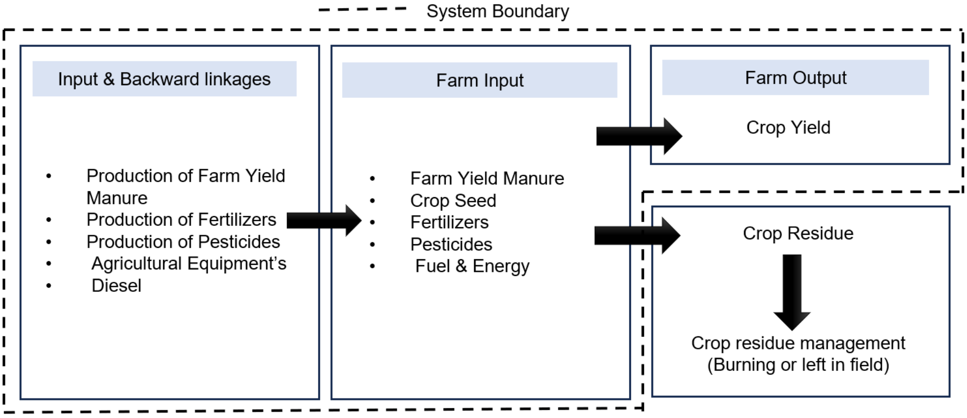

Figure 2. Schematic representation of the agricultural activities carried out as system boundaries. Source: Authors selected study boundaries.

Data quality: As inventory data plays an important role in the output emission results, the overall data quality in the study has been assessed to be fair using International Reference Life Cycle Data System (ILCD). Fairly representing the Time, Technology, Geography, Completeness, Precision and Methodological appropriateness. Data is used from authenticated government sources as a blanket requirement for the state, hence representing the geographic representativeness. As per the objective 'ReCiPe 2016 (Midpoint)' method was used to measure the other Environmental impacts along with Global warming Impact. This methodology is employed and preferred over other calculation methods because it can provide more comprehensive, specific, and coherent results regarding the environmental impacts than other methodologies. This is confirmed by the fact that most rice and wheat production LCA analysis has used the 'Recipe 2016

Midpoint' technique (Jimmy et al., 2017; Yodkhum et al., 2018; Habibi et al., 2019; Shew et al., 2019; Harun et al., 2021; Rezaei et al., 2021; Escobar et al., 2022; Xu et al., 2022) in last 5 years.

Functional unit: Defining a functional unit in life cycle assessment is essential specifying the amount or quantity of a product, process, or service that is being studied. It is a way to standardize the comparison of environmental impacts across different products, processes, or services. The functional unit determines the boundaries and scope of the assessment and helps to ensure that all environmental impacts are accounted for in a consistent and meaningful way. In this study, the functional unit for emission from wheat and rice production is measured in Crop production in kg ha^{-1}.

RESULTS AND DISCUSSION

Life Cycle Inventory

The research investigated the use of all inputs in the production of both wheat and rice, beginning with the preparation of the soil and continuing through planting, the management of fertilizer and pesticides, and the utilization of resources such as fossil fuel, energy, and water, along with pre farm activities linked to the input activities. Input inventory for the wheat and rice has been gathered from MP Govt (POP) reports. Due to the lack of specific data regarding the fuel consumed by agricultural apparatus, data exerted from Chen (2015). All inputs considered in producing rice & wheat in Madhya Pradesh are displayed in the Table 4 below. In rice production consideration was given to a hybrid DSR (Direct Seeded Rice) scenario of rice production. The fuel and energy data from Ranguwal & Singh (2022) have been extracted and converted.

Table 4. Inputs considered in Wheat & Rice LCI

Input	Unit	Crop		
		(IW)	(RW)	(DSR)
(01) Seed rate				
Seed rate	kg ha^{-1}	100	100	16
(02) Fertilizers & Soil Nutrients				
N	kg ha^{-1}	120	40	100
P	kg ha^{-1}	60	20	40
K	kg ha^{-1}	30	0	25
Zn	kg ha^{-1}	25	25	
Manure (FYM)	kg ha^{-1}	-	-	50
(03) Crop protection chemicals				
Pendimethalin	kg ha^{-1}	1	1	-
Sulfosulfuron	g ha^{-1}	33.5	33.5	-
Metribuzin	g ha^{-1}	250	250	
Dichlorophenoxyacetic acid	g kg^{-1} seed	0.5	0.5	
Carbendazim	g kg^{-1} seed	3	3	
Tebuconazole	g kg^{-1} seed	1	1	
Azospirillum	kg ha^{-1}			5
Carbendazim	g kg^{-1} seed			2.5
Mancozeb	g kg^{-1} seed			3
Imidacloprid (Guicho/Imidate)	kg ha^{-1}			
Dichlorophenoxyacetic acid	mL ha^{-1}			1,000
Pretilchlor	mL ha^{-1}			1,250
bensulfuran methyl	kg ha^{-1}			10
Fenoxaprop-p-ethyl	mL ha^{-1}			500
(04) Fuel				
Fuel (Diesel)	L ha^{-1}	37.2	63.9	144

* IW – Irrigated wheat; RW – Rainfed wheat; DSR – Direct seeded rice.

Source: Authors own data compilation from MP Govt (POP) reports.

LCA result

The study was performed in accordance with the ISO 14044 series procedural framework for carrying out LCA. The collected inventory data was analyzed using OpenLCA 1.11.0 software developed by GreenDelta. After the sensitivity analysis with 1,000 iterations of Monte Carlo simulation, the results are discussed below in 3 categories i.e., Emission to Ecosystem, Emission to Resources and Emission causing impact on Human Health.

Emission to Ecosystem. The LCA results of the study indicate that the emission from rice (DSR) has the highest GHG emission of 1,387.44 kg CO_2 eq. h^{-1} whereas irrigated and rainfed wheat production GHG emission was found to be 853.52 & 613.33 kg CO_2 eq. h^{-1} respectively based on the inputs given on the farm. ALU (Agriculture land use) represents the ecological devastation caused by the steady use of land for agricultural purposes. Every farming activity on a specific area of land used for agriculture over a specific time can cause damage. Therefore, when calculating ALU, all activities associated with agriculture's LCA are considered. Again, the ecological damage caused by land occupation is dependent on the level of environmental quality that is maintained throughout the occupation. Rice cultivation was observed with land use potential of 110.15 m^2 a crop eq (square meters per year of crop equivalent), whereas rainfed wheat requires slightly more land compared to irrigated wheat. Ozone formation (OFHH & OFTE) emission can negatively impact terrestrial vegetation by NO_2 emission which contributes to the formation of ground-level ozone. The study found that rice production has the highest impact on ozone formation in terrestrial ecosystems, followed by rainfed wheat and then irrigated wheat. Similar impacts were found in ozone formation impacting human health. Study results indicate that DSR cultivation methods result in higher emissions of NO_x {The term 'Nitrogen oxides' (NO_x) typically encompasses a combination of two distinct gases: Nitric oxide (NO) and Nitrogen dioxide (NO_2)}, which can contribute to the formation of ground-level ozone and potentially harm terrestrial plant life and ecosystems.

Terrestrial acidification (TEAF) is another major emission caused by the application of application of fertilizers which can harm plants, disrupt soil ecosystems, and affect nutrient availability. It can also indirectly impact other organism’s dependent on healthy plant communities. Rice (DSR) production found with the highest potential impact on TEAF, with a value of 6.56 kg SO_2 eq. Irrigated wheat follows with a value of 4.03 kg SO_2 eq, and rainfed wheat has the lowest potential impact with a value of 3.27 kg SO_2 eq. Agricultural production activities have potential to release various toxic substances/ pollutants during crop production cycle, causing ‘Terrestrial ecotoxicity’ (TETO). This study revealed that rice production has more than 3 times higher TETO potential than irrigated & rainfed wheat. The emissions among the produced illustrated in Fig. 3 below.

Madhya Pradesh is well resourced with river flows. Several small rivers get merged with major rivers in the state like ‘Narmada’ & ‘Tapti’ which lastly get merged in ocean. There is a risk that fertilizers & pesticides can run off agricultural sewage containing agricultural pollutants in nearby water bodies & rivers during rainfall. which eventually flow into the oceans. Once these pollutants get mixed in the marine environment, these contaminants can have adverse effects on marine organisms. The study found rice production with 389,969 kg 1,4-DCB (1,4-Dichlorobenzene) of indirect MET potential followed by wheat production; 22,795 & 186,328 kg 1,4-DCB of irrigated & rainfed wheat respectively. Additionally, these toxic pollutants possibly cause FWET potential of approximately 36 kg 1,4-DCB in wheat production, and more than 5 times higher (191 kg 1,4-DCB) in rice production. Whereas, when the excessive enrichment of nutrients, particularly N & P, gets mixed in marine water & freshwater resulted in Marine eutrophication (MEUT) and Freshwater eutrophication (FEUT).

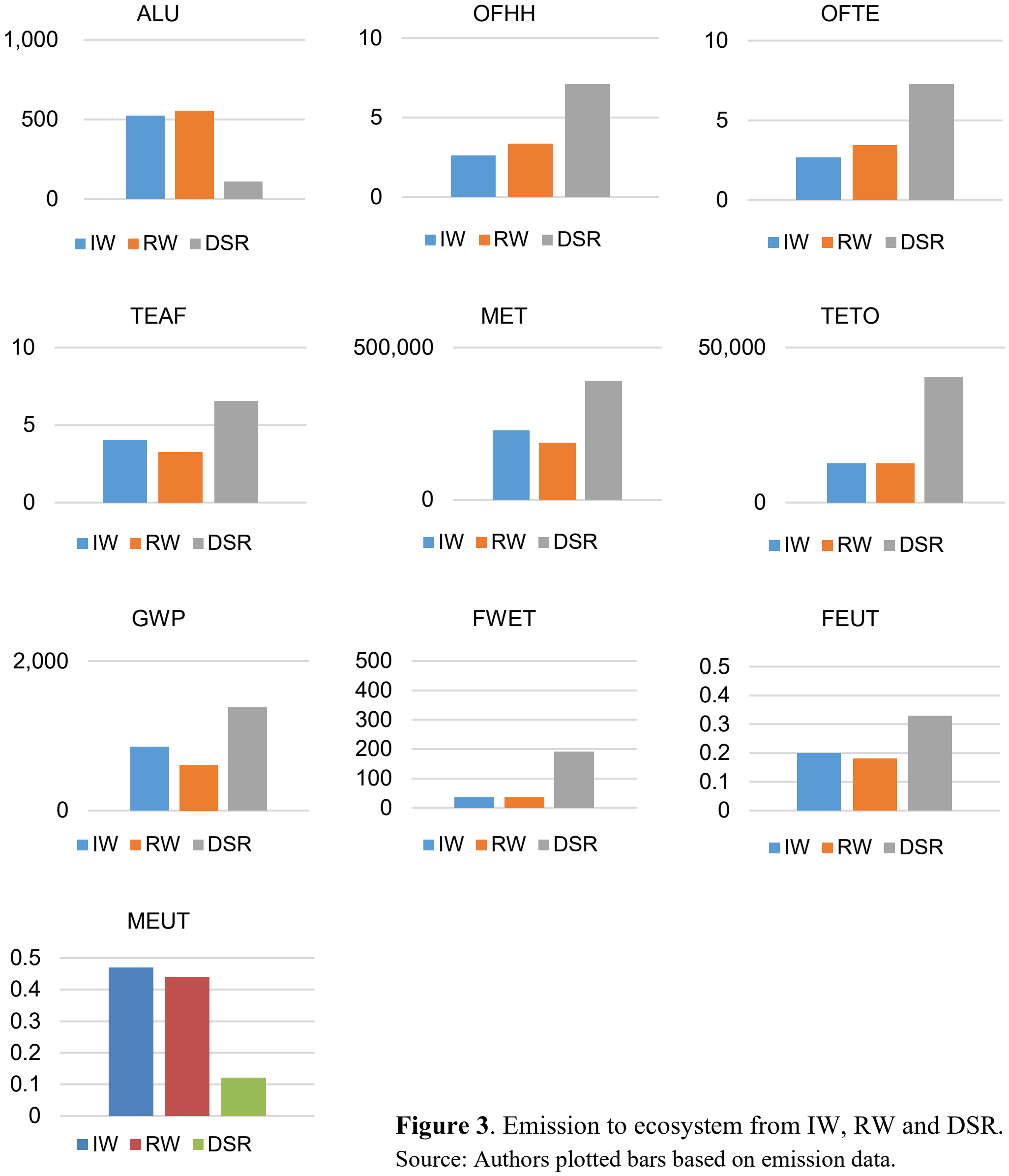

Figure 3. Emission to ecosystem from IW, RW and DSR.
Source: Authors plotted bars based on emission data.

Rice production potentially causes 0.32 kg P eq of FEUT potential and 0.12 kg N eq of MEUT potential. MEUT in wheat production was found higher as compared to rice. All the emissions from the production system of the crops are depicted below in Table 5.

Table 5. Environmental emissions per hectare

Emission categorization (ReCiPe 2016)	Impact category	Reference unit	IW*	RW*	DSR*
			Result	Result	Result
Emission to ecosystem	ALU	m^2 a crop eq	524.43	553.78	110.15
	OFHH	kg NO_x eq	2.61	3.35	7.09
	OFTE	kg NO_x eq	2.67	3.43	7.26
	TEAF	kg SO_2 eq	4.03	3.27	6.56
	MET	kg 1,4-DCB	2,27,975.63	1,86,328.58	3,89,969.43
	TETO	kg 1,4-DCB	12,658.07	12,719.23	40,474.92
	GWP	kg CO_2 eq	853.53	613.33	1387.45
	FWET	kg 1,4-DCB	36.36	36.22	191.67
	FEUT	kg P eq	0.2	0.18	0.33
	MEUT	kg N eq	0.47	0.44	0.12
Emissions affecting human health	PMF	kg PM2.5 eq	1.29	1.36	2.9
	HCT	kg 1,4-DCB	2,040.61	2,420.14	5,514.36
	HNCT	kg 1,4-DCB	2,08,032.68	1,71,148.54	3,46,564.03
	IR	kB_q Co-60 eq	55.03	64.73	143.16
	OD	kg CFC11 eq	0.02	0.01	0.02
Emission to resources	FRS	kg oil eq	265.98	188.5	429.2
	MRS	kg Cu eq	35.93	4.97	38.61

* IW – Irrigated wheat; RW – Rainfed wheat; DSR – Direct seeded rice.

{Agricultural land use (ALU), Global warming potential (GWP), Freshwater ecotoxicity (FWET), Freshwater eutrophication (FEUT), Marine ecotoxicity (MET), Marine eutrophication (MEUT), Terrestrial acidification (TEAF), Terrestrial ecotoxicity (TETO), Ozone formation emission affecting human health (OFHH), Ozone formation emission affecting terrestrial ecosystems (OFTE), Fossil resource scarcity (FRS), Mineral resource scarcity (MRS), Ionizing radiation (IR), Human carcinogenic toxicity (HCT), Human non-carcinogenic toxicity (HNCT), Stratospheric ozone depletion (OD), Fine particulate matter formation (PMF)}.

Source: Authors own calculations.

Emissions affecting Human Health. FPM (Fine particulate matter) can have detrimental effects on air quality and human health. Comparing the three productions, DSR (Direct-Seeded Rice) has the highest impact potential of PM2.5 formation, followed by rainfed wheat and then irrigated wheat. Irrigated and rainfed wheat production in Madhya Pradesh causes 1.28 & 1.36 kg PM2.5 eq of FPM potential whereas, DSR causes emission of 2.89 kg PM2.5 eq. similarly DSR has the higher potential {5,514 kg 1,4-DCB} to cause cancer decease (Human carcinogenic toxicity: HCT), followed by rainfed wheat and then irrigated wheat {2,420 & 2,040 kg 1,4-DCB respectively}. Whereas it was noticed that non-cancer deceases attributed by air pollution (HNCT) are majorly caused by Irrigated wheat, followed by rainfed wheat and then DSR. Radiological effects can damage our DNA and can be a major reason for Acute Radiation Syndrome (ARS) or Cutaneous Radiation Injuries (CRI). Selvaraj et al. (2021) witnessed that agricultural machinery production, tillage, diesel burned in building machines, and power sawing are the major factors contributing to IR. This study has measured Ionizing radiation potential (IR) to measure the impacts on human health. The potential radiological effects were found higher in DSR. DSR has potential of 143 kB_q Co-60 eq (kilobecquerels of Cobalt-60 equivalent) whereas wheat possesses the potential of 55 & 64 kB_q Co-60 eq in irrigated & rainfed wheat production respectively.

Emission from oxides of nitrogen (NOx) contribute to the formation of ground-level ozone, which can have negative impacts on human respiratory health as well as terrestrial ecosystems. Study found that DSR cultivation methods result in higher emissions of NOx, which can contribute to the formation of ground-level ozone and potentially have adverse effects on human respiratory health and ecosystem compared to rainfed and irrigated wheat production. Whereas, among the three cultivation methods, irrigated wheat has the relatively higher impact potential on stratospheric ozone depletion (OD) with a value of 0.024 kg CFC11 eq. and rainfed wheat has a lower impact on stratospheric ozone depletion with a value of 0.009 kg CFC11 eq. Direct-Seeded Rice has a slightly higher impact than rainfed wheat but lower than irrigated wheat. The emissions among the produced illustrated in Fig. 4 below.

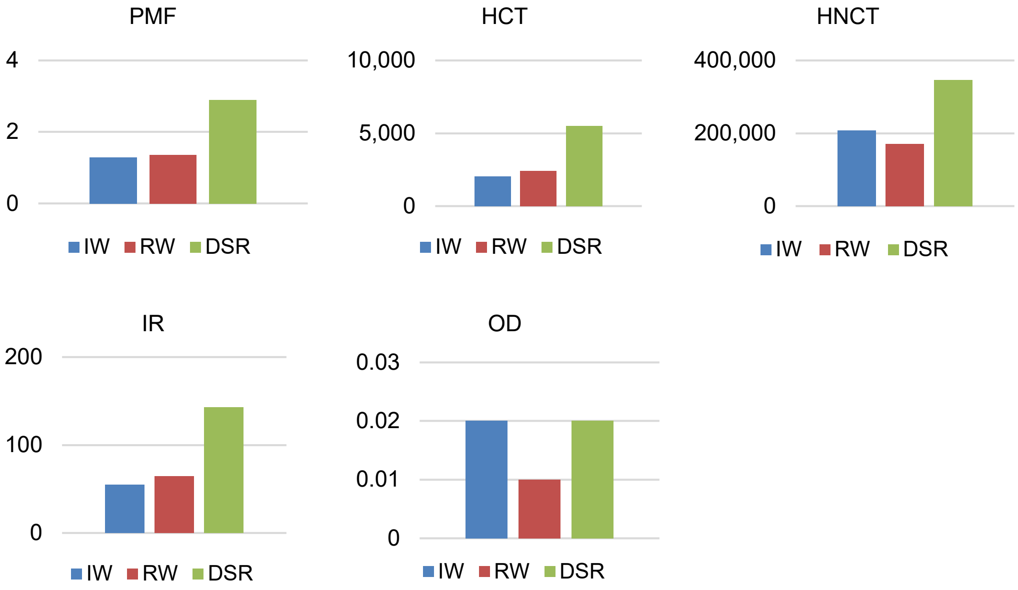

Figure 4. Emissions affecting human health from IW, RW and DSR.
Source: Authors plotted bars based on emission data.

These values suggest that irrigated wheat cultivation has the greatest potential for contributing to stratospheric ozone depletion among the three cultivation methods, followed by DSR and then rainfed wheat. Fig. 4 above depicts various emissions using bar graphs to facilitate comprehension of the differences.

Emission to resources. Agricultural activities not only influence the ecosys tem and have an impact on human health, but they also deplete the resources in terms of consumption of fossil resources specifically in terms of oil and consumption of mineral resources specifically in terms of copper equivalence with a focus on the limited availability of these resources. DSR, which was determined to have the possibly highest influence on fossil resource scarcity (FRS), had a value of 429.19 kg oil equivalent, whereas rainfed wheat had a value of 188.49 kg oil equivalent, making it the agricultural product with the lowest possible impact on FRS. With a value of 265.98 kg oil equivalent, the influence of irrigated wheat is the least significant one on the scarcity of fossil resources. According to these values, the DSR cultivation method has the most potential among the three cultivation methods to contribute to the shortage of fossil resources. Irrigated wheat comes in second, followed by rainfed wheat as the third. The research also showed that DSR cultivation has the largest potential for contributing to to mineral resource scarcity (MRS) among these three produced, followed by irrigated wheat and then rainfed wheat in that order (Fig. 5). This highlights the necessity for sustainable resource management practices as well as the research of alternative materials or recycling ways to decrease the effects of these impacts.

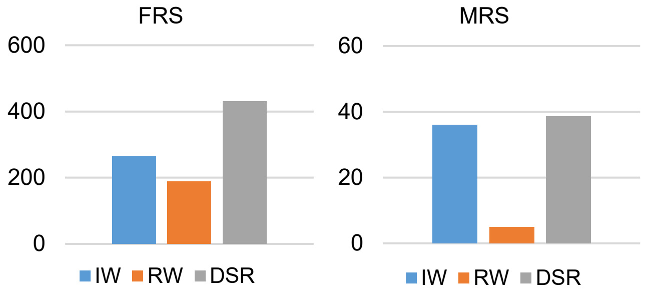

Figure 5. Emissions affecting resources from IW, RW and DSR.
Source: Authors plotted bars based on emission data.

Discussion

Agriculture follows a cyclical pattern, where agricultural production contributes in GHG emission (Jimmy et al., 2017; Tayefeh et al, 2018; Taki et al., 2018; Lynch et al., 2021) which is major reason for climate change and that changed climate in return negatively affects the productivity of the crop (Adams et al., 1998; Arora, 2019; Malhi et al., 2021) reflecting in thread to food security and sustainability. But agricultural production doesn't only contribute to affecting the climate, it creates a burden for the entire ecosystem including resources, and humans. The present study corroborates the findings of Fusi et al. (2014) by affirming that the primary environmental impacts associated with wheat and rice production stem from fuel consumption and emissions released in the field. Among the 17 emissions considered, approximately 06 burdens on the environment can be directly and indirectly attributed to the use of fossil fuels, particularly diesel fuel consumed by agricultural machinery. On the other hand, 09–10 emissions are generated by factors such as soil nutrients and crop protection chemicals. When converting the life cycle impact assessment (LCIA) results based on functional units, our study observed that the outcomes closely aligned with those reported by Fusi et al. (2014) & Korsaeth et al. (2012) in terms of global warming potential (GWP) and Fossil resource depletion (FRS). However, the categories of ozone depletion potential (OD), terrestrial acidification potential (TEAF), and freshwater ecotoxicity potential (FWET) exhibited higher values in the findings of Fusi et al. (2014). We attribute these differences to the study's limitation of not considering water-flooded paddy fields, which could account for the variances in results, particularly with higher methane emission. Furthermore, our investigation revealed that relative fuel consumption in rice production in the Madhya Pradesh region exceeded that of Vercelli (Italy), leading to comparatively higher carbon emissions. As for wheat production, our emission results closely resembled those reported by Roer et al. (2012), Achten et al. (2015), and the Indian study conducted by Selvaraj et al. (2021) across various emission categories. In terms of a comparative analysis, wheat production exhibited lower acidification potential and marine ecotoxicity potential but higher freshwater ecotoxicity potential.

In terms of carbon emissions, our study specifically examined the global warming potential (GWP) and found that the results for wheat production align closely with those reported by Nayak et al. (2022), Brock et al. (2012), Gan et al. (2012), Röös et al. (2011), and Fallahpour et al. (2012). Similarly, the GWP potential for rice production in our study closely resembles the findings of Cheng et al. (2014) and Pathak et al. (2010). It is important to note that due to limited data availability, our study did not account for water usage during the irrigation phase of rice cultivation & residue management of both crops. Consequently, the emission associated with rice production (paddy field flooding) was disregarded, resulting in variations compared to other studies. Recognizing the extensive environmental consequences associated with crop residue burning, which encompass its contribution to climate change, adverse effects on air quality and public health (Mittal et al., 2009; Zhang et al., 2011; Lohan et al., 2018), this study has excluded field burning of crop residue in its analysis due to unavailability of regional data.

This highlights that life cycle assessment (LCA) results are influenced by the system boundaries followed and inputs used, which can differ based on factors such as farming style, cropping patterns, seasons, geographic locations, and other variables. Furthermore, our study focused specifically on the Direct Seeded scenario, which is already considered a low-emission practice and presents a viable alternative to conventional puddle-based transplanted rice production (Pathak & Aggarwal, 2012). The present study conducted in Madhya Pradesh has revealed intriguing disparities in crop production emissions when compared to similar studies conducted globally. Notably, the TEAF and the MET in Madhya Pradesh's arising from crop production were found to be lower than those reported in other studies. Conversely, the FWET and TETO were observed to be higher in Madhya Pradesh (Roer et al., 2012; Achten et al., 2015). These observed differences can be attributed to multiple factors, including varying inputs and the influence of favorable climatic conditions. It is plausible that the specific combination of inputs employed in crop production practices within Madhya Pradesh diverges from region specific practices, leading to the observed discrepancies. Additionally, the region's climatic conditions, which are distinct from those found in other regions, may significantly impact the outcomes of agricultural production. Since Madhya Pradesh is a land locked state resulting in less MET potential whereas have higher FWET potential comparing to coastal region studies (Roer et al., 2012; Achten et al., 2015), (MET in landlocked state is unlikely, however Madhya Pradesh have rivers within its territory, which can affect the aquatic life due to runoff of the pollutants from rivers to sea). An intriguing aspect arising from this study is the revelation that Madhya Pradesh requires fewer soil nutrients and crop protection chemicals for wheat and rice production highlighting the potential efficiency and sustainability of agricultural practices. However, the study also detected a higher fuel consumption in rice production in Madhya Pradesh. This disparity highlights the need for a focused approach towards reducing fuel consumption and optimizing production practices for long-term sustainability.

CONCLUSION

The study exhibits that wheat and rice production in Madhya Pradesh contribute to environmental impacts in terms of harm to Ecosystem, Resources and Human health. In order to optimize production yield and maintain food security, the use of Nitrogen-based fertilizers has been enhanced, but this is having a serious negative impact on the environment. N - fertilizers and fuel are the most significant contributors to the emissions such as Freshwater eutrophication (FEUT), Fine particulate matter formation (PMF), Fossil resource scarcity (FSR), Freshwater ecotoxicity (FWET), Global warming (GWP), Human toxicity (HT) {Human carcinogenic toxicity & Human non-carcinogenic toxicity}, Terrestrial ecotoxicity (TETO) and Stratospheric ozone depletion (OD) etc. In all the categories, except for Marine eutrophication (MEUT) and Agricultural land use (ALU), rice production has relatively higher impact than wheat production. Irrigated wheat production has a higher potential of causing non-cancerous diseases caused by air pollution, whereas rice has the potential to contribute to cancer disease.

The study examines the environmental effects of Direct-Seeded Rice (DSR), irrigated wheat, and rainfed wheat. DSR cultivation's greenhouse gas (GHG) emissions, ozone generation, terrestrial acidification, and terrestrial ecotoxicity are the highest of the three production methods. It emits the most GHGs, contributes to terrestrial ozone production, and has the greatest potential for terrestrial acidification and ecotoxicity. Fine particulate matter (FPM), HCT, HNCT, IR, and ozone emissions vary by cultivation method. DSR emits the most FPM and HCT, exposing respiratory and cancer risks. Irrigated and rainfed wheat production increase HNCT. DSR cultivation has more IR-measured radiological impacts than wheat cultivation. FRS and MRS emissions show how agricultural activities stress scarce resources. DSR consumes the most oil equivalent, followed by rainfed and irrigated wheat. DSR cultivation has the most potential to contribute to MRS, highlighting the need for sustainable resource management and finding alternative methods & innovative technologies for sustainable agriculture.

In addition to considering the functional unit, when examining emissions for the entire state of Madhya Pradesh over the course of a year, significant environmental impacts are observed across all categories (as indicated in Table 6). The total global agrifood system emission of 7.4 Gt CO_2 eq. is primarily originated from all crop and livestock production activities within the farmgate (FAO, 2022). Notably, within this global total, the production of rice and wheat in Madhya Pradesh state alone contributes 0.0086 Gt CO_2eq (8056197.81 t CO_2 eq), that is 0.10 % of global total.

Agricultural production is a substantial source of greenhouse gas emissions, with the majority of these emissions arising in the sector's different activities. These emissions are principally caused by using fossil fuels in agricultural machinery, the application of soil nutrients such as fertilizers, and the use of crop protection chemicals such as herbicides and insecticides (Fig. 6). Fuel burning in agricultural machinery, such as tractors and harvesters, adds to greenhouse gas emissions, specifically carbon dioxide (CO_2). To power these machines, fossil fuels are burnt, releasing CO_2 into the atmosphere, and creating fossil resource scarcity on the other hand. Continuous and heavy use of agricultural machinery, particularly in large-scale farming operations, has resulted in increased fuel consumption and emissions. Soil nutrients, such as fertilizers, are critical for encouraging plant development and increasing agricultural productivity.

Table 6. Total Environmental emissions potential (tons/emission) in Madhya Pradesh for the year 2020–2021

Impact category	Reference unit	IW*	RW*	DSR*
		Overall impact	Overall impact	Overall impact
PMF	t PM2.5 eq	7,454.72	413.64	6,139.30
FRS	t oil eq	1,537,058.52	57,332.28	908,616.40
FWET	t 1,4-DCB	210,118.99	11,016.31	405,765.39
FEUT	t P eq	1,155.77	54.75	698.61
GWP	t CO_2 eq	4,932,421.84	186,544.32	2,937,231.65
HCT	t 1,4-DCB	11,792,379.10	736,085.58	11,673,900.12
HNCT	t 1,4-DCB	1,202,189,652.82	52,054,828.44	733,676,051.51
IR	tBq Co-60 eq	318,010.12	19,687.63	303,069.72
ALU	km^2 a crop eq	3,030,602.31	168,432.19	233,187.55
MET	t 1,4-DCB	1,317,436,969.43	56,671,837.61	825,565,283.31
MEUT	t N eq	2,716.06	133.83	254.04
MRS	t Cu eq	207,634.08	1,511.63	81,737.37
OFHH	t NOx eq	15,082.80	1,018.90	15,009.53
OFTE	t NOx eq	15,429.53	1,043.23	15,369.42
OD	t CFC11 eq	115.58	3.04	42.34
TEAF	t SO2 eq	23,288.77	994.57	13,887.52
TETO	t 1,4-DCB	73,149,087.82	3,868,553.80	85,685,405.64

Source: Authors own calculations.

However, unrestricted and inefficient fertilizer use can have negative environmental implications. Nitrous oxide (N_2O), a strong greenhouse gas, can be produced through the breakdown of nitrogen molecules in fertilizers. Nitrous oxide has a far larger potential for global warming than carbon dioxide. Furthermore, if fertilizers are not adequately handled and applied, they may leach into waterbodies, causing pollution and eutrophication. Herbicides and pesticides are crop protection agents that are used to manage pests, weeds, and illnesses that can harm crops. However, their broad use may result in environmental emissions. Some of these substances are volatile and may evaporate into the atmosphere, adding to pollution. Furthermore, when they wash off from fields or leak into groundwater, they can contaminate soil and water, posing dangers to ecosystems and human health.

To reduce agricultural emissions and ameliorate the ecological impact of crop production, it is critical to focus on the three activities that contribute to these emissions: fossil fuel combustion, soil nutrient management, and crop protection chemical application. Environmentally friendly practices and technologies can assist address these issues. One strategy is to reduce fuel usage in agricultural machinery or perhaps to phase out the use of fossil fuels entirely. This can be accomplished by using more fuel-efficient machinery, alternative energy sources like biofuels or electric power, and precision agriculture techniques. Precision agriculture entails using advanced technologies such as GPS and remote sensing to optimize input usage and decrease waste and reduce the need for unnecessary fuel consumption.

Efficient and responsible soil nutrient management is crucial for reducing emissions associated with fertilizer use. This includes adopting precision agriculture techniques to apply fertilizers only where and when they are needed, using slow-release or controlled-release fertilizers to minimize nutrient losses, and integrating organic farming practices that rely on natural sources of nutrients. In conclusion, addressing the environmental emissions associated with agricultural production requires a multi-faceted approach. By focusing on optimizing fuel consumption or transitioning to alternative energy sources, implementing efficient soil nutrient management practices, and promoting integrated pest management strategies, significant strides can be made in reducing the ecological impact of crop production and mitigating agricultural emissions. These efforts are essential to ensure sustainable food production while minimizing harm to the environment.

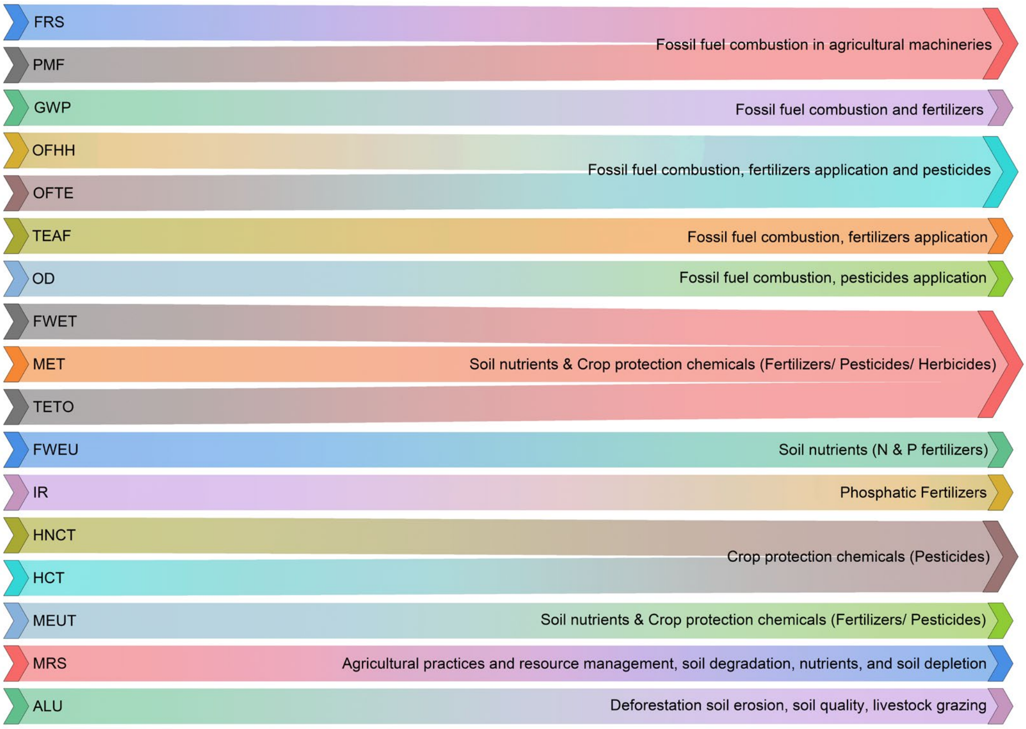

Figure 6. Environmental emissions from crop productions and causes.
Source: Authors own compilation.

In Madhya Pradesh Environmental Policy-1999, policymakers concentrated on agricultural practices and lowering emissions from fertilizers and herbicides; nevertheless, there is still a need to raise farmer awareness about sustainable farming and reducing emissions produced by fuel combustion. Keeping in mind the emissions that agriculture leaves behind and the goal of reaching 'Net Zero Emission by 2070,' it becomes apparent that the primary focus should be on the 'Sustainable production of crops' This can be accomplished through the implementation of sustainable practices such as 'Zero tillage,' the use of fuel-efficient or battery-operated technologies, and limiting the use of external nutrients, pesticides, and herbicides, or the discovery of sustainable alternatives to these things.

POLICY IMPLICATIONS & SUGGESTIONS

The study emphasizes sustainable farming practices to reduce agricultural production's environmental impact. Sustainable Ecosystem policies should reduce chemical fertilizer and pesticide consumption. Climate-smart agriculture and biofuel research can minimize agricultural emissions. Policy initiatives should reduce PMF and other pollutants that hinder human health. Air quality can be improved by regulating agricultural emissions and increasing biofuel use. For Madhya Pradesh's sustainable agro-food system, the following policy recommendations might be made.

- Increase investment in research and development to identify and promote environmentally friendly farming technologies and practices. Create research funds and encourage collaborations among research institutions, farmer collectives, and industry players to create and test sustainable agriculture solutions.
- Creating policies that encourage ecologically sustainable agriculture practices can be beneficial. To conserve ecosystems and minimize pollution, implementing emission limits and restricting the use of chemical fertilizers can help in emission reduction.
- In agrarian perspective ‘Madhya Pradesh Environmental Policy - 1999’ focused on application of bio fertilizers & pesticides ignoring the fact that fuel combustion also hampers ecosystem. As a result, the usage of biofuel should be encouraged in agricultural activities. Production & promotion of biofuel can contribute to environmental sustainability and add in aligning with the ‘National biofuel policy of India’, which aims to achieve 20% blending of ethanol in petrol and 5% blending of biodiesel in diesel by 2030 to reduce GHG emission from fuel.
- Tractors are enormous agricultural machines that use fossil fuels. The inclusion of an electric tractor in the ‘E-Krishi Yantra Yojana’ could be beneficial. Subsidies under the ‘E-Krishi Yantra Yojana- 2023’ can boost demand for electric tractors and can aid in the reduction of air pollution and other ecosystem and resource emissions.
- Encourage more environmentally responsible farming practices among state farmers with the help of farmers collectives (FPO’s), an awareness programs should be initiated focusing on spreading knowledge about environment & health-friendly farming practices and policies.

LIMITATIONS

The study was conducted on secondary database (Blanket requirement) for optimum production of grains by government, Meanwhile the on-farm scenario may refer across farm locations, soil, and climate. Data on fuel and energy use at the aggregate level has been re-used because it is not readily available for individual crops. Subsequent research endeavors may address this limitation by conducting primary surveys to acquire comprehensive data, thereby filling this critical research gap.

REFERENCES

Achten, W.M.J. & Van Acker, K. 2015. EU-Average Impacts of Wheat Production: A Meta-Analysis of Life Cycle Assessments. *Journal of Industrial Ecology* **20**(1), 132–144. doi:10.1111/jiec.12278

Adams, R.M., Hurd, B.H., Lenhart, S. & Leary, N. 1998, Effects of global climate change on agriculture: An interpretative review. *Clim. Res.* **11**, 19–30.

Agribalyse & Ecoinvent, https://nexus.openlca.org/databases

Area and production statistics, Ministry of Agriculture and Farmers welfare, accessed from: https://aps.dac.gov.in/APY/Public_Report1.aspx

Arora, N.K. 2019. Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability* **2**, 95–96. https://doi.org/10.1007/s42398-019-00078-w

Aryal, J.P., Sapkota, T.B., Jat, M.L. & Bishnoi, D.K. 2014. On-farm economic and environmental impact of zero-tillage wheat: a case of north-west india. *Experimental Agriculture* **51**(01), 1–16. doi:10.1017/s001447971400012x

Brentrup, F., Kusters, J., Kulmann, H. & Lamme, J. 2004b. Environmental impact assessment of agri-cultural production systems using the life cycle assessment methodology: I. Theoretical concept of a LCA method tailored to crop production. *European Journal of Agronomy* **20**(3), 246–247.

Brentrup, F., Kusters, J., Lamme, J., Barraclough, P. & Kulmann, H. 2004a. Environmental impact assessment of agricultural production systems using the life cycle assessment (LCA) methodology: II. The application to N fertilizer use in winter wheat production systems. *European Journal of Agronomy* **20**(3), 265–279.

Brock, P., Madden, P., Schwenke, G. & Herridge, D. 2012. Greenhouse gas emissions profile for 1 tonne of wheat produced in Central Zone (East) New South Wales: a life cycle assessment approach. *Crop and Pasture Science* **63**(4), 319. doi:10.1071/cp11191

Chen, M.B.B. 2015. Benchmarking Energy Use on Farm, *Barton, ACT: Rural Industries Research and Development Corporation*. Retrieved from: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0011/1321796/mov3ment-diesel-use-in-ag.pdf

Cheng, K., Yan, M., Nayak, D., Pan, G.X., Smith, P., Zheng, J.F. & Zheng, J.W. 2014. Carbon footprint of crop production in China: an analysis of National Statistics data. *J. Agric. Sci.* FirstView, 59–64.

Escobar, N., Bautista, I., Peña, N., Fenollosa, M.L., Osca, J.M. & Sanjuán, N. 2022. Life cycle thinking for the environmental and financial assessment of rice management systems in the Senegal river valley. *J. Environ Manage* **310**. https://doi.org/10.1016/j.jenvman.2022.114722

Falcone, G., Stillitano, T., Montemurro, F., De Luca, A.I., Gulisano, G. & Strano, A. 2019. Environmental and economic assessment of sustainability in Mediterranean wheat production, *Agronomy Research* **17**(1), 60–76. https://doi.org/10.15159/AR.19.011

Fallahpour, F., Aminghafouri, A., Ghalegolab Behbahani, A. & Bannayan, M., 2012. The environmental impact assessment of wheat and barley production by using life cycle assessment (LCA) methodology, *Environ Dev Sustain* **14**, 979–992. doi: 10.1007/s10668-012-9367-3

FAO, 2011. Looking ahead in world food and agriculture: perspectives to 2050. *Food and Agriculture Organization*, Italy. http://www.fao.org/docrep/014/i2280e/i2280e.pdf.

FAO, 2017. *FAOSTAT*. http://www.fao.org/faostat/en/#country/16

FAO, 2022. Greenhouse gas emissions from agrifood systems. Global, regional and country trends, 2000-2020. *FAOSTAT Analytical Brief Series No. 50*. Rome, FAO. https://www.fao.org/3/cc2672en/cc2672en.pdf

FAOSTAT Analytical Brief 18, 2021. Emissions due to agriculture, Global, regional and country trends 1990–2018. https://www.fao.org/3/cb3808en/cb3808en.pdf

Farmer Welfare and Agriculture Development Department, Madhya Pradesh (a), Package of Practice, n.d., Wheat, retrieved from https://mpkrishi.mp.gov.in/Englishsite_New/krishi_capsules_wheat_New.aspx, Accessed on April 10, 2023.

Farmer Welfare and Agriculture Development Department, Madhya Pradesh (b), Package of Practice, n.d., Paddy, retrieved from https://mpkrishi.mp.gov.in/Englishsite_New/krishi_capsules_dhan_New.aspx, Accessed on April 08, 2023.

Fusi, A., Bacenetti, J., González-García, S., Vercesi, A., Bocchi, S. & Fiala, M. 2014. Environmental profile of paddy rice cultivation with different straw management. *Science of The Total Environment* **494–495**, 119–128. doi:10.1016/j.scitotenv.2014.06.126

Gan, Y., Liang, C., Campbell, C.A., Zentner, R.P., Lemke, R.L., Wang, H. & Yang, C. 2012. Carbon footprint of spring wheat in response to fallow frequency and soil carbon changes over 25 years on the semiarid Canadian prairie. *Eur. J. Agron*. **43**, 175–184.

Giuliana, V., Lucia, M., Marco, R. & Simone, V. 2022. Environmental life cycle assessment of rice production in northern Italy: a case study from Vercelli, *The International Journal of Life Cycle Assessment.* https://doi.org/10.1007/s11367-022-02109-x

Gleick, P.H. 2014. The World's Water: The Biennial Report on Freshwater Resources. Washington, DC: *Island Press*. 335p. ISBN 1-55963-792-7

Green, R.F., Joy, E.J.M., Harris, F., Agrawal, S., Aleksandrowicz, L., Hillier, J., Macdiarmid, J.I., Milner, J., Vetter, S.H., Smith, P., Haines, A. & Dangour, A.D. 2018. Greenhouse gas emissions and water footprints of typical dietary patterns in India. *Science of The Total Environment* **643**, 1411–1418. doi:10.1016/j.scitotenv.2018.06.258

Habibi, E., Niknejad, Y., Fallah, H., Dastan, S. & Tari, D.B. 2019. Life cycle assessment of rice production systems in different paddy field size levels in north of Iran. *Environ Monit Assess* **191**(4). https://doi.org/10.1007/s10661-019-7344-0

Harun, S.N., Hanafiah, M.M. & Aziz, N.I.H.A. 2021. An LCA-based environmental performance of rice production for developing a sustainable agri-food system in Malaysia. *Environ Manage* **67**, 146–161.https://doi.org/10.1007/s00267-020-01365-7

Huijbregts, M.A.J., Steinmann, Z.J.N, Elshout, P.M.F., Stam, G, Verones, F, Vieira, M.D.M, Zijp, M, Hollander, A & van Zelm, R. 2017b. ReCiPe 2016 A harmonized life cycle impact assessment method at midpoint and endpoint level, *Int J Life Cycle Assess* **22**, 138–147. doi: 10.1007/s11367-016-1246-y

Huijbregts, M.A.J., Steinmann, Z.J.N, Elshout, P.M.F., Stam, G., Verones, F., Vieira, M.D.M, Hollander, A, Zijp, M. & Van Zelm, R. 2017a. ReCiPe 2016 A harmonized life cycle impact assessment method at midpoint and endpoint level Report I: Characterization. https://pre-sustainability.com/legacy/download/Report_ReCiPe_2017.pdf

IndiaStat. https://www.indiastat.com/madhya-pradesh-state/data/agriculture

Jimmy, A.N., Khan, N.A., Hossain, M.N. & Sujauddin, M. 2017. Evaluation of the environmental impacts of rice paddy production using life cycle assessment: case study in Bangladesh. *Model Earth Syst Environ* **3**(4), 1691–1705. https://doi.org/10.1007/s40808-017-0368-y

Kashyap, D. & Agarwal, T. 2021. Carbon footprint and water footprint of rice and wheat production in Punjab, India. *Agricultural Systems* **186**, 102959. doi:10.1016/j.agsy.2020.102959

Korsaeth, A., Jacobsen, A.Z., Roer, A.G., Henriksen, T.M., Sonesson, U., Bonesmo, H., Skjelvåg, A.O. & Strømman, A.H. 2012. Environmental life cycle assessment of cereal and bread production in Norway, Acta Agriculturae Scandinavica, Section A – *Animal Science* **62**(4), 242–253. doi:10.1080/09064702.2013.783619

Kumar, R., Karmakar, S., Minz, A., Singh, J., Kumar, A. & Kumar, A. 2021. Assessment of Greenhouse Gases Emission in Maize-Wheat Cropping System Under Varied N Fertilizer Application Using Cool Farm Tool. *Front. Environ. Sci*. **9**, 710108. doi: 10.3389/fenvs.2021.710108

Lechon, Y., Cabal, H. & Saez, R. 2005. Life cycle analysis of wheat and barley crops for bioethanol production in Spain. *International Journal of Agricultural Resources, Governance and Ecology* **4**(2), 113. doi:10.1504/ijarge.2005.007193

Lohan, S.K., Jat, H.S., Yadav, A.K., Sidhu, H.S., Jat, M.L., Choudhary, M., Jyotsna Kiran, P. & Sharma, P.C. 2018. Burning issues of paddy residue management in north-west states of India. *Renew. Sustain. Energy Rev.* **81**, 693–706.

Lynch, J., Cain, M., Frame, D. & Pierrehumbert, R. 2021. Agriculture's Contribution to Climate Change and Role in Mitigation Is Distinct From Predominantly Fossil CO2-Emitting Sectors. *Front. Sustain. Food Syst.* **4**, 518039. doi: 10.3389/fsufs.2020.518039

Malhi, G.S., Kaur, M. & Kaushik, P. 2021. Impact of Climate Change on Agriculture and Its Mitigation Strategies: A Review. *Sustainability* **13**, 1318. https://doi.org/10.3390/su13031318

Mancuso, T., Verduna, T., Blanc, S., Di Vita, G. & Brun, F. 2019. Environmental sustainability and economic matters of commercial types of common wheat. *Agricultural Economics* (Zemědělská Ekonomika) **65**(4), 194–202. doi:10.17221/172/2018-agricecon

Miranda, M.S., Fonseca, M.L., Lima, A., De Moraes, T.F. & Rodrigues, F.A. 2015. Environmental impacts of rice cultivation. *Am. J. Plant Sci.,* **6**(12), 2009

Mittal, S.K., Susheel, K., Singh, N., Agarwal, R., Awasthi, A. & Gupta, P.K. 2009. Ambient air quality during wheat and rice crop stubble burning episodes in Patiala. *Atmos. Environ* **43**, 238–244.

Mondani, F., Aleagha, S., Khoramivafa, M. & Ghobadi, R. 2017. Evaluation of greenhouse gases emission based on energy consumption in wheat Agroecosystems. *Energy Reports* **3**, 37–45. doi:10.1016/j.egyr.2017.01.002

Muthayya, S., Sugimoto, J.D., Montgomery, S. & Maberly, G.F. 2014. An overview of global rice production, supply, trade, and consumption. *Ann N Y Acad Sci* **1324**(1), 7–14. http://dx.doi.org/10.1111/nyas.12540

Nayak, A.K., Tripathi, R., Debnath, M., Swain, C.K., Dhal, B., Vijaykumar, S., Nayak, A.D., Mohanty, S., Shahid, M., Kumar, A., Rajak, M., Moharana, K.C., Chatterjee, D., Munda, C.S., Guru, P., Khanam, R., Lal, B., Gautam, P., Pattanaik, S., Shukla, A.K., Fitton, N., Smith, P. & Pathak, H. 2022. Carbon and water footprint of rice, wheat & maize crop productions in India, *Pedosphere* **33**(3), ISSN 1002-0160. https://doi.org/10.1016/j.pedsph.2022.06.045

Nesheim, M.C. 2015. 'Environmental Effects of the U.S. Food System.' A Framework for Assessing Effects of the Food System., *U.S. National Library of Medicine*. www.ncbi.nlm.nih.gov/books/NBK305182/

Pathak, H. & Aggarwal, P.K. 2012. Low Carbon Technologies for agriculture: A Study on Rice and Wheat system in the Indo-Gangetic Plains. *Indian Agricultural Research Institute*, P. xvii+78

Pathak, H., Jain, N., Bhatia, A., Patel, J. & Aggarwal, P.K. 2010. Carbon footprints of Indian food items, *Agriculture, Ecosystems and Environment*, August, doi:10.1016/j.agee.2010.07.002

Rajaniemi, M., Mikkola, H. & Ahokas, J. 2011. Greenhouse gas emissions from oats, barley, wheat and rye production. *Agronomy Research* **9**(S1: Biosystem Engineering Special), 189–195.

Ranguwal, S. & Singh, J. 2022. Energy Use Pattern and its Efficiency in Paddy Cultivation in Indian Punjab, *Indian Journal of Ecology* **49**(2), 629–635. doi: https://doi.org/10.55362/IJE/2022/3571

Rezaei, M., Soheilifard, F. & Keshvari, A. 2021. Impact of agrochemical emission models on the environmental assessment of paddy rice production using life cycle assessment approach. *Energy Source* Part A. https://doi.org/10.1080/15567036.2020.1864066

Roer, A.G., Korsaeth, A., Henriksen, T.M., Michelsen, O. & Strømman, A.H. 2012. The influence of system boundaries on life cycle assessment of grain production in central southeast Norway. *Agricultural Systems* **111**, 75–84. doi:10.1016/j.agsy.2012.05.007

Röös, E., Sundberg, C. & Hansson, P.A. 2011. Uncertainties in the carbon footprint of refined wheat products: a case study on Swedish pasta. *The International Journal of Life Cycle Assessment* **16**(4), 338–350. doi:10.1007/s11367-011-0270-1

Saravanakumar, V., Lohano, H.D. & Balasubramanian, R. 2022. A district-level analysis for measuring the effects of climate change on production of rice: evidence from Southern India. *Theoretical and Applied Climatology* **150**(3–4), 941–953. https://doi.org/10.1007/s00704-022-04198-y

Selvaraj, A., Gautam, J., Verma, S., Verma, G. & Jain, S. 2021. Life cycle sustainability assessment of crops in India. *Current Research in Environmental Sustainability* **3**, 100074. doi:10.1016/j.crsust.2021.100074

Sengupta, A. & Mohanasundari, T. 2023a. Analysis of the Effects of Climate Change on Cotton Production in Maharashtra State of India Using Statistical Model and GIS Mapping. *Journal of Sustainable Agriculture* **38**(1), 152–162. doi: http://dx.doi.org/10.20961/carakatani.v38i1.64377

Sengupta, A. & Mohanasundari, T. 2023b. Impact of climate change on sugarcane production in uttar pradesh, india: a district level study using statistical analysis and gis mapping, *Malaysian Journal of Sustainable Agriculture* **7**(1), 32–37. doi: http://doi.org/10.26480/mjsa.01.2023.32.37.

Shew, A.M., Durand-Morat, A., Putman, B., Nalley, L.L. & Ghosh, A. 2019. Rice intensification in Bangladesh improves economic and environmental welfare. *Environ Sci Policy* **95**, 46–57. https://doi.org/10.1016/j.envsci.2019.02.004

Singh, H., Singh, A.K., Kushwaha, H.L. & Singh, A. 2007. Energy consumption pattern of wheat production in India. *Energy* **32**(10), 1848–1854. doi:10.1016/j.energy.2007.03.001

Zhang, D., Shen, J., Zhang, F., Li, Y. & Zhang, W. 2017. Carbon footprint of grain production in China. *Scientific Reports* **7**(1). doi:10.1038/s41598-017-04182-x

Zhang, H., Hu, D., Chen, J., Ye, X., Wang, S.X., Hao, J., Wang, L., Zhang, R. & Zhi, A. 2011. Particle Size Distribution and Polycyclic Aromatic Hydrocarbons emissions from Agricultural Crop Residue Burning. *Environ. Sci. Technol.* **45**, 5477–5482.

Taki, M., Soheili-Fard, F., Rohani, A., Chen, G. & Yildizhan, H. 2018. Life cycle assessment to compare the environmental impacts of different wheat production systems. *Journal of Cleaner Production*. doi: 10.1016/j.jclepro.2018.06.173

Tayefeh, M., Sadeghi, S.M., Noorhosseini, S.A., Bacenetti, J. & Damalas, C.A. 2018. Environmental impact of rice production based on nitrogen fertilizer use. *Environmental Science and Pollution Research* **25**(16), 15885–15895. doi:10.1007/s11356-018-1788-6

Vetter, S.H., Sapkota, T.B., Hillier, J., Stirling, C.M., Macdiarmid, J.I., Aleksandrowicz, L., Green, R., Joy, E.J., Dangour, A.D. & Smith, P. 2017. Greenhouse gas emissions from agricultural food production to supply Indian diets: Implications for climate change mitigation. *Agric Ecosyst Environ* **16**(237), 234–241. doi: 10.1016/j.agee.2016.12.024

Xu, Q., Dai, L., Gao, P. & Dou, Z. 2022. The environmental, nutritional, and economic benefits of rice-aquaculture animal coculture in China. *Energy* **249**. https://doi.org/10.1016/j.energy.2022.123723

Yodkhum, S., Sampattagul, S. & Gheewala, S.H. 2018. Energy and environmental impact analysis of rice cultivation and straw management in northern Thailand. *Environ Sci. Pollut. Res.* **25**(18), 17654–17664. https://doi.org/10.1007/s11356-018-1961-y

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Adsorbent potential of cocoa pod husk activated charcoal to remove metals from the Ucayali River

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Abstract. The problem of river water contamination due to the presence of dangerous metals for ichthyological flora and fauna and human health has motivated the search for innovative and feasible solutions. Therefore, the production of activated carbon from cocoa pod husks was investigated to eliminate metals present in the Ucayali River. Response surface methodology was used to optimize the manufacturing of the adsorbent and test its effectiveness in removing metals from water using a factorial design of 3^3 and 3^2, with three replicates each. The optimal amount of activated carbon (18.41 g) was obtained from 200 g of fresh cocoa pod husks. It was converted into activated carbon under the following conditions: thermal modification at 100, 150, and 200 °C; activation time of 1.0, 1.5, and 2.0 h; and pyrolysis and activation at 400, 500, and 600 °C. This allowed the elimination by efficient adsorption of 56.8% Fe^{2+}, 68.4% Al^{3+}, 65.9% Cu^{2+}, and 55.5% Zn^{2+} from Ucayali River, thus demonstrating its adsorbent power. The results will make it possible to manufacture filters to decontaminate water containing heavy metals, thus guaranteeing its consumption.

Key words: adsorption, heavy metals, pyrolysis, thermal modification, water pollution.

INTRODUCTION

In most countries, the waters of rivers are effluent dumps, which carry pollutants, including heavy metals, that negatively affect the fish population by accumulating in the muscles, viscera, and other tissues (Adeyeye & Ashaolu, 2022; Akila et al., 2022; Zaghloul et al., 2022). They also affect the health of residents who use drink these waters and can have a carcinogenic effect. Therefore, it is considered a public health problem in Peru (Ccanccapa-Cartagena et al., 2021) and is also a prevalent problem worldwide (Mariana et al., 2021).

The development of cocoa (*Theobroma cacao*) cultivation has been increasing in the Ucayali-Peru region. In 2021, 20,046 t were harvested, and by February 2022, 1,213 t had been accumulated (López & Alva, 2022), of which 70–75% (Cruz et al., 2012) corresponded to the husks; therefore, between 14,032.2 and 15,034.5 t, and 849.1 and 909.8 t of residues, respectively, were left in the field, increasing environmental and landscape pollution.

Decontaminating the waters of the Ucayali-Peru river, due to the presence of metals, is approached from a perspective of reuse of lignocellulose waste from cocoa pod shells, which comprise 43.6% lignin, 34.4% cellulose, and 11.75% hemicellulose (Díaz-Oviedo et al., 2022), making them excellent raw materials for the production of activated carbon (Valdés-Rodríguez et al., 2022) using pyrolysis (Maulina & Iriansyah, 2018). They contribute to the formation of micropores of appropriate diameters, have a greater surface area, and have appropriate functional groups on their surface, making it a potential and efficient adsorbent for contaminant removal (González-García et al., 2013; Mariana et al., 2021).

Pyrolysis shows promise for dealing with agricultural waste by thermochemical conversion at high temperatures in the absence of oxygen (Mariana et al., 2021) into activated carbon, and has many important effects, particularly on its adsorption capacity, because increasing the temperature from 350 to 650 °C leads to a drastic increase in surface area and carbonisation with the loss of functional groups (Choi & Kan, 2019).

For the production of activated carbon, the values of surface area and micro pore volume, which are influenced by the type of raw material and pyrolysis temperature, obtained at 300–500 °C using coconut shell (*Cocos nucifera*) were 13.0 m^2 g^{-1} and 0.021 cm^3 g^{-1} (Solanki & Boyer, 2017), respectively; 120.0 m^2 g^{-1} and 2.43 cm^3 g^{-1}, respectively, using palm (*Elaeis guineensis*) activated carbon at 600 °C (Guo et al., 2016); 605.0 m^2 g^{-1} and 0.421 cm^3 g^{-1}, respectively, using wheat straw (*Triticum spp*) at 700 °C (Wu et al., 2018); and 904.1 m^2 g^{-1} and 0.506 cm^3 g^{-1}, respectively, using grapefruit peel (*Citrus paradisi*) at 600–900 °C (Chen et al., 2017).

The use of activated carbon made from lignocellulose waste to remove metals from water samples has been successfully tested; thus, Cu^{2+} has been removed from wastewater samples using activated carbon from green vegetables (Sabela et al., 2019). The removal of Fe^{2+} from the Nag-India River was achieved using activated carbon from orange (*Citrus sinensis*) peels (Nandeshwar et al., 2016).

Liu et al. (2020) removed Hg^{2+} from wastewater using activated carbon from rice husks (*Oriza sativa*); Yunus et al. (2020) removed Cr^{3+} and Zn^{2+} from mining effluents in the tile and electroplating industries using melon (*Cucumis melo*); and Olaoye et al. (2018) and Prastuti et al. (2019) removed Cu^{2+} and Cr^{6+} from textile wastewater, and Pb^{2+}, Cr^{3+}, and Cd^{2+} ions from wastewater in the cassava (*Manihot esculenta*) industry using banana (*Musa paradisiaca*).

In agroindustrial waste, which is converted into activated carbon, palm kernel (*Elaeis guineensis*) shells (Baby & Hussein, 2020) have excellent activity in removing metals such as Cr^{6+}, Pb^{2+}, Zn^{2+}, and Cr^{2+} from water samples. The use of cocoa (*T. cacao* L.) pod shells converted into an adsorbent has been shown to be effective in removing Pb^{2+} and Cu^{2+} from water samples from a processing plant containing a mixture of metals and from a refinery and petrochemical company (Odubiyi et al., 2012; Sadheesh et al., 2021).

The reported methodologies for manufacturing activated carbon include the use of chemical activators and the limited use of cocoa pod shells as lignocellulose biomass. The metals removed did not include aluminium (Al^{3+}), copper (Cu^{2+}), iron (Fe^{2+}), and zinc (Zn^{2+}), but were oriented towards chromium (Cr^{6+}) and cadmium (Cd^{2+}), which have not been discussed in depth in the literature.

Therefore, if cocoa pod shells are converted by thermochemical activation, followed by carbonisation, and the thermochemical modification time is controlled, activated carbon with excellent adsorbent characteristics to remove heavy metals from the waters of the Ucayali River will be obtained. Therefore, the purpose of this study was to manufacture activated carbon from cocoa pod shells (*T. cacao* L.) and determine its adsorption efficiency and removal of heavy metals in water samples from the Ucayali River, Peru.

MATERIALS AND METHODS

Manufacture of activated carbon by pyrolysis: experimental and statistical design

Activated carbon was obtained, as described by Tsai et al. (2018), with adaptations comprising: reception, selection, weighed (200 g) on Ohaus-PA512 scale, repeatability 0.01 g, cut into pieces, chopped to 5 mm, thermal modification at 100, 150, and, 200 °C for 1.0, 1.5, and 2.0 h, pyrolysis and thermal activation at 400, 500, and, 600 °C, in a muffle furnace (Thermolyne brand Thermo Fisher Scientific, MA, USA; FB1414M, ± 0.5 °C at 1,000 °C) for 1 h, ground to particles of 149 μm (0.149 mm), washed with distilled water until total decolourisation, dried at 150 °C in an oven (Memmert, precision up to 99.9 °C: 0.1/from 100 °C: 0.5) for 1 h, with 6.4% humidity (ASTM, 1997), and packaging in glass jars. Obtaining activated carbon followed a 3^3 factorial design with three repetitions, because it is a widely used technique to optimize activation conditions to minimize the number of experiments and obtain maximum information (Grich et al., 2024). The response surface methodology was used for its optimization because it was a multi-factor experiment, which allowed us to effectively distinguish the degree and difference of the influence of each factor (Aksoy & Sagol, 2016), the variables studied being the thermal modification temperature (X_1), thermal activation time (X_2), and the carbonization temperature (X_3), whose parameters are shown in Table 1.

Table 1. Variables and their levels for the production of activated carbon

Variable	Unit	Code	-1	0	+1
Change temperature	°C	X_1	100	150	200
Activation time	h	X_2	1.0	1.5	2.0
Pyrolysis/activation temperature	°C	X_3	400	500	600

The weight of the activated carbon obtained was considered the response variable, as represented in Eq. 1.

$$Y_1 = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{22}X_2^2 + B_{23}X_2X_3 + B_{33}X_3^2 \quad (1)$$

where Y_1 – response variable (amount of activated carbon); B_0 – coefficient of the constant; B_1, B_2, B_3 – linear coefficients; B_{12}, B_{13}, B_{23} – binary interaction coefficients; B_{11}, B_{22}, B_{33} – quadratic coefficients, and X_1, X_2, X_3 – levels of the variables for manufacturing activated carbon.

Evaluation of the adsorbent activity of activated carbon and statistical analysis

Water samples from the Ucayali River were taken 30 m from the shore at the Reloj Público port, using 1 L glass bottles, which were immediately insulated and stored under refrigeration using thermal boxes.

The effectiveness of the adsorbent was evaluated using atomic absorption spectroscopy, as recommended by Sibal & Espino (2018). For this purpose, 250 mL water samples were collected from the Ucayali River, with Fe^{+2}, Al^{+3}, Cu^{+2}, and Zn^{+2} of 1.37, 0.25, 0.041, and 0.011 mg L^{-1}, respectively. Water samples were spiked with 1, 3, or 5 g of washed and dried activated carbon, shaken at 300 rpm for 30, 60, or 90 min, filtered, packed in glass jars, hermetically sealed, coded, and stored under refrigeration. The adsorption of Fe^{+2}, Al^{+3}, Cu^{+2}, and Zn^{+2} on the water samples by activated carbon was measured by atomic absorption using a spectrophotometer (Labtron, model LAAS-A11, precision $\lambda \leq \pm 0.5$ nm), and a calibration curve was made with five concentrations and a reagent blank in the linear range for each element measured. To evaluate the adsorptive effectiveness of activated carbon in the adsorption of metals from water samples from the Ucayali River, a factorial 3^2 design with three replicates was used, and the adsorption was optimised using response surface methodology, considering the amount of activated carbon (X_1) and agitation time (X_2) as variables, as shown in Table 2.

Table 2. Factors, codes, and values determined in the adsorption of metals from water samples with activated carbon

Factors	Unit	Code	-1	0	+1
Amount of activated carbon	g	X_1	1	3	5
Stirring time	min	X_2	30	60	90

Statistical analysis of the generated data was performed using the Statgraphics CENTURION XIX software, version 19.1.2 (StatPoint, 2020).

The response variables ($Y_{Fe,\ Al,\ Cu,\ Zn}$), show the adsorption of the metals (Eq. 2).

$$Y_{Fe,Al,Cu,Zn} = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{12}X_1X_2 + B_{22}X_2^2 \qquad (2)$$

where $Y_{Fe,Al,Cu,Zn}$ – response variable (adsorption of Fe^{+2}, Al^{+3}, Cu^{+2}, Al^{+2}); B_0 – coefficient of the constant; B_1 and B_2 – linear coefficients; B_{12} – binary interaction coefficient; B_{11} and B_{22} – quadratic coefficients, and X_1 and X_2 – coded values of the variables responsible for the adsorbed metal.

RESULTS AND DISCUSSION

Characterization of the raw material, production of activated carbon, pyrolysis, and ANOVA

The analysis of variance (ANOVA) of the data obtained for the amount of activated carbon as a function of the modification temperature, activation time, and pyrolysis and activation temperatures is reported in Table 3.

The statistical significance was observed in factors C and B^2 ($p \leq 0.05$), which corresponds to the activation time and the quadratic effect of the pyrolysis and activation temperature.

Table 3. ANOVA for the fitted quadratic polynomial model for activated carbon manufacture

Source of variation	Sum of squares	gl	Mean square	*F*-ratio	*P*-value
A: Modification temperature	0.1701	1	0.1701	0.05	0.8257^{ns}
B: Pyrolysis/activation temperature	5.4891	1	5.4891	1.61	0.2212^{ns}
C: Activation time	85.4996	1	85.4996	25.12	0.0001*
A^2	2.4876	1	2.4876	0.73	0.4045^{ns}
AB	2.1168	1	2.1168	0.62	0.4412^{ns}
AC	1.2936	1	1.2936	0.38	0.5457^{ns}
B^2	62.1675	1	62.1675	18.27	0.0005*
BC	0.3571	1	0.3571	0.10	0.7500^{ns}
C^2	0.2576	1	0.2576	0.08	0.7865^{ns}
Error	57.8564	17	3.4033		
CV (%)	16.7000				
R^2	0.7342				

ns and *, not significant and significant, respectively, $p \leq 0.05$ using the *F* test; CV: coefficient of variation; R^2: coefficient of determination.

After optimising the variables used in the production of activated carbon, 18.41 g was determined as the optimum quantity, obtained at 100 °C modification temperature, 400 °C pyrolysis and activation temperature, and 1 h of activation time. Eq. 3 shows the optimal variables generated by the software:

$$\hat{y}_1 = 115.74 - 10.5406^{*}X_3 + 0.00032189^{*}X_2^2 \qquad (3)$$

The graph of the optimisation of obtaining activated carbon from cocoa pod husks (in g) is shown in Fig. 1, A, which corresponds to the response surface, while Fig. 1, B shows the analysis of the main effects. At lower modification and pyrolysis temperatures, there was a higher yield of activated carbon. The activation time had a negative effect because increasing its value decreased the production of activated carbon.

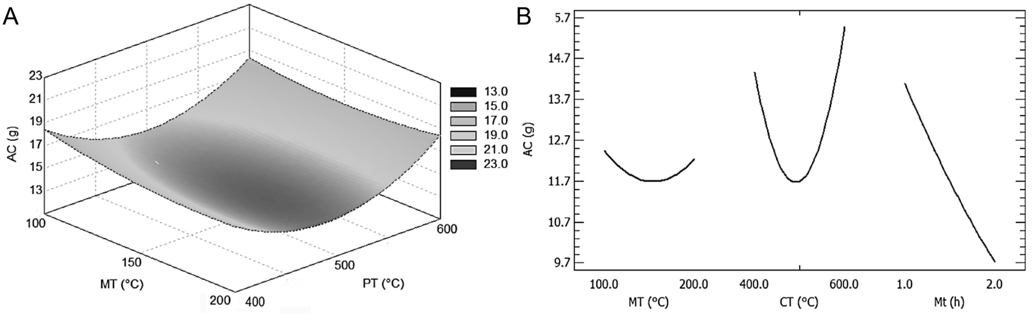

Figure 1. Effect of modification temperature, pyrolysis and activation temperature (A), and activation time (B) on obtaining activated carbon from cocoa pod husks.

MT – Modification Temperature; PT – Pyrolysis and Activation Temperature; AC – Activated Carbon; Mt – Modification Time.

Using lignocellulose materials from forestry and food industries as raw materials to manufacture activated carbon using pyrolysis, to be used as an adsorbent (Martelo et al., 2022), reduces costs, revaluates and minimises industrial waste, and becomes a viable alternative for carbon capture, thereby optimising environmental management and taking advantage of the benefits of its chemical composition.

Cocoa pods, depending on the variety, and according to Díaz-Oviedo et al. (2022) and Lara et al. (2016), have 43.6 and 12.66% lignin, 34.4 and 19.82% cellulose, and 11.75 and 9.45% hemicellulose, respectively, the latter two being carbohydrates, which decompose independently during pyrolysis (Kim et al., 2022), because hemicellulose is the first component to degrade between 220 and 315 °C (Yang et al., 2007). In contrast, at temperatures between 300 and 400 °C, cellulose undergoes decarbonisation, ring-opening polymerisation, and aromatisation (Hoang et al., 2021) to form aromatic groups (Lara et al., 2016). According to Ma et al. (2016), the pyrolysis of lignin leads to the transformation of methoxyl groups ($-O-CH_3$), and total phenol groups and G-type phenols (guaiacol) increase at 500 °C; however, from 400 to 700 °C, S-type phenols (syringol) decrease, while P-type phenols (phenol) and C-type phenols (catechol) increase, which are important for giving activated carbon its adsorbent characteristics.

Rincón et al. (2015) reported that the production of activated carbon typically involves two stages: the carbonisation (pyrolysis) of the raw material, and the activation of the carbonised material at temperatures of ≥ 500 °C. In the current study, it was carbonised and activated at 400, 500, and 600 °C. The high carbonisation and activation temperatures (700 °C) give the activated carbon larger pores (mesopores), making it suitable for water treatment (Rincón et al., 2015). Wang et al. (2021) stated that the production of activated carbon from lignocellulosic biomass comprises two main processes, activation and pyrolysis, which were applied in this study.

In the production of activated carbon, the pyrolysis temperature a considerable influence on its texture, which in turn influences its adsorbent properties depending on its pore type. Rodríguez-Sánchez et al. (2019), when processing chestnut (*Castanea sativa Mill*) waste activated carbon at low-temperature (220 °C) carbonisation, obtained BET areas (specific surface areas) of 3 $m^2\ g^{-1}$, whereas at 800 °C, the BET area was 568 $m^2\ g^{-1}$, and the micropore and mesopore volumes were 0.133 $cm^3\ g^{-1}$ and 0.033 $cm^3\ g^{-1}$, respectively. Notably, the temperature, residence time, and heating rate have a substantial impact on pyrolysis (Yogalakshmi et al., 2022).

In this regard, Chen et al. (2022) indicated that during pyrolysis, lignocellulosic components were degraded, generating the first volatile hemicellulose compounds, followed by cellulose, and then volatile lignin.

The pyrolysis of lignocellulose feedstocks leads to the formation of carbon, depolymerisation, and fragmentation of molecules via various mechanisms. In this regard, Chen et al. (2022) indicated that during pyrolysis, lignocellulosic components were degraded, generating the first volatile hemicellulose compounds, followed by cellulose, and then volatile lignin. Pyrolysis of lignin generates hydrocarbons due to aromatic rings and methoxy groups. With increasing temperature, the carboxy-C and O-alkyl-C structures in biochar decrease, whereas aryl-C increases due to deoxygenation reactions such as dehydroxylation, decarboxylation, decarbonylation, and demethoxylation, which reduce the amount of oxygen-containing functional groups (such as -OH, -C=O, -COOH, and $-OCH_3$), and polycondensation reactions, leading to the formation of more polycyclic aromatic hydrocarbon units during pyrolysis. According to Nyirenda et al. (2022), the heat treatment produced by pyrolysis develops oxide compounds on the surface of activated carbon, which directly influences its ability to remove metals from water samples.

Adsorbent activity, metal adsorption mechanisms of activated carbon, and ANOVA

The results of the statistical analysis of the data generated by the adsorptive action of the activated in removing Fe^{2+}, Al^{3+}, Cu^{2+}, and Zn^{2+} as water pollutants, as well as their significance, are presented in Table 4.

Table 4. Summary of removal of Fe^{+2}, Al^{+3}, Cu^{+2}, and Zn^{+2} according to ANOVA

Sources of variation	*P*-value			
	Fe^{2+}	Al^{3+}	Cu^{2+}	Zn^{2+}
A: Amount of activated carbon	0.0000*	0.0000*	0.0000*	0.0000*
B: Stirring time	0.0000*	0.0001*	0.0000*	0.0000*
AA	0.0002*	0.0000*	0.2014^{ns}	0.6955^{ns}
AB	0.0000*	0.0048*	0.0020*	0.5082^{ns}
BB	0.9912^{ns}	0.0001*	0.6326^{ns}	0.8754^{ns}
CV (%)	37.52	36.95	32.28	38.34

ns and *, not significant and significant, respectively, $p \leq 0.05$ using the *F* test; CV: coefficient of variation.

A 5% significance level was set for the removal of Fe^{2+}, Al^{3+}, Cu^{2+}, and Zn^{2+} as the amount of activated carbon used correlated with the stirring time at $p \leq 0.05$. Table 5 lists the adjusted metal removal models.

Table 5. Coefficient of determination and fitted models of metal removal

Metal removed	R^2	Adjusted R^2	Equation	
Fe^{2+}	0.9787	0.9711	$\hat{y}_{Fe^{2+}} = 1.06911 - 0.090333^{*}X_1 - 0.0067889^{*}X_2 - 0.002425^{*}X_1X_2 - 0.000167^{*}X_1^2$	(4)
Al^{3+}	0.9937	0.9913	$\hat{y}_{Al^{3+}} = 0.1560 - 0.035^{*}X_1 + 0.002478^{*}X_2 - 0.0001417^{*}X_1X_2 + 0.0095^{*}X_1^2 - 0.0000339^{*}X_2^2$	(5)
Cu^{2+}	0.9224	0.8938	$\hat{y}_{Cu^{2+}} = 0.033889 + 0.001733^{*}X_1 - 0.000233^{*}X_2 - 0.00008417^{*}X_1X_2$	(6)
Zn^{2+}	0.9266	0.8996	$\hat{y}_{Zn^{2+}} = 0.0084556 - 0.0007333^{*}X_1 - 0.00005389^{*}X_2$	(7)

* denotes significance at $p \leq 0.05$ using the *F* test; R^2: coefficient of determination.

It is considered that there was a good fit in the models because the coefficients of determination (R^2), including the adjusted R^2, ranged from 0.9224 to 0.9937 and 0.8938 to 0.9913, respectively (Table 5), in all the tests performed at the laboratory level, owing to the type of experimental design, which allowed us to determine the influence of the independent variables on the removal of metals from the water samples of the Ucayali River (Fig. 2).

The optimum removal value, according to the fitted model, was 0.778 ppm for Fe^{2+}, with 1.0 g of activated carbon and a stirring time of 90.0 min (Eq. 4); 0.171 ppm for Al^{3+}, with 1.0 g of activated carbon and a stirring time of 34.5 min (Eq. 5); 0.027 ppm for Cu^{2+}, with 1.0 g of activated carbon and a stirring time of 30.0 min (Eq. 6);

and 0.0061 ppm for Zn^{2+}, with 1.0 g of activated carbon and a stirring time of 30.0 min (Eq. 6; Fig. 2).

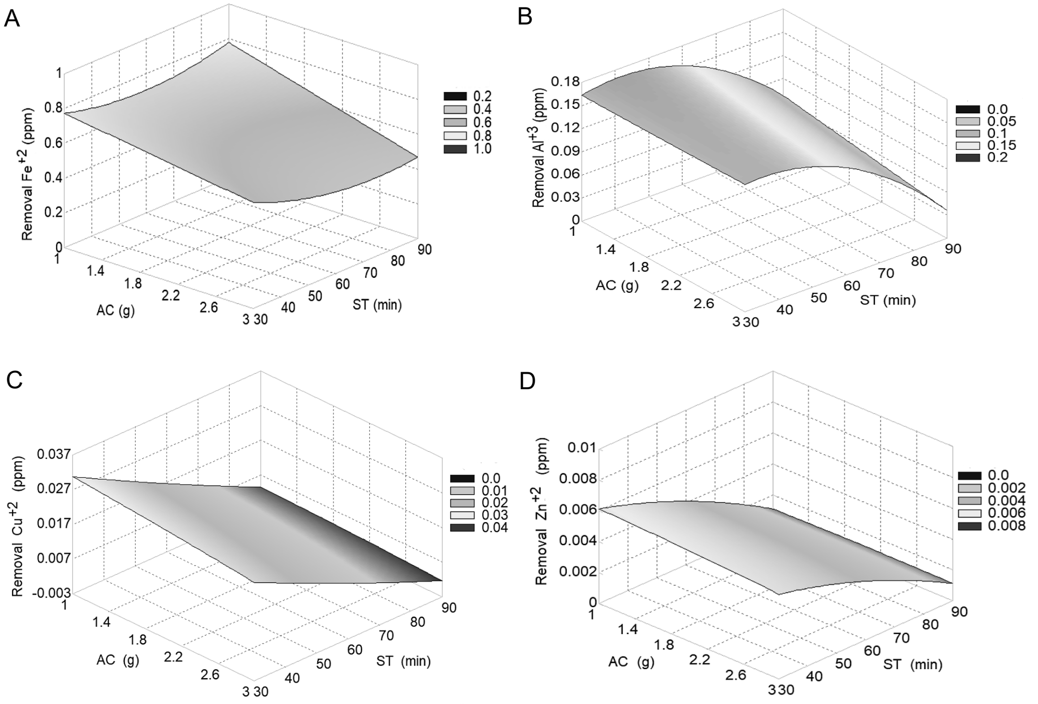

Figure 2. Effect of the amount of activated carbon and the agitation time on the removal of Fe^{2+}, Al^{3+}, Cu^{2+}, and Zn^{2+} from water samples from the Ucayali River, Peru.
ST – Stirring Time; AC – Activated Carbon.

The adsorptive effect of activated carbon on the removal of metals from water samples from the Ucayali River was obtained from the optimization of the models, which were developed under ideal conditions, and 1 g of activated carbon was established as the effective amount to remove Fe^{2+}, Al^{3+}, Cu^{2+}, y, and Zn^{2+} (Table 6).

Table 6. Percentage of metals removed from water samples with 1 g of activated carbon from cocoa pod shells

	Metal concentration (ppm)			
	Fe^{2+}	Al^{3+}	Cu^{2+}	Al^{2+}
Initial content	1.370	0.250	0.041	0.0110
Amount adsorbed	0.778	0.171	0.027	0.0061
% removed	56.8	68.4	65.9	55.5

The adsorptive effectiveness of activated carbon depends on the carbonisation temperature and the activation method. Narvekar et al. (2021) obtained activated carbon with a pore volume of 0.08 m^3 g^{-1} and a surface area of 425 m^2 g^{-1} at 800 °C in the presence of N_2. These are fundamental factors to guarantee the adsorptive power of biochar, whose functionality is provided by C=C bonds (alkene group) and the $-SO_3H$ group (sulphone). The sulfone group has surfactant properties and is used for the removal of metals from wastewater by chemisorption, for example, of Cd^{2+} and Pb^{2+} (Gul et al., 2022).

Ahmad et al. (2018) manufactured activated carbon from banana peels (*Musa paradisiaca*) and cauliflower leaves (*Brassica oleracea* var. botrytis) by thermal modification in an oven at 200 °C for 1 h, then by pyrolysis and activation at 600 °C for 2 h. Subsequently, they were crushed to particles of 0. 452–1 mm, with which they removed metals from water by adsorption. The banana peels had the highest adsorption efficiency, being in the order of removal of $Pb^{2+} > Cu^{2+} > Cd^{2+}$, with the electrostatic attraction being the predominant mechanism governing the sorption process.

Lara et al. (2016) used washed, dehydrated, and ground cocoa shells as a bioadsorbent to remove Pb^{2+} (91.32%) and Cd^{2+} (87.80%) from wastewater samples, attributing the bioadsorption to the action of aromatic groups, specifically C-H and C=C.

Valdés-Rodríguez et al. (2022) obtained activated carbon from jacaranda (*Jacaranda mimosifolia*) and guava (*Psidium guajava*) seed residues and grape pomace (*Vitis vinifera*) and sugar cane bagasse (*Saccharum officinarum*), resulting in the jacaranda seed biochar being the most efficient for adsorbing Hg^{2+} and Pb^{2+} from wastewater samples at pyrolysis at 800 °C for 4 h, attributing the adsorption mechanism to the oxygenated functional groups present in the activated carbon.

Namasivayam et al. (2007) used activated carbon from pine nut (*Jatropha curcas*) shells, pyrolysed at 700 °C in a muffle furnace for 1 h, with particles of 250–500 μ, and successfully removed Cr^{6+}, V^{5+}, and Ni^{2+} from water samples, attributing the adsorption capacity to its large surface area, its microporous characteristic, and the chemical nature of its surface.

Using plant-derived activated carbon with additives to improve adsorption capacity has been demonstrated by Nyirenda et al. (2022), who employed a silver-silica nanocomposite using activated carbon as a support, with which they were able to adsorb 84.75, 81.30, 87.72, and 81.97 mg g^{-1} for Cu^{2+}, Pb^{2+}, Cd^{2+}, and Zn^{2+}, respectively. The removal of these metals by activated carbon adsorption occurs via electrostatic interactions between the dissolved cations and negatively charged silanolate surface sites; however, ion exchange is the main adsorption mechanism (Ahmad et al., 2017).

New alternatives to improve the adsorption capacity of activated carbon from cellulose are being experimented to maximise its capacity to remove metals present in water. Mubarak et al. (2022) experimented with activated carbon and a nanocomposite of carborundum and microcrystalline cellulose, which acted as an exceptional active adsorbent for the adsorption of As^{3+} and Cu^{2+} ions for water treatment. The main mechanism of adsorption of the ions by the nanocomposite was electrostatic interaction at moderate pH values (above 6), while at high acidity conditions (pH 2–6), the dominant mechanism was chemical complexation.

CONCLUSION

The production of activated carbon from cocoa pod husks by pyrolysis can be applied to various lignocellulose raw materials because the activation and modification of cellulose, hemicellulose, and lignin provides a large surface area, suitable surface functional groups, and appropriate pore diameters that confer adsorbent potential. The adsorption and heavy metal removal efficiencies of the activated carbon was a function of the parameters used for its production, such as a modified temperature of 100 °C, activation time of 1 h, and pyrolysis and activation temperatures of 400 °C, with which 18.41 g of activated carbon was obtained from cocoa husks. Moreover, 56.8%, 68.4%, 65.9%, and 55.5% of iron, aluminium, copper, and zinc was removed, verifying its use in the manufacture of filters to decontaminate water with heavy metals.

REFERENCES

Adeyeye, S.A.O. & Ashaolu, T.J. 2020. A Study on Polycyclic Aromatic Hydrocarbon and Heavy Metal Concentrations of Commercial Grilled Meat (Suya) and Smoked Catfish (Clarias gariepinus Burchell, 1822) Fish from South-West, Nigeria. *Polycyclic Aromatic Compounds* **42**(6), 3281–3290. doi:10.1080/10406638.2020.1858883

Ahmad, I., Siddiqi, W.A. & Ahmad, T. 2017. Synthesis, Characterization of Silica Nanoparticles and Adsorption Removal of Cu 2+ Ions in Aqueous Solution. *International Journal of Emerging Technology and Advanced Engineering* **7**(8), 439–445.

Ahmad, Z., Gao, B., Mosa, A., Yu, H., Yin, X., Bashir, A., Ghoveisi, H. & Wang, S. 2018. Removal of Cu(II), Cd(II) and Pb(II) ions from aqueous solutions by biochars derived from potassium-rich biomass. *Journal of Cleaner Production* **180**, 437–449. doi:10.1016/j.jclepro.2018.01.133

Akila, M., Anbalagan, S., Lakshmisri, N., Janaki, V., Ramesh, T., Merlin, R.J. & Kamala-Kannan, S. 2022. Heavy metal accumulation in selected fish species from Pulicat Lake, India, and health risk assessment. *Environmental Technology and Innovation* **27**, 102744. doi:10.1016/j.eti.2022.102744

Aksoy, D. & Ercan Sagol. 2016. Application of central composite design method to coal flotation: Modelling, optimization and verification. *Fuel* **183**, 609–616. doi:10.1016/j.fuel.2016.06.111

ASTM. 1997. Standard test method for determination of moisture content of particulate wood fuels using a microwave oven E 1358–97 (Reapproved 2006). *ASTM International* **11**(10), 1–2. doi:10.1520/E1358 -97R13.2

Baby, R. & Hussein, M.Z. 2020. Ecofriendly Approach for Treatment of Heavy-Metal-Contaminated Water Using Activated Carbon of Kernel Shell of Oil Palm. *Materials* **13**(11), 2627. doi:10.3390/ma13112627

Ccanccapa-Cartagena, A., Paredes, B., Vera, C., Chavez-Gonzales, F.D., Olson, E., Welp, L.R. & Jafvert, C.T. 2021. Occurrence and probabilistic health risk assessment (PRA) of dissolved metals in surface water sources in Southern Peru. *Environmental Advances* **5**, 100102–100102. doi:10.1016/j.envadv.2021.100102

Chen, D., Cen, K., Zhuang, X., Gan, Z., Zhou, J., Zhang, Y. & Zhang, H. 2022. Insight into biomass pyrolysis mechanism based on cellulose, hemicellulose, and lignin: Evolution of volatiles and kinetics, elucidation of reaction pathways, and characterization of gas, biochar and bio-oil. *Combustion and Flame* **242**, 112142–112142. doi:10.1016/j.combustflame.2022.112142

Chen, D., Xie, S., Chen, C., Quan, H., Hua, L., Luo, X. & Guo, L. 2017. Activated biochar derived from pomelo peel as a high-capacity sorbent for removal of carbamazepine from aqueous solution. *RSC Advances* **7**(87), 54969–54979. doi:10.1039/c7ra10805b

Choi, Y. & Kan, E. 2019. Effects of pyrolysis temperature on the physicochemical properties of alfalfa-derived biochar for the adsorption of bisphenol A and sulfamethoxazole in water. *Chemosphere* **218**, 741–748. doi:10.1016/j.chemosphere.2018.11.151

Cruz, G.L., Pirilä, M., Huuhtanen, M., Carrión, L., Alvarenga, E. & Keiski, R.L. 2012. Production of Activated Carbon from Cocoa (Theobroma cacao) Pod Husk. *Journal of civil & environmental engineering* **2**(2). doi:10.4172/2165-784x.1000109

Díaz-Oviedo, A.F, Ramón-Valencia, B.A. & Moreno-Contreras, G. 2022 Physical- chemical characterization of the cocoa pod husk as a possible use in the production of agglomerated boards. *Revista de Investigación, Desarrollo e Innovación* **12**(1), 97–106 (in Spanish). doi:10.19053/20278306.v12.n1.2022.14211

Fathy, M., Zayed, A.I. & Ahmed, H.B. 2022. Activated Carbon/Carborundum@Microcrystalline Cellulose core shell nano-composite: Synthesis, characterization and application for heavy metals adsorption from aqueous solutions. *Industrial Crops and Products* **182**, 114896. doi:10.1016/j.indcrop.2022.114896

González-García, P., Centeno, T.A., Urones-Garrote, E., Ávila-Brande, D. & Otero-Díaz, L. 2013. Microstructure and surface properties of lignocellulosic-based activated carbons. *Applied Surface Science* **265**, 731–737. doi:10.1016/j.apsusc.2012.11.092

Grich, A., Taoufiq Bouzid, T., Aicha Naboulsi, Abdelmajid Regti, Abdelaaziz Aloui Tahiri, Mamoune El Himri, & Mohammadine El Haddad. 2024. Preparation of low-cost activated carbon from Doum fiber (Chamaerops humilis) for the removal of methylene blue: Optimization process by DOE/FFD design, characterization, and mechanism. *Journal of Molecular Structure* **1295**, 136534–136534. doi:10.1016/j.molstruc.2023.136534

Gul, S., Ahmad, Z., Asma, M., Ahmad, M., Rehan, K., Munir, M., Bazmi, A.A., Ali, H.M., Mazroua, Y., Salem, M.L., Akhtar, M., Khan, M.S., Chuah, L.F. & Asif, S. 2022. Effective adsorption of cadmium and lead using SO3H-functionalized Zr-MOFs in aqueous medium. *Chemosphere* **307**, 135633. doi:10.1016/j.chemosphere.2022.135633

Guo, X., Dong, H., Yang, C., Zhang, Q., Liao, C., Zha, F. & Gao, L. 2016. Application of goethite modified biochar for tylosin removal from aqueous solution. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **502**, 81–88. doi:10.1016/j.colsurfa.2016.05.015

Hoang, A.T., Show, P.L., Fattah, I.R., Chong, C.T., Cheng, C.K., Sakthivel, R. & Kim, K. 2021. Progress on the lignocellulosic biomass pyrolysis for biofuel production toward environmental sustainability. *Fuel Processing Technology* **223**, 106997. doi:10.1016/j.fuproc.2021.106997

Kim, H., Yu, S., Kim, M. & Ryu, C. 2022. Progressive deconvolution of biomass thermogram to derive lignocellulosic composition and pyrolysis kinetics for parallel reaction model. *Energy* **254**, 124446. doi:10.1016/j.energy.2022.124446

Lara, J., Tejada, C., Villabona, A. & Arrieta, A. 2016. Adsorption of lead and cadmium in continuous of fixed bed on cocoa waste. *Revista Ion* **29**(2), 111–122 (in Spanish). doi:10.18273/revion.v29n2-2016009

Liu, Z., Sun, Y., Xu, X., Qu, J. & Qu, B. 2020. Adsorption of Hg(II) in an Aqueous Solution by Activated Carbon Prepared from Rice Husk Using KOH Activation. *ACS omega* **5**(45), 29231–29242. doi:10.1021/acsomega.0c03992

López, M. & Alva, F. 2022. UCAYALI: Síntesis de Actividad Económica FEBRERO 2022. Banco Central de Reserva del Perú. Departamento de Estudios Económicos, Iquitos, Perú (in Spanish). https://www.bcrp.gob.pe/docs/Sucursales/Iquitos/2022/sintesis-ucayali-02-2022.pdf. Accessed 12.09.2023

Ma, Z., Sun, Q., Ye, J., Yao, Q. & Zhao, C. 2016. Study on the thermal degradation behaviors and kinetics of alkali lignin for production of phenolic-rich bio-oil using TGA–FTIR and Py–GC/MS. *Journal of Analytical and Applied Pyrolysis* **117**, 116–124. doi:10.1016/j.jaap.2015.12.007

Mariana, M., Khalil, H.P.S.A., Mistar, E.M., Yahya, E.B., Alfatah, T., Danish, M. & Amayreh, M.Y. 2021. Recent advances in activated carbon modification techniques for enhanced heavy metal adsorption. *Journal of water process engineering* **43**, 102221. doi:10.1016/j.jwpe.2021.102221

Martelo, N., Antxustegi, M., Corro, E., Baloch, M., Volpe, R., Gagliano, A., Fichera, A. & Alriols, M.G. 2022. Use of residual lignocellulosic biomass for energetic uses and environmental remediation through pyrolysis. *Energy Storage and Saving* **1**(3), 129–135. doi:10.1016/j.enss.2022.04.004

Maulina, S. & Iriansyah, M. 2018. Characteristics of activated carbon resulted from pyrolysis of the oil palm fronds powder. *IOP Conference Series: Materials Science and Engineering* **309**, 012072. doi:10.1088/1757-899x/309/1/012072

Mubarak, M.F., Zayed, A.M. & Ahmed, H.A. 2022. Activated Carbon/Carborundum@Microcrystalline Cellulose core shell nano-composite: Synthesis, characterization and application for heavy metals adsorption from aqueous solutions. *Industrial Crops and Products* **182**, 114896–114896. doi:10.1016/j.indcrop.2022.114896

Namasivayam, C., Sangeetha, D. & Gunasekaran, R. 2007. Removal of Anions, Heavy Metals, Organics and Dyes from Water by Adsorption onto a New Activated Carbon from Jatropha Husk, an Agro-Industrial Solid Waste. *Chemical Engineering Research and Design* **85**(2), 181–184. doi:10.1205/psep05002

Nandeshwar, S.N., Mahakalakar, A.S., Gupta, R. & Kyzas, G.Z. 2016. Green activated carbons from different waste materials for the removal of iron from real wastewater samples of Nag River, India. *Journal of Molecular Liquids* **216**, 688–692. doi:10.1016/j.molliq.2015.12.065

Narvekar, A.A., Fernandes, J.O., Naik, S.N. & Tilve, S.G. 2021. Development of glycerol based carbon having enhanced surface area and capacitance obtained by KOH induced thermochemical activation. *Materials Chemistry and Physics* **261**, 124238. doi:10.1016/j.matchemphys.2021.124238

Nyirenda, J., Kalaba, G.M. & Munyati, O. 2022. Synthesis and characterization of an activated carbon-supported silver-silica nanocomposite for adsorption of heavy metal ions from water. *Results in engineering* **15**, 100553. doi:10.1016/j.rineng.2022.100553

Odubiyi, O.A., Awoyale, A.A. & Eloka-Eboka, A.C. 2012. Wastewater Treatment with Activated Charcoal Produced from Cocoa Pod Husk. *International Journal of Environment and Bioenergy* **4**(3), 162–175.

Olaoye, R.A., Afolayan, O.D., Mustapha, O.I. & Adeleke, H.O. 2018. The Efficacy of Banana Peel Activated Carbon in the Removal of Cyanide and Selected Metals from Cassava Processing Wastewater. *Advances in research* **16**(1), 1–12. doi:10.9734/air/2018/43070

Prastuti, O.P., Septiani, E., Kurniati, Y., Widiyastuti, P. & Setyawan, H. 2019. Banana Peel Activated Carbon in Removal of Dyes and Metals Ion in Textile Industrial Waste. *Materials Science Forum* **966**, 204–209. doi:10.4028/www.scientific.net/msf.966.204

Rincón, J.S., Rincón, S., Guevara, P., Ballén, D.A.S., Morales, J.C. & Monroy, N. 2015. Production of activated carbon by physical methods from El Cerrejon coal and its application in the treatment of wastewater from dyeing plants. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* **39**(51), 171–175 (in Spanish). doi:10.18257/raccefyn.138

Rodríguez-Sánchez, S., Ruiz, B.A., Martínez-Blanco, D., Sánchez-Arenillas, M., Diez, M.A., Suárez-Ruiz, I., Marco, J.F., Blanco, J.E. & Fuente, E. 2019. Sustainable Thermochemical Single-Step Process To Obtain Magnetic Activated Carbons from Chestnut Industrial Wastes. *ACS Sustainable Chemistry & Engineering* **7**(20), 17293–17305. doi:10.1021/acssuschemeng.9b04141

Sabela, M.I., Kunene, K., Kanchi, S., Xhakaza, N.M., Bathinapatla, A., Mdluli, P.S., Sharma, D. & Bisetty, K. 2019. Removal of copper (II) from wastewater using green vegetable waste derived activated carbon: An approach to equilibrium and kinetic study. *Arabian Journal of Chemistry* **12**(8), 4331–4339. doi:10.1016/j.arabjc.2016.06.001

Sadheesh, S., Shankar, R., Kumar, S. & Prasath, B.G. 2021. Contaminated water treatment with Activated charcoal build from cocoa pod husk. *IOP conference series* **1145**(1), 012027. doi:10.1088/1757-899x/1145/1/012027

Sibal, L.N. & Espino, M.P.B. 2018. Heavy metals in lake water: a review on occurrence and analytical determination. *International Journal of Environmental Analytical Chemistry* **98**(6), 536–554. doi:10.1080/03067319.2018.1481212

Solanki, A. & Boyer, T.H. 2017. Pharmaceutical removal in synthetic human urine using biochar. *Environmental science* **3**(3), 553–565. doi:10.1039/c6ew00224b

Tsai, C., Tsai, W., Liu, S. & Lin, Y. 2018. Thermochemical characterization of biochar from cocoa pod husk prepared at low pyrolysis temperature. *Biomass Conversion and Biorefinery* **8**(2), 237–243. doi:10.1007/s13399-017-0259-5

Valdés-Rodríguez, E., Mendoza-Castillo, D.I., Reynel-Avila, H.E., Aguayo-Villarreal, I.A. & Bonilla-Petriciolet, A. 2022. Activated carbon manufacturing via alternative Mexican lignocellulosic biomass and their application in water treatment: Preparation conditions, surface chemistry analysis and heavy metal adsorption properties. *Chemical engineering research and design* **187**, 9–26. doi:10.1016/j.cherd.2022.08.039

Wang, H., Chen, D., Huang, L., Zhang, Y., Zhang, Y., Wang, A., Xiao, G., Zhang, W., Lei, H. & Ruan, R. 2021. Activated carbon from lignocellulosic biomass as catalyst: A review of the applications in fast pyrolysis process. *Journal of Analytical and Applied Pyrolysis* **158**, 105246. doi:10.1016/j.jaap.2021.105246

Wu, L., Yang, N., Li, B. & Bi, E. 2018. Roles of hydrophobic and hydrophilic fractions of dissolved organic matter in sorption of ketoprofen to biochars. *Environmental Science and Pollution Research* **25**(31), 31486–31496. doi:10.1007/s11356-018-3071-2

Yang, H., Yan, R., Chen, H., Lee, D.H. & Zheng, C. 2007. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **86**(12–13), 1781–1788. doi:10.1016/j.fuel.2006.12.013

Yogalakshmi, K.,T, Sivashanmugam, P.D., Kavitha, S., Kannah, R.Y., Varjani, S., Adishkumar, S., Aslam, M. & Rajesh, B.J. 2022. Lignocellulosic biomass-based pyrolysis: A comprehensive review. *Chemosphere* **286**, 131824. doi:10.1016/j.chemosphere.2021.131824

Yunus, Z.M., Al-Gheethi, A., Othman, N., Hamdan, R. & Ruslan, N.N. 2020. Removal of heavy metals from mining effluents in tile and electroplating industries using honeydew peel activated carbon: A microstructure and techno-economic analysis. *Journal of Cleaner Production* **251**, 119738. doi:10.1016/j.jclepro.2019.119738

Zaghloul, G., El-Din, H.M.S., Mohamedein, L.I. & El-Moselhy, K.M. 2022. Bio-accumulation and health risk assessment of heavy metals in different edible fish species from Hurghada City, Red Sea, Egypt. *Environmental Toxicology and Pharmacology* **95**, 103969. doi:10.1016/j.etap.2022.103969

Agronomy Research **22**(S2), 672–684, 2024
https://doi.org/10.15159/AR.24.035

Effects of chemical seed priming on germination performance and seedling growth of *Lycopersicon esculentum (Mill.)* under salt stress

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Abstract. As an important economic plant, *Lycopersicon esculentum (Mill.)* faces salinity stress from germination to all growth stages. The aim of this study is to ride salt-induced agriculture difficulties of tomato by applying different chemical seed priming: ascorbic acid (ASA), potassium nitrate (KNO_3) and calcium nitrate ($CaNO_3$) during two time periods which are 24 and 48 hours. In the current case, the seeds were pre-treated with previously mentioned chemicals for varying periods of time before germination in a salt solution (100 mM NaCl). The treatments were replicated three times. For no primed seeds, salt treatment decreased germination parameters as well as seedling growth parameters (fresh weight, epicotyl and root length and chlorophyll content). Different chemical seed priming alleviated the salt harmful effect on germination and growth parameters. In saline conditions, the priming agents, had more significant effect in comparison with normal conditions. Significantly, the treatment including $CaNO_3$-48-h priming, had high efficacy in promoting germination and plant growth and is associated with reduced levels of leaf proline and malondialdehyde (MDA) content.

Key words: ascorbic acid, calcium nitrate, potassium nitrate, tomato, priming, salinity, seed germination.

INTRODUCTION

Salt exposure is one of major environmental stress that affect plant growth and agricultural crop (Mbarki et al., 2020; Shahid et al., 2020). Like several arid regions, in Saoudi Arabia, tomato growth suffered from sub-optimal conditions, especially salinity (Al-Harbi et al., 2017). Germination stage is one of the most sensitive stages of plant growth to salinity, and as shown by Rajabi Dehnavi et al. (2020), salinity decrease the germination rate due to an increase in the osmotic pressure of the soil solution, resulting in a slowdown in the uptake and a decrease in the absorption of water required for the movement of various metabolic processes. The study conducted by Wang et al. (2020) showed that salt stress leads to a reduction in germination rate and this is mainly related to seed inability to take in the required amount of water, and also because of the poisoning of the foetus by high concentrations of some ions such as chlorine (Hakim et al., 2010).

The used technique is ‘seed priming’, it’s a commonly used approach that involves submerging the seeds in water and/or a chemical solution (Pawar & Laware, 2018). Seed priming technique has been discovered as a successful approach to enhance stress tolerance in several plant species. Several physiological treatments have been extensively studied to enhance seed germination and emergence of seedlings under various stress conditions (Ghoohestani et al., 2012; Moaaz et al., 2020; Ikram et al., 2023; Mangal et al., 2023). In addition, the combination of growth regulators of plant and different pre-sowing seed treatments could improve seed performance in a number of vegetable crops. Favorable results of KNO_3 priming have been obtained for several plant species, including soybean, tomato and wheat (Ghassemi-Golezani et al., 2011; Feghhenabi et al., 2020; Moaaz et al., 2020; Hadia et al., 2023). Salles et al., 2019 demonstrated that calcium nitrate priming increased the germination rate of eggplant seeds. Previously, Ghoohestani et al., 2012 demonstrated the effect of ASA, and salicylic acid on tomato seed germination improvement.

Hence, the aim of this research is to examine the effect of chemical priming (ASA, KNO_3 and $CaNO_3$) on germination performance and seedling growth of tomato in salt conditions within different priming periods (24 and 48 hours).

MATERIALS AND METHODS

Tomato seeds (GRObite Desi Tomato Vegetable Seeds) were sterilized with 0.25% sodium hypochlorite solution for 10 minutes. Seeds were firstly divided into 7 groups: (No pre-treated seed group,3 groups were pre-treated with different priming solutions (KNO_3 2%, ASA 150 mg L and $CaNO_3$ 0.2%) for 24 hours, and 3 groups were pre-treated with the same priming solutions for 48 hours, with gentle shaking (Ghoohestani et al., 2012; Salles et al., 2019; Moaaz Ali et al., 2020). After that, the priming seeds were placed for germination in Petri dishes soaked with distilled water or NaCl solution (100 mM). Finally, each priming agent resulted in 4 treatments (2 time periods of priming and 2 irrigation solutions). Two controls are designed: Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. For each treatment, 3 petri dishes were used with twenty seeds in each. The treatments were replicated three times.

Seeds were considered germinated when the radicle and epicotyl emerged. Samples were sorted by epicotyl and radicle for determination of fresh weight, photosynthetic pigments, malondialdehyde (MDA) and proline content.

According Wu et al., 2019, we determined Final germination percentage (FGP).

1. $FGP = NGS/NTS \times 100$

Also mean germination time (MGT)

2. $MGT=\Sigma\ (N1T1 + N2T2 + \ldots + NiTi)\ /\Sigma\ (\mathrm{N1} + \mathrm{N2} \ldots + \mathrm{Ni})$

We analyzed Germination rate index (GRI) and germination index (GI) using the formula proposed by Shah et al. (2021).

3. $GRI = N1/T1 + N2/T2 + \ldots. + Ni/Ti$
4. $GI = (10 \times N1) + (9 \times N2) + \ldots + (1 \times Ni)$

Abbreviations used in above equations were:

NGS: number of final germinated seeds (in the end of experiment: 6 days)

NTS: number of total tested seeds

Ni: number of seeds germinated in the i^{th} time

Ti: time taken for seed germination at i^{th}

The methodology utilized to determine chlorophyll was that of Arnon (1949). At 663 nm and 645 nm, the absorbance of each sample was measured. Using the formula provided by MacKinney (1941), the chlorophyll content was computed and expressed in mg per g FW.

Total Chlorophylls (mg L) = $20.2 \times A645 + 8.02 \times A663$

A represents the extract's absorbance at the specified wavelength.

According to the ninhydrin method, we estimated proline content (Pro) in tomato seedlings (Bates et al., 1972).

In epicotyl, the total amount of malondialdehyde (MDA) was determined using the approach outlined by Heath & Packer in 1968. An extraction procedure was conducted on leaf samples utilizing a solution comprising 10% trichloroacetic acid (TCA) and 0.65% 2-thiobarbituric acid. The MDA concentration in epicotyl sample was expressed as nmol g^{-1} FW.

ANOVA analysis and Tukey's HSD tests were used to ascertain significant variations between the means of different treatments at probability level ≤ 0.05.

RESULTS AND DISCUSSION

Germinations parameters

Globally, tomatoes are ingested due to their rich nutrient and bioactive compound content (Li et al., 2021; Ali et al., 2021). Recent studies declared that salt is one of major factors that affect plant yield and fruit quality (Zhang et al., 2022). Although salinization of soil and water is a natural process, it is exacerbated by anthropogenic activities such as land clearance and improper irrigation techniques.

In the absence of seed priming, salt treatment inhibited seed germination (Fig. 1, A). Various chemical priming methods were shown to enhance seed germination under salt stress, with the most effective treatment being seed priming for 48 hours (as depicted in Fig. 1, A, Fig. 1, B, and Fig. 1, C). In normal conditions, KNO_3 and $CaNO_3$priming had no effect on final germination percentage (FGP) (Fig. 1, D). However, ASA agent induced a slight decrease of FGP (10%). In absence of seed priming, salt reduced FGP by 25% (Fig. 1, D). With 24-h chemical priming, salt reduced too FGP by 28%, 13 and 10% in ASA, KNO_3 and $CaNO_3$, respectively. In 48-h pre-treated seeds, ASA and KNO_3 agents decreased FGP by about 20% and 11% respectively, however $CaNO_3$ priming gave 100% FGP (Fig. 1, D). Found result is supported by several studies which reported the effect of seed priming in improving germination rates, increase grain production, and improve seedling growth (Goiba et al., 2018; Nouri & Haddioui, 2021). Different methods were used to mitigate harmful effect of salt: as seed priming (Ben Youssef et al., 2023), salt co-treatment in media (Moghaddam et al., 2023) or foliar spray (El-Hawary et al., 2023).

Recently Ben Youssef et al., 2023 revealed the effectiveness of seed priming with calcium chloride on germination and seedling growth in barley species. Exogenous KNO_3 application alleviates salt effect on glasswort growth (Moghaddam et al., 2023) and foliar application of ASA improved growth of wheat under salt treatment (El-Hawary et al., 2023). The current study found that different chemicals used in seed priming improved germination under salt stress; the most effective treatment for alleviating salt stress seems to be the $CaNO_3$-48-hour priming treatment (Fig. 1, D). For ASA agent, more research should be done to identify an effective ASA concentration that improve salt stress mitigation in tomato.

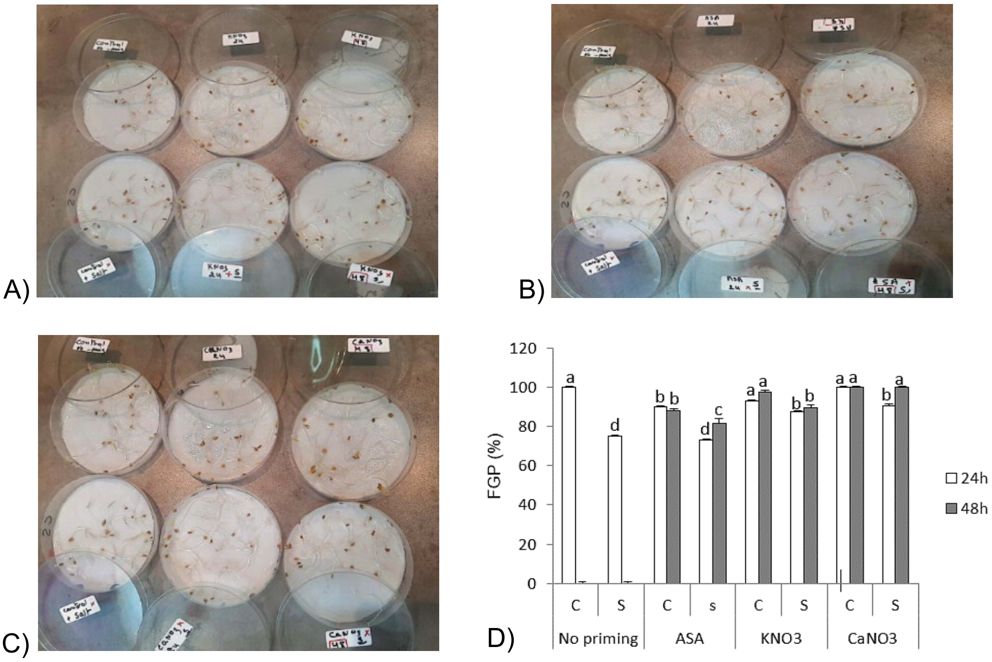


Figure 1. Germination test of tomato seeds in Petri dishes with different chemical priming: Ascorbic Acid priming (ASA) (A), potassium nitrate (KNO_3) (B) and calcium nitrate priming ($CaNO_3$) (C) within 24 h and 48 h. Effect of different chemical priming on Final germination percentage (FGP (D) within 24 and 48 hours after 6 days of germination under salt conditions. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

In normal conditions, chemical priming had minor effect on mean germination time (MGT) (Fig. 2, A). Under salt treatment, MGT increased in all chemical priming agents by about 28% that which was close to MGT in positive control both in 24-h and 48-h treated seeds (Fig. 2, A). So, different priming agents had no significant effect on germination time in normal conditions (Fig. 2, A). However, under salinity, priming agents decreased MGT in comparison with positive control, particularly with a 48-h $CaNO_3$ treatment (11%).

In normal conditions, KNO_3 and $CaNO_3$ increased germination rate index (GRI) in both 24-h primed seeds and 48-h primed seeds. GRI increase was most significant in KNO_3 and $CaNO_3$-48-h primed seeds by 13% and 29%, respectively (Fig. 2, B). In no primed seeds, salt reduced enormously GRI by 75%. In primed seeds, salt reduced less GRI especially in 48-h treated seeds. In ASA and KNO_3 pretreated seeds, salt reduced

GRI by more than 60% referring to positive control. In 48-h-$CaNO_3$ primed seeds, salt treatment reduced GRI only by 54% referring to positive control (Fig. 2, B).

In normal conditions, different chemical priming had no significant effect on germination index (GI) (Fig. 2, C). In no primed seeds, salt reduced GI by 47% referring to negative control. In ASA primed seeds, salt reduced GI by 37% and 27% after 24 h and 48 h of seed pretreatment, respectively. In KNO_3 primed seeds, salt reduced GI by about 25% in both 24 and 48 h of seed priming. In $CaNO_3$ primed seeds, salt reduced GI respectively by 26% and 16% in 24-h and 48-h seed priming. So, $CaNO_3$ priming restored more GI after 48 h of treatment in comparison with others priming agents (Fig. 2, C).

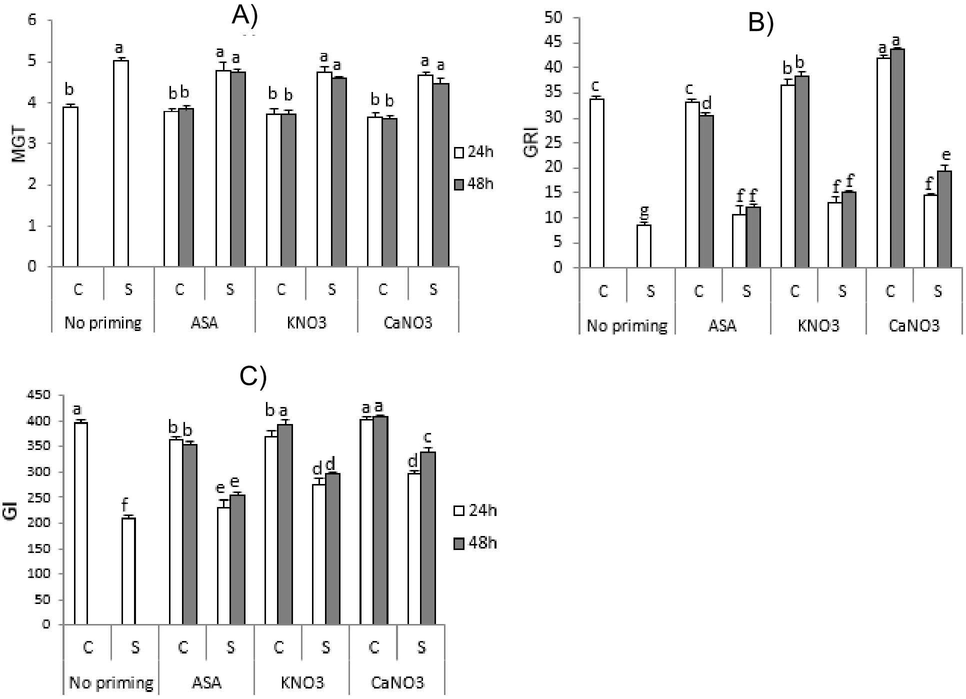


Figure 2. Effect of different chemical priming (ASA/KNO_3/$CaNO_3$) and time (24 h/48 h) on mean germination time (MGT) (A), Germination rate index (GRI) (B) and germination index (GI) (C) in normal and salt conditions. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

The current study revealed that KNO_3 and $CaNO_3$ seed priming increased GRI in normal conditions. Khoshvaghti et al., 2013 reported same results in *Anethum graveolents*. In addition, KNO_3 seed priming has been reported to enhance the germination rate of pepper seeds (Tu et al., 2022).

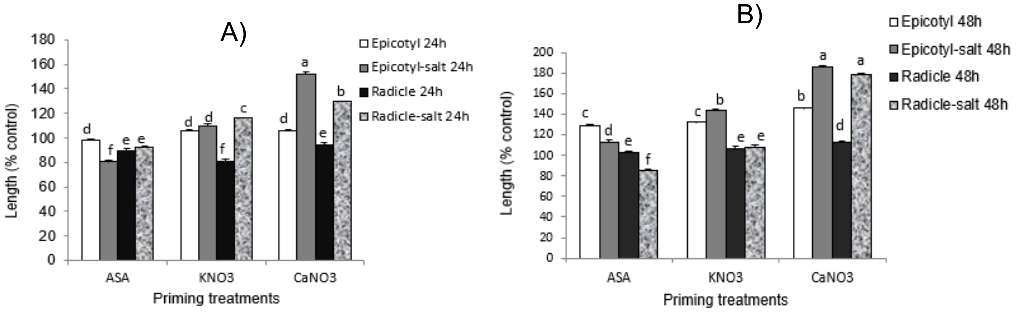
Chemical priming had no significant effect on MGT in normal conditions (Fig. 2, A). However, in several studies, seed priming reduced MGT, indicating that primed seeds are capable of germination in a shorter period of time (Nazari et al., 2017; Arun et al., 2022).

Found result revealed that without priming, salt treatment restrained germination seed (Fig. 1, A). Salt reduced FGP and GRI by 25% and 75%, respectively (Fig. 1, C; Fig. 2, B). This result is supported by Chakma et al. (2019) which demonstrated that salt stress decreased the rate of germination in tomato plant.

In current study, different chemical priming agents restored germination under salt treatments, especially 48-h seed priming. In $CaNO_3$-48-h pretreated seeds, FGP remain equal to normal conditions (100%) as shown in Fig. 1, C. Results showed that GRI increase was most significant in 48-h primed seeds by $CaNO_3$ (29%) referring to positive control (Fig. 2, B). $CaNO_3$ priming restored more GI after 48 hours of treatment in comparison with other chemical priming agents (Fig. 2, C).

Growth parameters

The chemical priming agents induced growth enhancement of tomato seedlings under both normal and salt conditions, as demonstrated in Fig. 3, C, Fig. 3, D, and Fig. 3, E. Notably, the 48-h $CaNO_3$ priming agent exhibited particularly significant effects.



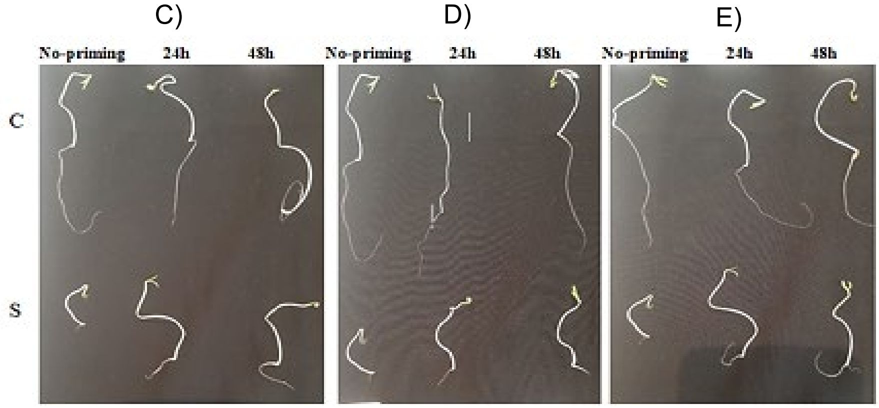


Figure 3. (A–B). Effect of different chemical priming (ASA/KNO_3/$CaNO_3$) on epicotyl and root length (% controls) after 24 h (**A**) and 48 h (**B**). Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.
Figure 4. (C–E): Effect of different chemical priming ASA, KNO_3 and $CaNO_3$, respectively, on seedling growth within 24 and 48 hours after 6 days of germination.

Under normal conditions, the 24-h seed priming with different chemicals had no significant effect on epicotyl length (Fig. 3, A). While in roots, KNO_3 and $CaNO_3$ treatments induced length by about 5% referring to negative control. Under salt treatment, 24-h chemical priming, especially $CaNO_3$, increased epicotyl and root length by 50% and 29%, respectively, referring to positive control.

Under normal conditions, 48-h priming with ASA, KNO_3 and $CaNO_3$ treatments induced epicotyl length by 28%, 32% and 45%, respectively, refer to negative control (Fig. 3, B). In roots, the effect was less than those in epicotyl. Under salt treatment, ASA induced epicotyl length, while radicle length was decreased. Especially, $CaNO_3$ priming induced both epicotyl and radicle length by 85% and 78%, respectively, referring to positive control (Fig. 3, B).

In normal conditions, the 24-h seed priming with different chemicals increased mainly epicotyl fresh weight (FW) and especially with $CaNO_3$ priming (21%) refer to negative control (Fig. 4, A). Under salt treatment, chemical priming, especially $CaNO_3$, increased both epicotyl and root FW by 46% and 24%, respectively, referring to positive control.

In normal conditions, 48-h priming with ASA had no effect on epicotyl and root FW (Fig. 4, B). KNO_3 and $CaNO_3$ treatments induced epicotyl FW by respectively 16%, 34% refer to negative control (Fig. 4, B). Under salt treatment, KNO_3 and $CaNO_3$ priming increased both epicotyl and radicle FW (Fig. 4, B). Especially, $CaNO_3$ priming increased both epicotyl and radicle FW by 85% and 80%, respectively, referring to positive control (Fig. 4, B).

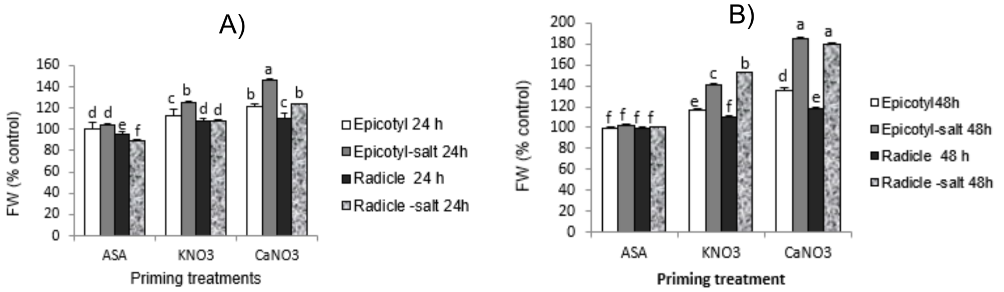


Figure 4. Effect of different chemical priming (ASA/KNO_3/$CaNO_3$) on epicotyl and root fresh weight (% controls) after 24 h (**A**) and 48 h (**B**). Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

Although used chemicals have no significant effect on seed germination in normal conditions, ASA, KNO_3 and $CaNO_3$ treatments induced an increase in both epicotyl and root length (Fig. 3, B). Seed priming with different chemicals increased epicotyl fresh weight (FW); especially with $CaNO_3$ priming within 24 h and 48 h (Fig. 4, B).

The current results suggested that under salinity, the decrease in plant growth, specifically the length and fresh weight of the epicotyl and root, (Fig. 3, A, Fig. 3, B, Fig. 4, A and Fig. 4, B), could be attributed to the osmotic effect caused by salt stress. This effect leads to a decrease in growth promoters, an increase in growth inhibitors and a disruption in the water balance (Rady, 2012; Rady et al., 2019a; Semida & Rady, 2014). Under salt treatment, both time of priming and all chemical agents, especially 48-h $CaNO_3$, increased epicotyl and root length and FW (Fig. 3, B; Fig. 4, B).

Previously, Oliveira et al. 2019, demonstrated that KNO_3 has the potential to effectively mitigate the detrimental impacts of salt treatment during the first stages of melon seed germination and plantlet growth. As in Papaya, results demonstrated that calcium ions had a more significant salt-alleviation effect on seedling growth in comparison to potassium ions (Maneesha et al., 2019). While ASA has demonstrated efficacy as a compound in wheat cultivation amidst saline conditions, its impact on tomato plants remains relatively insignificant when compared to KNO_3 and $CaNO_3$ El-Hawary et al., (2023).

The Fig. 5 illustrated the effect of 48-h chemical priming on chlorophyll content. In normal conditions, there was no significant effect on chlorophyll content (Fig. 5). Under salt treatment, chlorophyll content decreased by 6.7% refers to positive control.

Different chemical priming restored chlorophyll content referring to positive control. The decrease of chlorophyll level was more alleviated by $CaNO_3$ priming: the chlorophyll content decreased by about only 1% refers to control $CaNO_3$ primed seedlings.

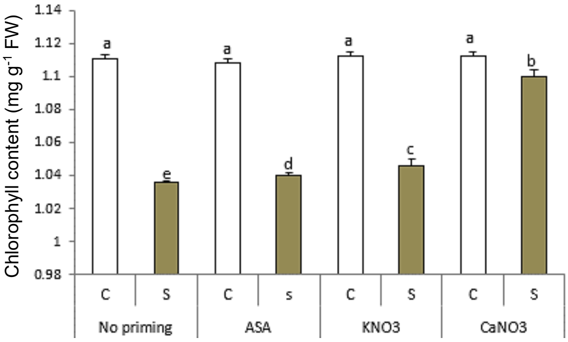


Figure 5. Effect of 48-h chemical priming type (ASA/KNO_3/$CaNO_3$) on chlorophyll content (mg g^{-1} FW) in leaves of tomato seedlings. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

In general, salt decreased the chlorophyll content of an extensive variety of plant species as in canola (Iqbal et al., 2022), alfalfa (Wang et al., 2020) and tomato (Shin et al., 2020). In several studies, priming technique mitigates harmful salt effect and restored chlorophyll content as in cowpea with $CaCl_2$ priming (Farooq et al., 2020) and in wheat with ASA priming (Baig et al., 2021).

In normal conditions, the 48-h seed priming with different chemicals had no significant effect on leaf MDA content (Fig. 6, A). With no priming, salt increased the MDA content in leaves by 77%. All chemical priming reduced the salt induced-MDA increase. Under salt treatment, leaf MDA content in ASA, KNO_3 and $CaNO_3$ pretreated seedlings was respectively 54%, 41% and 14% referring to control chemical primed seedlings (Fig. 6, A). Recently, several studies demonstrated that $CaCl_2$ and KNO_3 seed priming reduced effectively MDA accumulation caused by salt stress (Abdelhamid et al., 2019; Ben Youssef et al., 2021).

In normal conditions, ASA priming increased proline content in leaves while KNO_3 and $CaNO_3$ priming had no significant effect on proline content (Fig. 6, B). In no primed seedlings, salt increased proline content more than 8-fold refers to control. The chemical priming alleviated salt effect especially $CaNO_3$ agent. In stressed $CaNO_3$ primed seedlings, salt increased only 3-fold proline content refers to control $CaNO_3$ primed seedlings.

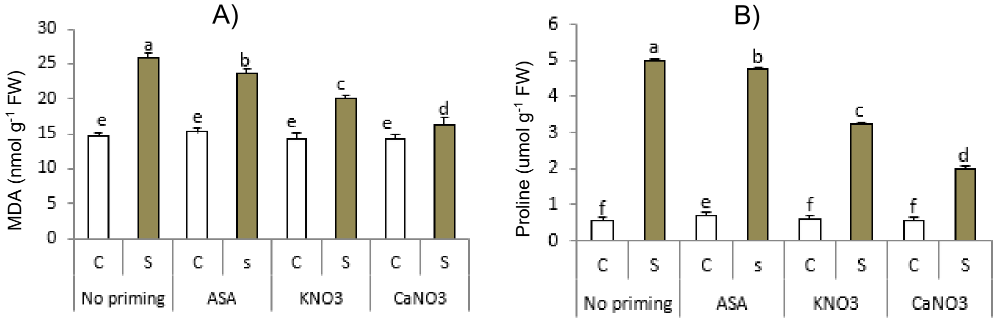


Figure 6. Effect of 48-h chemical priming type (ASA/KNO_3/$CaNO_3$) on malondialdehyde (MDA) content (A) and Proline content (B) in leaves of tomato seedlings. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

Generally, salt treatment induced proline accumulation in plant tissues. Plants accrue compatible solutes, such as proline, to withstand salt stress. By reducing the cytoplasmic osmotic potential, this process promotes water absorption and removes reactive oxygen species (ROS) molecules (Rady et al. 2019b). Recently, Abdelhamid et al revealed that seed priming alleviates detrimental salt effect by inducing proline accumulation (Abdelhamid et al., 2019).

However, in current study, the application of ASA, KNO_3 and $CaNO_3$ under salinity decreased the undesirable effect of salinity on seed germination and plant growth while decreasing proline accumulation. These results are supported by other studies where several chemical seed priming reduced proline content under salinity stress as in wheat (Salama et al., 2015) in tomato (Mimouni et al., 2016), sweet peppers (Abdelaal et al., 2020) and soybeans (Hasanuzzaman et al., 2022). Chemical used in seed priming could mitigate salt effect by the restriction of sodium and/or chlore absorption. For example, in salt-stressed wheat, calcium improve plant growth via its apoplastic effects on the transport of Na and K across the root plasma membrane (Reid & Smith, 2000).

CONCLUSIONS

In tomato, salt treatment reduced seed germination parameters and seedling growth. In normal conditions, the germination parameters (FGP, GI, GRI, and MGT) and seedling growth were not significantly affected by the applications of used chemical priming. The duration of seed priming and used chemical agent are crucial in determining chemical efficacy in salt stress alleviation. ASA, KNO_3 and $CaNO_3$ demonstrated efficiency in alleviation of salt effect on FGP and GI, GRI and MGT, especially 48-h as priming duration. Under salinity, chemical priming improved more germination parameters, growth of issued seedlings and leaf chlorophyll content, particularly after 48 hours of $CaNO_3$ priming.

The effectiveness of the mitigation strategy is traduced by low level of MDA indicating less significant oxidative stress. The improvement in seedling growth was concurrent with a decrease in the level of proline, which is an indicator of salt-induced osmotic stress, which suggests that used priming agents possibly reduced the absorption of salt ions and consequently the osmotic stress. It is determined that these priming agents could be categorized into three ranges according to their effectiveness on the various studied parameters. Among these, $CaNO_3$ is the most effective, closely followed by KNO_3. Whereas ASA agent needs more studies to determine an effective concentration that mitigate effectively salt stress on tomato seeds.

REFERENCES

Abdelaal, K.A., EL-Maghraby, L.M., Elansary, H., Hafez, Y.M., Ibrahim, E.I., El-Banna, M., El-Esawi, M. & Elkelish, A. 2020. Treatment of Sweet Pepper with Stress Tolerance-Inducing Compounds Alleviates Salinity Stress Oxidative Damage by Mediating the Physio-Biochemical Activities and Antioxidant Systems. *Agronomy* **10**, 26. doi:10.3390/agronomy10010026

Abdelhamid, M.T., El-Masry, R.R., Darwish, D.S., Abdalla, M.M., Oba, S., Ragab, R., ... & Omer, E. 2019. Mechanisms of seed priming involved in salt stress amelioration. *Priming and Pretreatment of Seeds and Seedlings: Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants*, 219–251. doi.org/10.1007/978-981-13-8625-1_11

Al-Harbi, A., Hejazi, A. & Al-Omran, A. 2017. Responses of grafted tomato (*Solanum lycopersicon L.)* to abiotic stresses in Saudi Arabia. *Saudi Journal of Biological Sciences* **24**(6), 1274–1280. doi.org/10.1016/j.sjbs.2016.01.005

Ali, M., Kamran, M., Abbasi, G.H., Saleem, M.H., Ahmad, S., Parveen, A., ... & Fahad, S. 2021. Melatonin-induced salinity tolerance by ameliorating osmotic and oxidative stress in the seedlings of two tomato (*Solanum lycopersicum L.*) cultivars. *Journal of Plant Growth Regulation* **40**, 2236–2248. doi.org/10.1007/s00344-020-10273-3

Arnon, D.I. 1949. Copper enzyme in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**, 1–15. doi: 10.1104/pp.24.1.1

Arun, M.N., Hebbar, S.S., Senthivel, T., Nair, A.K., Padmavathi, G., Pandey, P. & Singh, A. 2022. Seed priming: The way forward to mitigate abiotic stress in crops. *London, UK: IntechOpen* **11**, 173. doi: 10.5772/intechopen.102033

Baig, Z., Khan, N., Sahar, S., Sattar, S. & Zehra, R. 2021. Effects of seed priming with ascorbic acid to mitigate salinity stress on three wheat (*Triticum aestivum L.*) cultivars. *Acta Ecologica Sinica* **41**(5), 491–498. doi.org/10.1016/j.chnaes.2021.08.010

Bates, L.S., Walden, R.P. & Teare, I.D.1972. Rapid determination of free proline for water stress studies. *Plant Soil* **39**, 205–207. doi.org/10.1007/BF00018060

Ben Youssef, R., Boukari, N., Abdelly, C. & Jelali, N. 2023. Mitigation of salt stress and stimulation of growth by salicylic acid and calcium chloride seed priming in two barley species. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 1–11. doi.org/10.1080/11263504.2023.2200792

Chakma, P., Hossain, M. M. & Rabbani, M.G. 2019. Effects of salinity stress on seed germination and seedling growth of tomato: Salinity stress on seed germination and seedling growth. *Journal of the Bangladesh Agricultural University* **17**(4), 490–499. doi.org/10.3329/jbau.v17i4.44617

El-Hawary, M.M., Hashem, O.S. & Hasanuzzaman, M. 2023. Seed priming and foliar application with ascorbic acid and salicylic acid mitigate salt stress in wheat. *Agronomy* **13**(2), 493. doi.org/10.3390/agronomy13020493

Farooq, M., Rehman, A., Al-Alawi, A.K., Al-Busaidi, W.M. & Lee, D.J. 2020. Integrated use of seed priming and biochar improves salt tolerance in cowpea. *Scientia Horticulturae* **272**, 109507. doi.org/10.1016/j.scienta.2020.109507

Feghhenabi, F., Hadi, H., Khodaverdiloo, H. & Van Genuchten, M.T. 2020. Seed priming alleviated salinity stress during germination and emergence of wheat (Triticum aestivum L.). *Agricultural Water Management* **231**, 106022. doi.org/10.1016/j.agwat.2020.106022

Ghassemi-Golezani, K., Farshbaf-Jafari, S. & Shafagh-Kolvanagh, J. 2011. Seed priming and field performance of soybean (Glycine max L.) in response to water limitation. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **39**(2), 186–189. doi.org/10.15835/nbha3926122

Ghoohestani, A., Gheisary, H., Zahedi, S.M. & Dolatkhahi, A. 2012. Effect of Seed Priming of Tomato with Salicylic Acid, Ascorbic Acid and Hydrogen Peroxideon Germination and Plantlet Growth in Saline Conditions. *International journal of Agronomy and Plant Production* **3**(S), 700–704. http:// www.ijappjournal.com

Goiba, P.K., Durgude, A.G., Pharande, A.L., Kadlag, A.D., Chauhan, M.R. & CA Nimbalkar, C.A. 2018. Effect of seed priming with iron and zinc on yield contributing parameters as well as the nutrient uptake of the soybean (*Glycine max*) in calcareous soil. *Int. J. Chem. Stud.* **6**(2).

Hadia, E., Slama, A., Romdhane, L., Cheikh M'Hamed, H., Fahej, M.A.S. & Radhouane, L. 2023. Seed priming of bread wheat varieties with growth regulators and nutrients improves salt stress tolerance particularly for the local genotype. *Journal of Plant Growth Regulation* **42**(1), 304–318. doi.org/10.1007/s00344-021-10548-3

Hakim, M.A., Juraimi, A.S., Begum, M., Hanafi, M.M., Ismail, M.R. & Selamat, A. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology* **9**, 1911–1918. doi:10.5897/AJB09.1526

Hasanuzzaman, M., Ahmed, N., Saha, T., Rahman, M., Rahman, K., Alam, M.M., Rohman, M.M. & Nahar, K. 2022. Exogenous Salicylic Acid and Kinetin Modulate Reactive Oxygen Species Metabolism and Glyoxalase System to Confer Waterlogging Stress Tolerance in Soybean (*Glycine max L.*). *Plant Stress* **3**, 100057. doi.org/10.1016/j.stress.2022.100057

Heath, R.L, Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys***125**, 180–198. doi.org/10.1016/0003-9861(68)90654-1

Ikram, N.A., Ghaffar, A., Khan, A.A., Nawaz, F. & Hussain, A. 2023. Optimizing iron seed priming for enhanced yield and biofortification of tomato. *Journal of Plant Nutrition* **46**(12), 2796–2810. doi.org/10.1080/01904167.2022.2160751

Iqbal, W., Afridi, M.Z., Jamal, A., Mihoub, A., Saeed, M.F., Székely, Á., ... & Pompelli, M.F. 2022. Canola seed priming and its effect on gas exchange, chlorophyll photobleaching, and enzymatic activities in response to salt stress. *Sustainability* **14**(15), 9377. doi.org/10.3390/su14159377

Khoshvaghti, H., Hoseini, M. & Baser-Kouchehbagh, S. 2013. Does priming improve dill (Anethum graveolens) seed germination and yield? *International Journal of Biosciences (IJB)* **3**(7), 126–131. doi.org/10.12692/ijb/3.7.126-131

Li, Y., Liu, C., Shi, Q., Yang, F. & Wei, M. 2021. Mixed red and blue light promotes ripening and improves quality of tomato fruit by influencing melatonin content. *Environmental and Experimental Botany* **185**, 104407. doi.org/10.1016/j.envexpbot.2021.104407

MACKINNEY, G. 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem* **40**, 315–322. doi.org/10.1016/S0021-9258(18)51320-X

Maneesha, S.R. 2019. Effect of calcium nitrate and potassium nitrate priming on seed germination and seedling vigour of papaya (*Carica papaya* L.). *Journal of Horticultural Sciences* **14**(2), 149–154.

Mangal, V., Lal, M.K., Tiwari, R.K., Altaf, M.A., Sood, S., Kumar, D., ... & Aftab, T. 2023. Molecular insights into the role of reactive oxygen, nitrogen and sulphur species in conferring salinity stress tolerance in plants. *Journal of Plant Growth Regulation* **42**(2), 554–574. doi.org/10.1007/s00344-022-10591-8

Mbarki, S., Skalicky, M., Vachova, P., Hajihashemi, S., Jouini, L., Zivcak, M., ... & Zoghlami Khelil, A. 2020. Comparing salt tolerance at seedling and germination stages in local populations of *Medicago ciliaris* L. to *Medicago intertexta* L. and *Medicago scutellata* L. *Plants* **9**(4), 526. doi.org/10.3390/plants9040526

Mimouni, H., Wasti, S., Manaa, A., Gharbi, E., Chalh, A., Vandoorne, B., Lutts, S., Ahmed, H.B. 2016. Does Salicylic Acid (SA) Improve Tolerance to Salt Stress in Plants? A Study of SA Effects on Tomato Plant Growth, Water Dynamics, Photosynthesis, and Biochemical Parameters. *OMICS J. Integr. Biol.* **20**, 180–190. doi.org/10.1089/omi.2015.016

Moaaz, Ali, M., Javed, T., Mauro, R.P., Shabbir, R., Afzal, I., Yousef, A.F. 2020. Effect of Seed Priming with Potassium Nitrate on the Performance of Tomato. *Agriculture* **10**, 498. doi.org/10.3390/agriculture10110498

Moghaddam, A., Larijani, H.R., Oveysi, M., Moghaddam, H.R.T. & Nasri, M. 2023. Alleviating the adverse effects of salinity stress on *Salicornia persica* using sodium nitroprusside and potassium nitrate. *BMC Plant Biology* **23**(1), 166. doi.org/10.1186/s12870-023-04179-x

Nazari, S., Aboutalebian, M.A. & Golzardi, F. 2017. Seed priming improves seedling emergence time, root characteristics and yield of canola in the conditions of late sowing. *Agronomy Research* **15**(2), 501–514.

Nouri, M. & Haddioui, A. 2021. Improving seed germination and seedling growth of *Lepidium sativum* with different priming methods under arsenic stress. *Acta Ecologica Sinica* **41**(1), 64–71. doi.org/10.1016/j.chnaes.2020.12.005

Oliveira, C.E.D. S., Steiner, F., Zuffo, A.M., Zoz, T., Alves, C.Z. & Aguiar, V.C.B.D. 2019. Seed priming improves the germination and growth rate of melon seedlings under saline stress. *Ciência Rural* **49**. doi.org/10.1590/0103-8478cr2018058

Pawar, V.A. & Laware, S.L. 2018. Seed priming a critical review. *International Journal of Scientific Research in Biological Science* **5**(5), 94–101. https://api.semanticscholar.org/CorpusID:150331735

Rady, M.M. 2012. A novel organo-mineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil. *South African journal of botany* **81**, 8–14. doi.org/10.1016/j.sajb.2012.03.013

Rady, M.M., Desoky, E.S., Elrys, A.S. & Boghdady, M.S. 2019a. Can licorice root extract be used as an effective natural biostimulant for salt-stressed common bean plants? *South African Journal of Botany* **121**, 294–305. doi.org/10.1016/j.sajb.2018.11.019

Rady, M.M., Kuşvuran, A., Alharby, H.F., Alzahrani, Y. & Kuşvuran, S. 2019b. Pretreatment with proline or an organic bio-stimulant induces salt tolerance in wheat plants by improving antioxidant redox state and enzymatic activities and reducing the oxidative stress. *Journal of Plant Growth Regulation* **38**, 449–462. doi.org/10.1007/s00344-018-9860-5

Rajabi Dehnavi, A., Zahedi, M., Ludwiczak, A., Cardenas Perez, S. & Piernik, A. 2020. Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy* **10**(6), 859. doi.org/10.3390/agronomy10060859

Reid, R.J. & Smith, F.A. 2000. The limits of sodium/calcium interactions in plant growth. *Functional Plant Biology* **27**(7), 709–715. doi.org/10.1071/PP00030

Salama, K.H.A., Mansour, M.M.F., Al-Malawi, H.A. 2015. Glycinebetaine priming improves salt tolerance of wheat. – *Biologia* (Poland) **70**(10), 1334–1339. doi.org/10.1515/biolog-2015-0150

Salles, J.S., de Lima, A.H., Binotti, F.F.D.S., Costa, E., Binotti, E.D., Salles, J.S, Gustavo, H.C.V. & Andreia, F.G.O. de Souza. 2019. Calcium Nitrate Priming Increases the Germination Rate of Eggplant Seeds. *Journal of Agricultural Science* **11**, 15. doi.org/10.5539/jas.v11n15p181

Semida, W.M. & Rady, M.M. 2014. Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and anti-oxidant capacity in Phaseolus vulgaris L. grown under NaCl stress. *The Journal of Horticultural Science and Biotechnology* **89**(3), 338–344. doi.org/10.1080/14620316.2014.11513088

Shah, S., Ullah, S., Ali, S., Khan, A., Ali, M. & Hassan, S. 2021. Using mathematical models to evaluate germination rate and seedlings length of chickpea seed (*Cicer arietinum* L.) to osmotic stress at cardinal temperatures. *PLoS ONE* **16**(12), e0260990. doi: 10.1371/journal.pone.0260990

Shahid, M.A., Sarkhosh, A., Khan, N., Balal, R.M., Ali, S., Rossi, L., Gómez, C., Mattson, N., Nasim; W. & Garcia-Sanchez, F. 2020. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy* **10**(7), 938. doi.org/10.3390/agronomy10070938

Shin, Y.K., Bhandari, S.R., Cho, M.C. & Lee, J.G. 2020. Evaluation of chlorophyll fluorescence parameters and proline content in tomato seedlings grown under different salt stress conditions. *Horticulture, Environment, and Biotechnology* **61**, 433–443. doi.org/10.1007/s13580-020-00231-z

Tu, K., Cheng, Y., Pan, T., Wang, J. & Sun, Q. 2022. Effects of seed priming on vitality and preservation of pepper seeds. *Agriculture* **12**(5), 603. doi.org/10.3390/agriculture12050603

Wang, Y., Diao, P., Kong, L., Yu, R., Zhang, M., Zuo, T., ... & Wuriyanghan, H. 2020. Ethylene enhances seed germination and seedling growth under salinity by reducing oxidative stress and promoting chlorophyll content via ETR2 pathway. *Frontiers in plant science* **11**, 1066. doi.org/10.3389/fpls.2020.01066

Wu, L., Huo, W., Yao, D. & Li, M. 2019. Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. *Scientia Horticulturae* **255**, 161–168. doi: 10.1016/j.scienta.2019.05.007

Zhang, H., Zhu, J., Gong, Z., Zhu, J.K. 2022. Abiotic stress responses in plants. *Nat Rev Genet* **23**, 104–119. doi.org/10.1038/s41576-021-00413-0

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Physiological mechanisms in *Ficus carica* L. genotypes in response to moisture stress

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Abstract. The genus *Ficus* comprises cultivated and wild species that vary in phenotypic characteristics of both the plant and the fruit. This genus is considered to originate from Mediterranean regions and arid lands of Europe and Africa, known as the Fertile Crescent. *Ficus carica* L. (fig) is a globally emerging fruit crop due to its increasing production trends and capacity to produce in low water availability. Understanding the fig tree's responses to water deficit is essential for adapting to sustainable production and climate change. In this study, we investigated the water deficit tolerance of native *Ficus carica* accession and the Black Mission commercial variety. This research aimed to define the relationship between resistance to water deficit and plant physiological and biochemical markers (physiological and biochemical). Those markers considered relative water content (RWC), photosynthesis (P_N), stomatal conductance (gs), intercellular CO_2 (Ci), transpiration (E), proline (Pro), and soluble sugar content (SSC). The results revealed that fig genotypes exhibit various adaptive mechanisms and physiological responses to water deficit, including osmotic adjustment, stomatal regulation, and proline accumulation. The water deficit condition was confirmed by measuring the soil water potential; the maximum values were in the range of -2.1 to -3.6 MPa. The 'Guadalupe Victoria' accession demonstrated significant water deficit resilience by maintaining higher P_N values in low water availability. Additionally, the study highlighted the role of osmotic adjustments in maintaining water balance and cellular function during stress periods. These findings will provide valuable insights for the selection process of genotypes with enhanced drought tolerance in water-limited environments.

Key words: osmotic response, native accession, photosynthesis, proline.

INTRODUCTION

Moraceae is an angiosperm plant family characterized by milk latex, unisexual flowers, anatropous ovules, and aggregated drupes (Barolo et al., 2014). The *Ficus* genus is an important group to which *Ficus carica* belongs. This family produces a 'fruit' (borne from a complex inflorescence called syconium). According to available data in recent years, fig production has steadily been increasing for 2021; the gross production was about 1,057,349,000 US$ (FAOSTAT, 2021). The top fig-producing countries from this production are Turkey, Egypt, and Morocco (FAOSTAT, 2021). In addition, the economic value of fig production is reflected in employment opportunities generated throughout the value chain, benefiting farmers, processors, and exporters (Caliskan, 2015). Moreover, fig cultivation supports local economies in many regions, particularly in arid and semi-arid areas where few other crops can thrive. As the importance and consumption of figs continue to grow, it is expected that fig production will remain a vital sector within the global agricultural industry. For producers and consumers, the fig nutrient value is a desirable characteristic (Soni et al., 2014; Caliskan, 2015; Bougiouklis et al., 2020). The levels of nutrients in fig plants are influenced by factors such as absorption, transportation, and internal movement of nutrients (Bougiouklis et al., 2020). All these processes are influenced by various conditions such as genetics, physiology, and climate.

As climate change progresses, there is a growing number of reports indicating water scarcity in agricultural settings (Nikolaou et al., 2020; Tzanakakis et al., 2020). This trend is expected to have ramifications for the availability of food (Paulus et al., 2020). An important focus is the scarcity of water resources because of the efficient use of water in agricultural systems (Kartal et al., 2019). Fig is also grown in different ecologies due to its drought resistance (Gholami et al., 2012). Fig's ability to tolerate water stress and adapt to varying soil conditions allows it to thrive in different ecosystems, contributing to ecosystem stability and biodiversity. This species has evolved several adaptive mechanisms and physiological processes to cope with limited water availability (Ammar et al., 2020, 2023). One of the main strategies employed by figs is drought avoidance, which involves shedding leaves in response to substantial water deficits (Ammar et al., 2023). By reducing leaf surface area, fig trees minimize water loss through transpiration and conserve moisture within their tissues (Akınci & Lösel, 2012). They accomplish this through mechanisms such as adjusting stomatal conductance and regulating gas exchange, reducing water loss while maintaining essential physiological processes (Ammar et al., 2020, 2023).

It is important to highlight the study of the fig for arid and semi-arid zones through the selection of genetic materials that have developed tolerance to water deficiency (Çalişkan & Aytekin Polat, 2011¸ Ammar et al., 2020). In this context, such knowledge can contribute to the selection of fig trees to explore their full potential and face climate change. Therefore, we hypothesize that native *Ficus carica* genotypes will present differences in their ability to tolerate water deficit in contrast to the Black Mission commercial variety. This research aimed to define the relationship between resistance to water deficit and plant physiological and biochemical markers. The analysis of relative water content (RWC), photosynthesis (P_N), stomatal conductance (gs), intercellular CO_2 (Ci), transpiration (E), proline (Pro), and soluble sugar content (SSC), as Gholami et al. (2012) suggest, can be used to identify potentially valuable traits in breeding programs.

Moreover, knowing the fig materials' responses under limited and unlimited water availability in deficit and recovery conditions is essential.

MATERIALS AND METHODS

Study site

The experiment was carried out in the experimental areas of the Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, which is located at 103°36´07” N and 25°53´43” W. Its altitude is 1,109 m above sea level (INEGI, 2014). The climate is a desert with rains during the summer and winter (258 mm of annual rainfall and 2,000 mm of annual evaporation) (Köppen, 1948).

Plant material

Plants were collected in the Comarca Lagunera, which corresponds to a region located between the Durango and Coahuila states in México. The fig materials were obtained from backyards or wild locations by layers. The fig materials were propagated by cutting the layers after 40 days of rotting. The layers in the mother plant presented a rooting rate of 94 %. Those layers were planted in pots and developed for 30 days. For this experiment process, three layers were grown in 10 kg capacity pots filled with 9.5 kg of soil (soil characteristics: organic matter of 2.68 mg kg^{-1}; pH of 8.8, and electric conductivity of 3.61 dS m^{-1}). The used soil presented a field capacity (FC) of 33% and a Permanent Wilting Point (PWP) of 20%. The fig accessions were acclimatized to pot conditions for six months; pots were irrigated according to their water requirements (around 80% of the FC). During the experiment, the daytime mean air temperature was in the range of 29–46 ± 1 °C, the night-time mean air temperature was in the range of 20–24 ± 1 °C, and the daily mean relative humidity was measured in the range of 24–54 ± 3%. Fig. 1 shows the air temperature and relative humidity that were monitored during the experimental period using a high-accuracy humidity and temperature sensor (ORIA OUS-WA62®) (Fig. 1).

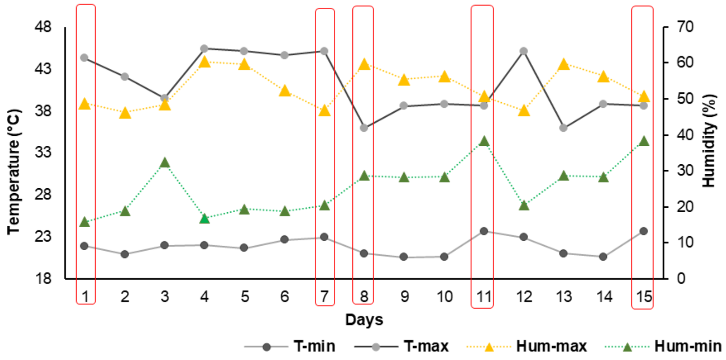

Figure 1. Daily mean minimum and maximum air temperature (T-Min and T-Max, °C) and mean minimum and maximum ambient humidity (Hum-min and Hum-Max, %) at the experimental site. The red rectangles represent the evaluation days of the experimental variables.

Experimental Design

The experiment was established as a randomized block design and arranged into a divided plot layout with three replications in semi-controlled conditions. It was developed using 6-month-old local fig accessions (Arista, Ceballos, Fortuna, Guadalupe Victoria, and San Antonio) and one commercial variety (Black Mission) (Table 1). To group accessions and variety, we call them ‘genotypes’. The plants were divided into two groups the water deficit (WD) and the field capacity (FC). The soil water content was recorded as soil water potential (SWP).

Table 1. Identification and origin of fig accessions of *Ficus carica* L. genotypes

Identification	Origin of fig accessions	Origin
Arista	282°71′73.26” N; 65°74′27.50” E	Backyard
Ceballos	293°36′38.29” N; 58°65′62.21” E	Backyard
Fortuna	293°25′64.71” N; 59°00′43.26” E	Wild
Guadalupe Victoria	282°16′00.44” N; 64°88′06.03” E	Backyard
San Antonio	291°33′54.86” N; 56°05′00.71” E	Wild
Black Mission	NA	Commercial

NA: Not available.

The experiment was conducted from June to July 2022, corresponding to the vegetative plant stage. The experimental process started with all the plants at FC. The WD condition was produced in the plants by interrupting the irrigation. This condition lasted from day 1 to day 7; during this period, we call days after irrigation suspension (DAIS). The WD plants were subjected to a dehydrating period due to evapotranspiration. The pots were weighed all days at the same hour (10:00 h), and the FC pots were rehydrated as needed. Plants were evaluated during this time to determine the daily water loss. The WD condition lasted 7 days; at this time, at least 50% of the plants showed evidence of physical water stress (leaves wilting and turgidity loss). The WD condition was confirmed by measuring the soil water potential at day 7, which was in the range of -2.1 to -3.6 MPa with a mean value of -2.7 MPa, far below PWP (-1.5 MPa).

After the maximum WD was observed, the plants were irrigated. Then, those were evaluated in a recovery period at 8, 11, and 15 days after the experiment was started and the irrigation was recovered (i.e. 1, 4, and 8 days at field capacity moisture). During this recovery period, the plants were maintained at FC. The response variables were measured on day 1 (beginning condition), day 7 (the maximum stress condition), day 8 (24 h recovery), day 11 (medium recovery period), and finally, day 15 (maximum recovery period). All those measures were done in recently matured leaves; the sampling was done between 10:00 and 11:00 h on sunny and clear days.

Relative Water Content (RWC)

A wet chamber was used to collect leaves from each treatment to avoid water loss during transportation to the laboratory. The RWC was determined by considering fresh weight (FW), dry weight (DW), and turgent weight (TW) by the (FW-DW)/(TW-DW) x 100. Foliar sections of 2 cm^2 were measured (fresh weight), and those were submerged in water at 4 °C for 12 h without light. Passed this time, the weight was determined, and the turgent weight was considered. Those turgent leaf sections were dried at 80 °C for

24 h, and the dry weight (DW) was determined. (U.S. SOLID, model USS-DSS) (Barrs & Weatherley, 1962).

Gas exchange measurements

Leaf samples were used for measuring net photosynthetic assimilation rate (P_N, μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), intercellular CO_2 content (Ci, μmol CO_2 mol air^{-1}), and transpiration rate (E, mol H_2O m^{-2} s^{-1}). All the evaluations were developed on sunny and clear days (10:00 to 11:00 h) using a portable infrared gas exchange analyzer (LI-COR 6400XT, LI-COR Inc., Lincoln, NE, USA). The operative conditions were at 400 ppm CO_2 in the camera and active photosynthetic radiation (PAR) of 750 μmol m^{-2} s^{-1} at 25 °C cuvette temperature. The leaf samples were placed in the cuvette for about one minute for data collection (Evans & Santiago, 2014). Three early mature leaves from each of the three replicants, exposed to sunlight, were measured from each accession and genotype during the evaluation time.

Soluble sugar content (SSC)

The SSC was determined by the method of Dubois et al. (1956). A total of 100 mg of nitrogen-frozen leaf tissue was diluted in distilled water (5 mL). This extract was mixed with phenol and concentrated H_2SO_4. The mix was measured using a spectrophotometer at A 490 nm (UV-VIS, Model 721, Shanghai Precision and Scientific Instrument Co; Ltd). SSC was calculated using a D-glucose standard curve and expressed as SSC mg g^{-1} FW.

Proline concentration (Pro)

The Pro was determined according to Bates et al. (1973) with minor modifications. The nitrogen-frozen material (500 mg) was mixed with 5 mL of 3% of sulfosalicylic acid solution. The mix was homogenized and centrifuged (6,000 rpm for 30 min at 10 °C). The reaction solution was mixed with 1 mL of glacial acid and ninhydrin (previously warmed). The mixture was incubated at 100 °C for 1 h, and the reaction was stopped on an ice bath before extraction with 3 mL of toluene. The organic phase (pink-red) was measured at A 530 nm. The proline concentration was determined using L-Proline (Sigma Aldrich) and expressed as μMol Proline g^{-1} FW.

Statistical Analysis

Factorial variance analysis was done. The mean comparison values were made by T-test of independent samples as well as one-way ANOVA and using Tukey´s multiple range test at the $p \leq 0.05$ level. Those procedures were carried out by the software SPSS 18.0 Version (Inc., Chicago IL) and ©2013 Minitab 16.2.4 Inc.

RESULTS AND DISCUSSION

Utilizing breeding programs to choose drought-resistant genetic materials is a strategy to mitigate the impact of drought stress on crop yield. As well, the resilience to drought stress is affected by complex genetic factors and diverse environmental conditions (Arshadi et al., 2018). Additionally, the level of tolerance may be influenced by the severity and duration of drought stress, as well as its interaction with other environmental variables (Anjum et al., 2011; Sachdev et al., 2021). The study provided twelve treatments, taking into account five accessions and one genotype, and two soil water contents, to understand the responses of young fig plants to drought tolerance. Then, responses were measured using RWC, P_N, gs, Ci, E, Pro, and SSC, as Gholami et al. (2012) suggested. All the means of soil water potential (SWP) were lower than the value of PWP (-1.5 MPa) at 7 Days After Irrigation Suspension (DAIS) under the WD condition. The mean value of SWP was -2.7 MPa. The only statistical difference in SWP among fig plants at 7 DAIS corresponds to the 'Ceballos' accession (Fig. 2). In other words, the 'Ceballos' accession was submitted to the lowest SWP during the experiment (-3.5 MPa).

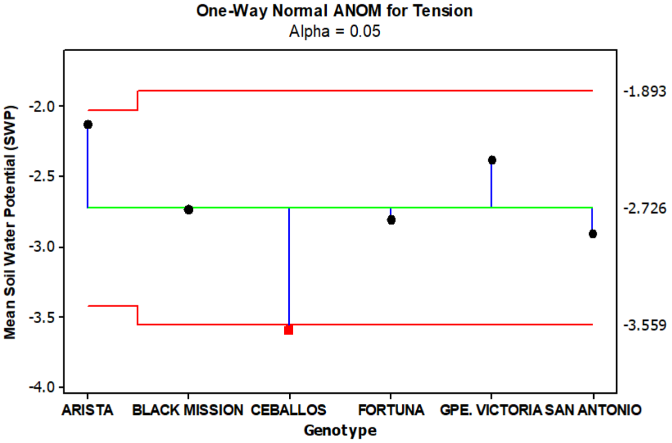

Figure 2. Comparison of means of Soil Water Potential (SWP, MPa) belonging to genotypes at 7 DAIS (Days After Irrigation Suspension) under WD (Water deficit) condition. The green line indicates the mean value, the red line indicates the decision limits, and the blue lines indicate the distance in relation to the mean.

Some water stress adaptative mechanisms and processes are associated with root plasticity, water use efficiency, osmotic adjustment, drought avoidance strategies, and drought resistance traits (Gao et al., 2018). In our study, *Ficus carica* materials could tolerate SWC below the Permanent Wilting Point (PWP) due to various adaptive mechanisms and physiological processes (Fig. 1). One of the main mechanisms of water avoidance strategies in fig is to shed leaves in a substantial water deficit (Ammar et al., 2020); however, all the evaluated fig materials tolerated a mean SWP of -2.7 MPa without shed leaves (Fig. 1). Those fig materials continued their photosynthesis activity even with low soil water potential. In the case of all evaluated fig materials, some plants reduced water loss by a low RWC and recovered their RWC when water became available again.

The evaluated variables were examined by a factorial analysis (FA). The FA presented significative effects of individual factors such as genotypes (G), Soil Water Content (SWC), and Time (T) at Days After Irrigation Suspension (DAIS) and Days at Field Capacity Moisture (DFCM) cases. Moreover, the interaction effect represents the joint influence of factors on the response variable that cannot be explained by the primary effects alone. The interaction effects between GxSWC, GxT, SWCxT, and Gx SWCxT presented statistical differences in some of the evaluated variables (Table 2).

Table 2. *P* values of Factorial Analyses of the Genotype (G), Soil Water Content (SWC), and Time (T) and their interactions on physiological (RWC, PN, gs, Ci, E, Pro, and SSC evaluations in 6 fig genotypes during DAIS (Days after Irrigation Suspension) and DFCM (Days at Field Capacity Moisture) evaluations

	DAIS (Stress period)							DFCM (Recovery period)						
	RWC	P_N	g_s	Ci	E	Pro	SSC	RWC	P_N	g_s	Ci	E	Pro	SSC
Model	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
G	0.339	0.001	0.815	0.786	0.478	0.977	0.494	0.036	0.080	0.117	0.366	0.276	0.923	0.332
SWC	0.001	0.001	0.012	0.001	0.004	0.001	0.001	0.208	0.001	0.555	0.292	0.508	0.001	0.001
T	0.001	0.001	0.001	0.231	0.001	0.001	0.017	0.178	0.996	0.001	0.001	0.001	0.001	0.001
CxSWC	0.050	0.051	0.080	0.045	0.028	0.043	0.038	0.007	0.001	0.018	0.010	0.665	0.017	0.035
CxT	0.331	0.238	0.051	0.232	0.012	0.047	0.776	0.171	0.142	0.002	0.322	0.003	0.457	0.639
SWCxT	0.001	0.001	0.057	0.050	0.022	0.029	0.001	0.098	0.001	0.041	0.001	0.059	0.001	0.054
CxSWCxT	0.329	0.888	0.926	0.221	0.754	0.050	0.952	0.335	0.828	0.068	0.352	0.071	0.018	0.438

Interaction G x SWC

The interaction between G x SWC presented significant effects in all the evaluations, except in gs in DAIS and E in DFCM cases. In these interactions at the DAIS period in RWC evaluation, we observed that the 'Arista' accession exhibited statistical differences between WD and FC conditions (Fig. 3, a). During the DFCM period, the 'Ceballos' accession showed statistical differences between the SWC. The 'Ceballos' accession exhibited a great recovery capacity in the RWC from the DAIS to the DFCM condition (Fig. 3a). Those changes showed the accessions' recovery capacity (Fig. 3). The statistical differences in the RWC reflected the metabolic activity in tissues, and it was used as the most meaningful index for dehydration tolerance (Sallam et al., 2019). The RWC in Black Mission (69.85%), Brown Turkey (69.75 %), and Brunswick (68.82%) in stress conditions showed a decrease of around 20% in comparison with the control condition (Rabei Metwali et al., 2016).

When the interaction between factors G x SWC was analyzed in the DAIS context, P_N showed statistical differences for most cases; the only exception was the 'Ceballos' accession. The highest P_N in the DAIS period was also observed in the 'Guadalupe Victoria' accession in the FC condition (Fig. 3, b). In the DFCM situation, the P_N differences were significant for 'Arista' and 'Ceballos' accessions.

In the case of gs evaluation, the interaction C x SWC showed statistical differences in the DFCM case but not in DAIS evaluations (Fig. 3, c). Notably, the 'Black Mission' and 'San Antonio' genotypes presented statistical variations. Most of the genotypes presented higher gs in WD condition during the DFCM case, except in 'Arista' and 'San Antonio' accessions. Moreover, the 'Guadalupe Victoria' accession showed the highest gs under the WD condition and the lowest under the FC condition; the highest gs belong to 'Guadalupe Victoria' (WD) under DFCM (Fig. 3, c).

Notably, significant differences in Ci evaluation belong to the 'Black Mission' and 'Guadalupe Victoria' genotypes in the DAIS period (Fig. 3, d). Also, it was observed that Ci was not different among genotypes under the two SWC considering DFCM. At the same time, the Ci was markedly low in the 'Fortune' accession at FC.

In general, lower E values belonging to 'Black Mission', 'Fortuna', and 'Guadalupe Victoria' genotypes at FC condition at DAIS. However, G x SWC differences were insignificant (Fig. 3, e). In the DAIS case, the E in the WD was higher than the FC condition in most cases, except in the ‘San Antonio’ accession. All G presented non-significant variation in the DFCM cases.

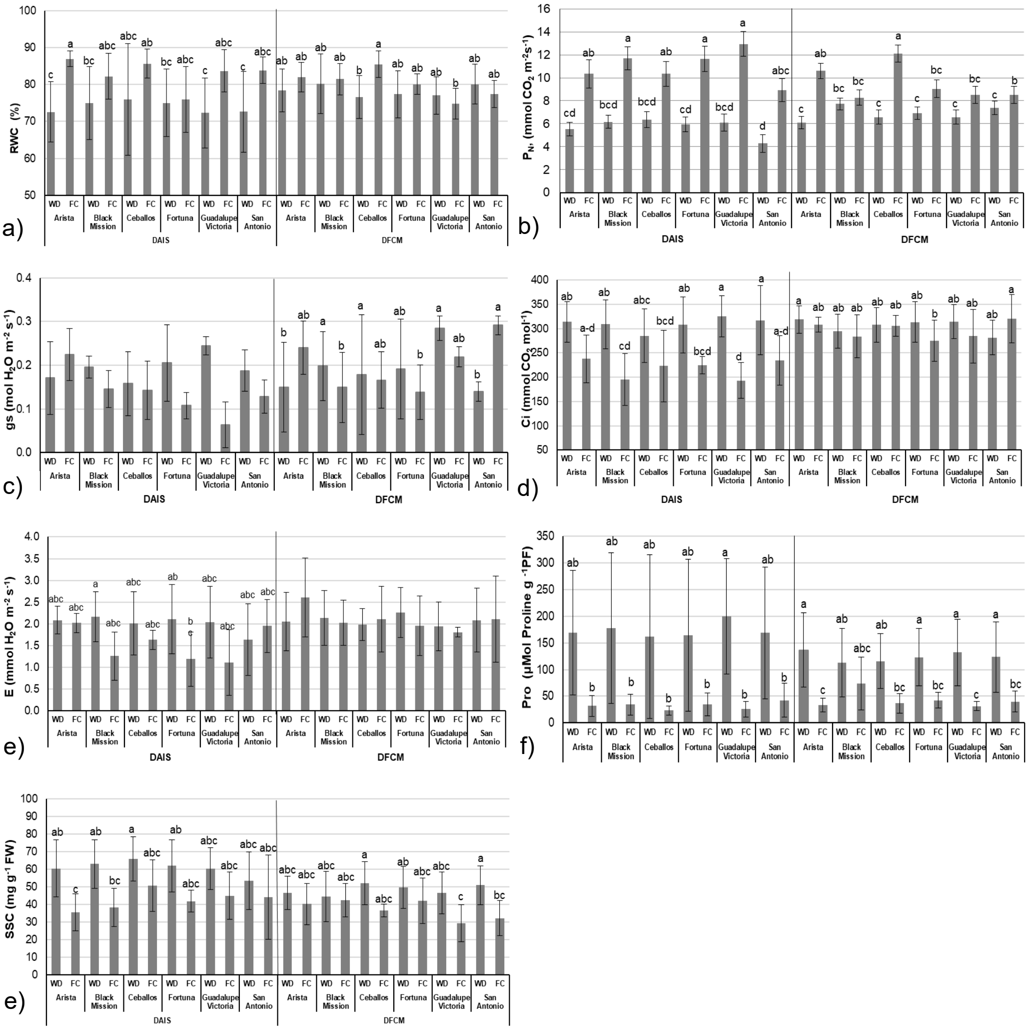

Figure 3. Mean values of the interaction between G x SWC at days after irrigation suspension (DAIS) and at Days at Field Capacity (DFCM) measured in *Ficus carica* mature leaves of six genotypes (Arista, Ceballos, Fortuna, Guadalupe Victoria, and San Antonio, and Black Mission) under two Soil Water Contents (SWC) (Water deficit, WD, i.e. -2.7 MPa; and Field Capacity, FC). a) Relative Water Content (RWC); b) Photosynthetic rate (P_N); c) Stomatal Conductance (gs); d) Intercellular CO_2 concentration (Ci); e) Transpiration rate (E); f) Proline content (Pro); g) Soluble Sugar Content (SSC) in the interaction Bars with different letters indicates statistical differences by Tukey multiple range test at $p \leq 0.05$.

Markedly, the highest increase of the Pro in the WD condition was observed in the ‘Guadalupe Victoria’ accession (368.1 µMol Proline g^{-1} FW) during the DAIS period (Fig. 3, f). In addition, the Pro content was higher in most of the genotypes in WD evaluations at the DFCM case, except for the ‘Black Mission’ and ‘Ceballos’ genotypes.

Also, statistical differences in the SSC were found in the 'Arista' accession between WD and FC conditions at DAIS (Fig. 3, g). The higher SSC was observed in the WD condition in all genotypes in both DAIS and DFCM cases. Also, the SSC showed statistical differences in the 'San Antonio' accession between SWC conditions in the DFCM case.

The 'Black Mission' and 'Fortuna'genotypes presented the highest RWC in the WD condition (Fig. 3, a). Those RWC can be associated with cuticles, latex, and trichomes, which act as a water loss barrier (Kunjet et al., 2013; Hernandez & Bae Park, 2022). In response to low RWC, plants may produce a thicker cuticle or modulate the deposition of latex (Arya et al., 2017). The fig leaf structure may enhance their leaves' tolerance for water loss.

Plant's osmotic adjustment is activated by distinct mechanisms to maintain cellular turgor and minimize water loss. They accumulate compatible solutes such as sugars, amino acids, and other organic compounds within their cells (Moradi, 2016). These solutes help maintain water potential, preventing excessive water loss and maintaining RWC, cell structure, and function (López-Galiano et al., 2019; Seleiman et al., 2021). In this research, the fig accession presented a high recovery capacity in the RWC, as observed in Fig. 3, a. The quick RWC recovery, together with the increase of Pro, suggests that this amino acid response to WD is in close relation with RWC. The quick RWC recovery permits fig materials to maintain essential physiological processes (Ammar et al., 2020, 2022, 2023).

In contrast, the 'Ceballos' accession did not exhibit a statistical difference in P_N between SWC at the DAIS period (Fig. 3b), even though this accession presented the lowest SWP (-3.6 MPa). This accession may have the ability to acclimate to WD conditions photosynthetically. The acclimation process can be altered or adjusted to optimize resource use efficiency and minimize damage from water deficit (Vincent et al., 2020). Those changes in P_N may be subtle or not immediately apparent. However, that performance can be observed during the DFCM period in the 'Ceballos' accession since P_N did not completely recover from the FC condition.

Physiological resilience can activate stress response pathways and the production of protective compounds, antioxidants, and osmolytes to mitigate the negative impacts of stressors (Sachdev et al., 2021). As we notice, a remarkable recovery was observed in P_N at 'Black Mission', 'Fortuna', 'Guadalupe Victoria', and 'San Antonio' genotypes. This performance is related to the resilience response in fig genotypes (Ammar et al., 2020). This process is also observed with the decrease of Pro in the DFCM period (Fig. 3, f).

Interaction G x T

We observed statistical differences in the interaction G x T in the gs and E variables analysis; however, those differences were not significant in the rest of the evaluations. All the evaluations decreased from 1 DAIS to 7 DAIS (Fig. 4, a). In the case of DFCM evaluations, the gs presented an increased-decreased performance during this recovery period. The gs in the 'Guadalupe Victoria' accession presented a significant increase at 4 DFCM (Fig. 4, a). While water availability decreases, the stress gets progressed by ROS production, and some other responses are active in plants (Ammar et al., 2020). When the stress production and recovery period are evaluated, the physiological functions are also affected. Moreover, the differences in the recovery rate among plat materials might provide valuable clues to selecting proper planta for periodic droughts in arid and semiarid lands (Rostami & Rahemi, 2013).

In the case of E, we observed that 'Arista' and 'Ceballos' accessions showed statistical differences from the 1 to 7 DAIS condition (Fig. 4, b). The higher E was observed at 1 DAIS in the stress period, while in the recovery period, the highest was in the 8 DFCM. In the DFCM period, the 'Guadalupe Victoria' and 'San Antonio' accessions presented a strong recovery at 4 DFCM (Fig. 4, b).

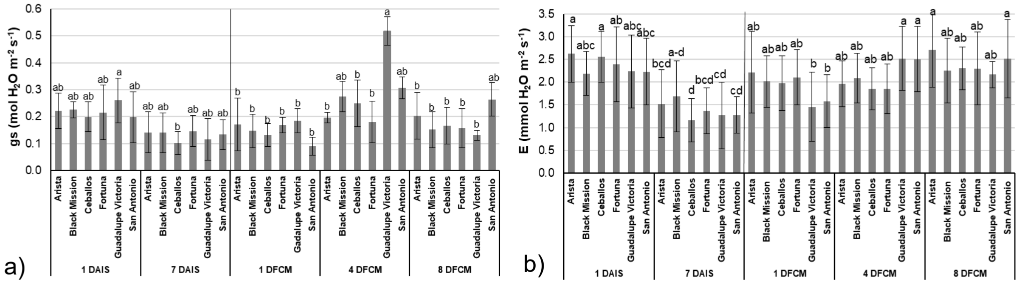

Figure 4. Means of the interaction G x T at 0 and 7 days after irrigation suspension (DAIS) and 1, 4, and 8 Days at Field Capacity Moisture (DFCM) measured in *Ficus carica* mature leaves of six genotypes(Arista, Ceballos, Fortuna, Guadalupe Victoria, San Antonio, and Black Mission). a) Means of stomatal conductance (gs); b) Transpiration rate (E). Bars with different letters indicate statistical differences by Tukey multiple range test at $p \leq 0.05$.

Interaction SWC x T

As a general response, the RWC decreased significantly in the plants under WD conditions (Fig. 5, a). Their RWC decreases 22% in plants at WD in comparison with FC condition at the 7 DAIS. The RWC showed statistical differences between the WD condition from 1 to 7 DAIS; however, the WD and FC evaluations did not differ at the DFCM period in the interaction between SWC x T (Fig. 5, a). In the evaluations of the DFCM period, the genotypes showed a quick recovery at 1 DFCM (Fig. 5, a). Also, fig plants growing in WD reached RWC levels like those growing in FC conditions at 8 DFCM.

Interestingly, P_N was lower in the genotypes growing under the WD condition (Fig. 5, b). In general, P_N decreased 30.3% from 1 to 7 DAIS. Notably, the P_N increased significantly in most genotypes (Fortune accession was the exception) under the FC condition from 1 to 7 DAIS (Fig. 5, b). At 1 DAIS, WD and FC did not show a significant P_N difference; conversely, the P_N difference belonging to 7 DAIS is significant. In addition, the interaction between SWC x T suggested that P_N differed at 1 and 4 but not at 8 DFCM between the SWC.

The gs showed a general diminishing trend from 1 to 7 DAIS (Fig. 5, c). The gs presented statistical differences in WD condition between 1 and 7 DAIS. The trend increased from 1 to 4 DFCM and then diminished to 8 DFCM in both SWCs.

The Ci was lower in general in the FC than in the WD condition by considering DAIS (Fig. 5, d). The Ci presented statistical differences at 1 and 7 DAIS between SWC. In the case of DFCM, a general pattern of increases from 1 to 4 DFCM and a decrease to 8 DFCM was observed in both SWC (Fig. 5, d). In the DAIS case, the E had essential differences in the WD and FC conditions from 1 to 7 days. Also, the lower E was observed at 7 DAIS in FC condition (Fig. 5, e). Besides, the E decreased 46% in FC conditions from 1 to 7 DAIS. In addition, the E in the FC condition presented an improvement from 1 to 8 DFCM.

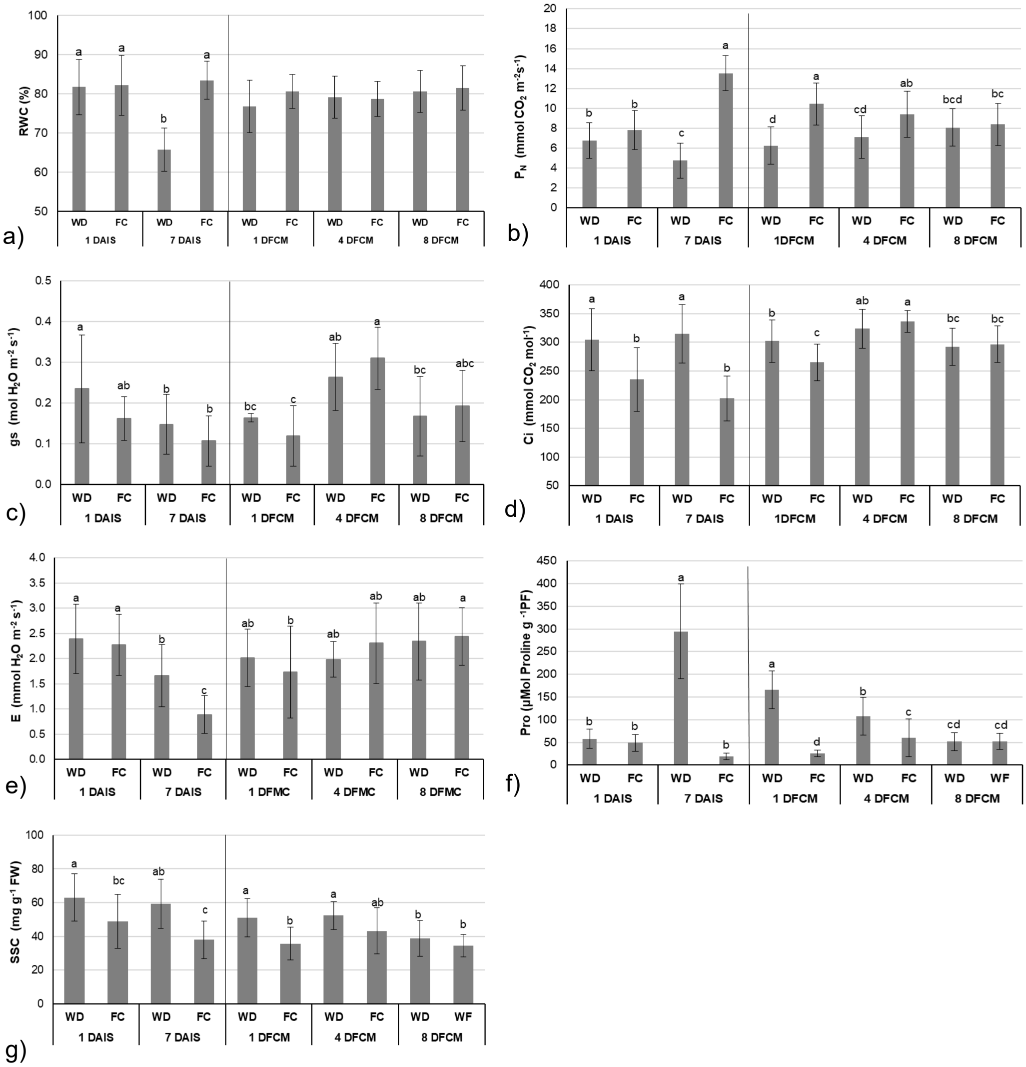

Figure 5. Mean values of the interaction between SWC x T at 0 and 7 days after irrigation suspension (DAIS) and 1, 4, and 8 Days at Field Capacity (DFCM) measured in *Ficus carica* mature leaves of six genotypes (Arista, Ceballos, Fortuna, Guadalupe Victoria, San Antonio, and Black Mission) under two Soil Water Contents (SWC) (Water deficit, WD, i.e. -2.7 MPa; and Field Capacity, FC).

a) Relative Water Content (RWC); b) Photosynthetic rate (P_N); c) Stomatal Conductance (gs); d) Intercellular CO_2 concentration (Ci); e) Transpiration rate (E); f) Proline content (Pro); g) Soluble Sugar Content (SSC). Bars with different letters indicate statistical differences by Tukey multiple range test at $p \leq 0.05$.

In the interaction between SWC x T, the initial condition did not present statistical differences (Fig. 5, f). The Pro showed that WD at 7 DAIS increased significantly compared to FC condition or the ones at 1 DAIS (Fig. 5, f). In the recovery period, the statistical differences persisted at 1 and 4 DFCM. The Pro content was similar in both SWC at 8 DFCM. Notably, SSC showed statistical differences in the DAIS evaluations between SWC (Fig. 5, g). Lower SSC was observed in the FC condition in both evaluation periods (DAIS and DFCM). Some other fig accessions observed responses such as leaf loss during the stress period as well as the new leaf regeneration after a rewatering period (Rostami & Rahemi, 2013); however, none of those responses were observed in evaluated figgenotypes during this experiment, even substantial water deficit was induced.

The RWC is commonly used for knowing the water status in the plant (Parkash & Singh, 2020). The RWC depends on many factors, such as plant species, growth stage, and environmental conditions; in addition, there are general levels that indicate different stress categories. An RWC above 70% is considered a non-stressful condition; a range of 60 to 70% is associated with mild water stress, moderate stress in the range of 50 to 60%, and severe stress below 50% (Laxa et al., 2019). We observed that the evaluated genotypes presented a mean RWC of 65% in the WD condition at 7 DAIS (Fig. 5, a), corresponding to mild water stress. The ‘Ceballos’ accession presented the lowest RWC in WD at 7 DAIS (60%). Those values of RWC represent a plant’s ability to regulate water balance under a water deficit condition.

Our results showed a decrease of 30% in the P_N in WD at 7 DAIS (Fig. 5, b), which may have some consequences for fig plants. Actually, P_N is closely linked to a plant's ability to withstand and recover from various stressors (Mareri et al., 2022). Besides, when there is a decrease in photosynthesis, it weakens the plant's ability to tolerate and recover from environmental stress, such as drought or heat (Tan et al., 2020; Sherin et al., 2022). In addition, the reduced production of energy and metabolites can compromise the plant's defense mechanisms, making it more susceptible to stress and increasing the risk of damage (dos Santos et al., 2022; Fontanetti-Rodrigues et al., 2019; Sachdev et al., 2021).

The reduction in gs helps maintain proper gas exchange and temperature regulation and prevent water loss, which can optimize photosynthetic efficiency (Lawson & Blatt, 2014). We could observe that the gs was lower in the FC condition than WD during the DAIS period (Fig. 5, b). This may be related to the saturation of water uptake, which is detected when the soil moisture is abundant and plant roots can access water easily; the uptake of water by the roots may exceed the plant´s immediate transpiration needs (Gavrilescu, 2021; Gul et al., 2023). As a result, the stoma regulates its opening to reduce water loss through transpiration, leading to a decrease in gs (Taiz & Zeiger, 2002). In addition, under FC conditions, plants can operate at their maximum P_N capacity without experiencing water stress. Our results showed a low gs and E but a high P_N under FC at the stress period (Fig. 5, b; 5, c, and 5, e).

Feedback regulation in *Ficus carica* has been reported to control the stomatal aperture to regulate gas exchange and water loss (Gholami et al., 2012). Furthermore, the increase and decrease in performance in gs and Ci evaluations in WD and FC conditions during the DFCM period (Figs 5, c and 5, d) could be associated with feedback regulation. This process in *Ficus carica* material is a dynamic and intricate system that helps the plant maintain equilibrium, adjust its physiological processes, and maximize its chances of survival and reproductive success in a changing environment.

Also, the decrease in Ci is less pronounced or absent because the primary limitation to P_N under water deficit is not the availability of CO_2 (Flexas et al., 2006). In our study, the Ci did not present significant changes in WD condition at DAIS and DFCM periods (Fig. 5, d). Likewise, a limited gs during the WD condition. Even so, this response of fig genotypes can be related to the reduction of gs; the limited diffusion of CO_2 into the leaf can result in a minimal decrease in Ci, which results from a stomatal limitation (Engineer et al., 2016).

In our study, fig plants reduced E in FC condition in DAIS evaluation, probably associated with a partially or entirely stomatal closure (Fig. 5, e). This occurs as part of the plant's natural response to conserve water and avoid problems such as root hypoxia (lack of oxygen to the roots) or waterlogging, which can be detrimental to its survival (Tan & Zwiazek, 2019). In addition, in the DFCM case fig materials presented a recovery performance. This can also be associated with the growth of adventitious roots (Tan & Zwiazek, 2019).

The stomatal closure restricts the entry of external CO_2 into the leaf, resulting in a decrease in Ci (Engineer et al., 2016). Consistently, all evaluated genotypes presented lower Ci at FC than at WD during the DAIS evaluation (Fig. 5, d). The response of fig materials can result from increased stomatal closure in FC conditions. The stomata tend to close partially or wholly when plants experience reduced transpiration rates due to decreased evaporative demand. With reduced E, less CO_2 is drawn into the leaf, leading to a decrease in Ci (Mareri et al., 2022; Sherin et al., 2022). This performance is observed in fig genotypes in the DAIS period (Figs 3, d and 3, e).

Fig genotypes generally presented higher E in WD than in FC at the DAIS period. Also, some plants can adjust their osmotic potential by accumulating solutes (i.e., sugars and amino acids) during WD conditions (Mareri et al., 2022; Seleiman et al., 2021). Higher E can facilitate the removal of excess solutes from the cells, preventing osmotic imbalances and maintaining proper cellular functioning (Lawson & Blatt, 2014). The increase of E in WD conditions at the DAIS period (Fig. 5e) can be correlated with the critical increase of Pro content (Fig. 6a).

Interaction G x SWC x T

Interestingly, Pro increased by 247.5 μMol Proline g^{-1}FW on average in all genotypes in the WD condition; in contrast, the Pro decreased by 29.3 μMol Proline g^{-1} FW on average in all genotypes in the FC condition from 1 to 7 DAIS (Fig. 6, a). The Pro decreased 65% from 7 DAIS to 1 DFCM. The subsequent Pro evaluation decreased by 50% from the previous quantification until the 8 DFCM, when the Pro at WD was like the FC condition (Fig. 6, a).

The Pro is an amino acid implicated in response to WD. The Pro in all fig genotypes increased around 550% from 1 to 7 DAIS condition in WD condition. This Pro accumulation is related to osmotic adjustment acting as an osmolyte by accumulating in the cytoplasm and vacuoles, attracting water molecules and counteracting the effects of water stress-induced osmotic imbalance (Laxa et al., 2019; Sachdev et al., 2021). In addition, Pro accumulation during water stress also plays a role in stabilizing subcellular structures and proteins. It can protect enzymes and cellular structures from denaturation or damage caused by dehydration and osmotic stress (Hayat et al., 2012; Meena et al., 2019).

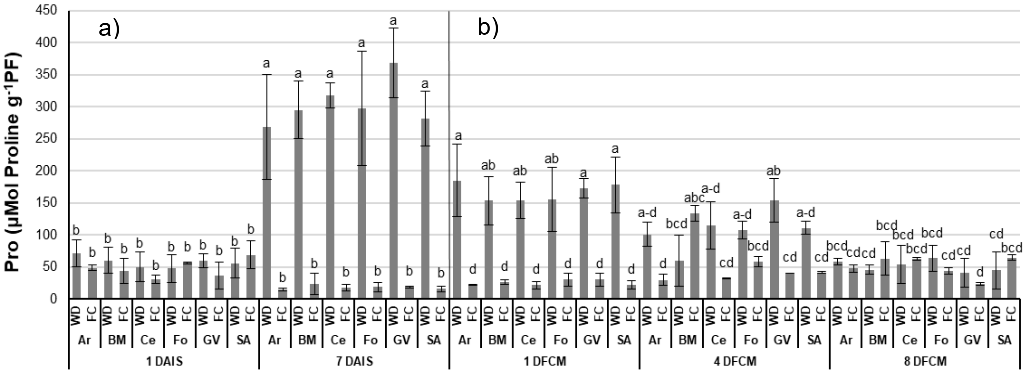

Figure 6. Means of Proline content (Pro) of the interaction between C x SWC x T measured in *Ficus carica* mature leaves of six genotypes (Arista, Ar; Ceballos, Ce; Fortuna, Fo; Guadalupe Victoria, G, San Antonio, SA, and Black Mission, BM) at 0 and 7 days after irrigation suspension (DAIS), and 1, 4, and 8 Days at Field Capacity moisture (DFCM) under two Soil Water Contents (SWC) (Water deficit, WD, i.e. -2.7 MPa; and Field Capacity, FC). Bars with different letters indicate statistical differences by Tukey multiple range test at $p \leq 0.05$.

The fig genotypes could use the enzymes proline dehydrogenase (PDH) and pyrroline-5-carboxylate (P5C) to convert into glutamate, which can be used in various metabolic pathways (Sallam et al., 2019). This conversion allows the reutilization of Pro as a carbon and nitrogen source and facilitates the decrease in Pro levels (Hosseinifard et al., 2022). This could be the reason for a significant decrease of Pro in the DFCM related to the proline degradation when the stress condition was relieved (Figs 3, f; 5, f, and 6, f). In addition, the Pro in the recovery phase restores osmotic balance by regulating water potential and preventing cellular dehydration (Fig. 3, f). Actually, Pro supports the refolding and reactivation of denatured proteins that may have been damaged during stress (Hosseinifard et al., 2022).

As part of the osmotic adjustment, SSC increased in the DAIS period (Fig. 3, g). These SSC help lower the water potential in the cell, enabling the plant to retain water and prevent excessive water loss through osmotic regulation (Yang et al., 2021). Also, SSC serves as a readily available energy source for cellular metabolism and respiration. They can be rapidly mobilized and broken down to provide carbon skeletons for essential cellular processes. The lowest SSC in the FC condition in the DAIS and DFCM period could be related to the response of fig plants with no limiting water availability (Fig. 5, g). Generally, plants under FC have less need to accumulate high levels of soluble solids. Consequently, the soluble solids may dilute within the plant tissues, decreasing SSC (Soberanes-Pérez et al., 2020).

CONCLUSIONS

Our study can confirm that the use of young genetic fig materials can express their water deficit tolerance potential. Also, we demonstrated that native *Ficus carica* genotypes present differences in their ability to tolerate water deficit in comparison to the 'Black Mission' commercial genotype. The adaptative mechanisms and physiological processes observed in *Ficus carica* genotypes that enable them to tolerate soil water content below PWP (-2.7 MPa) and recovery to WD conditions were presented in this report. The fig plants' plasticity is a response to water deficit, allowing them to use different mechanisms to use the available water for their physiological processes. For this reason, the P_N response to water deficit diminished by 30% in comparison to FC plants. The 'Guadalupe Victoria' accession presented higher P_N values in WD and FC conditions during the DAIS period; this is an ability to adjust their photosynthetic process under WD to optimize resource use and minimize drought damage. 'Guadalupe Victoria,' 'Black Mission,' and 'Ceballos' genotypes presented necessary stomatal regulations to control gas exchange and water loss, helping them maintain water equilibrium and adjust their physiological process.

'Guadalupe Victoria' and 'Fortuna' accession showed a significant proline accumulation in response to WD, which acts as an osmolyte that attracts water molecules and counteracts the effects of osmotic imbalance. In this context, the 'Ceballos' accession increases SSC under WD conditions to lower water potential in cells and retain water. These mechanisms and processes allow fig plants to cope with mild water stress, maintain RWC, and quickly recover when water becomes available again. They also help the plants optimize their photosynthetic efficiency and physiological functions under varying water availability conditions. This research defines the relationship between resistance to water deficit and plant physiological and biochemical markers in fig materials. Selecting genetic materials tolerant to deficits in juvenile stages is feasible through physiological criteria, such as proline accumulation, soluble solids accumulation, photosynthesis, relative water content, and thereby shortening the time in the selection process.

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REFERENCES

Akinci, S., Akıncı, Ş. & Lösel, D.M. 2012. Plant Water-Stress Response Mechanisms Plant Water-Stress Response Mechanisms. www.intechopen.com

Ammar, A., Aissa, I., Ben, Zaouay, F. & Gouiaa, M. 2023. Physiological Behaviour of Fig Tree (*Ficus carica* L.) Under Different Climatic Conditions. In Fig (*Ficus carica*): Production, Processing, and Properties, 247–25.

Ammar, A., Ben Aissa, I., Mars, M. & Gouiaa, M. 2020. Comparative physiological behavior of fig (*Ficus carica* L.) cultivars in response to water stress and recovery. *Scientia Horticulturae*, **260**(108881), 1–7. https://doi.org/10.1016/j.scienta.2019.108881

Ammar, A., Ben, I., Gouiaa, M. & Mars, M. 2022. Fig (*Ficus carica* L.) vulnerability to climate change : Combined effects of water stress and high temperature on ecophysiological behaviour of different cultivars. *South African Journal of Botany* **147**, 482–492. https://doi.org/10.1016/j.sajb.2022.02.014

Anjum, S.A., Xie, X.-Y., Wang, L.-C., Saleem, M.F., Man, C. & Lei, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research* **6**(9), 2026–2032. https://doi.org/10.5897/AJAR10.027

Arshadi, A., Karami, E., Sartip, A., Zare, M. & Rezabakhsh, P. 2018. Genotypes performance in relation to drought tolerance in barley using multi-environment trials. *Agronomy Research* **16**(1), 5–21. https://doi.org/10.15159/AR.18.004

Arya, S., Rawal, S., Luthra, S.K., Sharma, N., Gupta, V.K. & Kadian, M.S. 2017. Participatory evaluation of advanced potato (*Solanum tuberosum*) clones for water stress tolerance. *Indian Journal of Agricultural Sciences* **87**, 11.

Barolo, M.I., Ruiz Mostacero, N. & López, S.N. 2014. *Ficus carica* L. (*Moraceae*): An ancient source of food and health. *Food Chemistry* **164**, 119–127. Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2014.04.112

Bates, L.S. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* **207**(39), 205–207. doi.org/10.1007/BF0001806

Bougiouklis, J.N., Karachaliou, Z., Tsakos, J., Kalkanis, P., Michalakos, A. & Moustakas, N. 2020. Seasonal variation of macro-and micro-nutrients in leaves of fig (*Ficus carica* L.) under mediterranean conditions. *Agronomy Research* **18**(4), 2328–2339. https://doi.org/10.15159/AR.20.201

Caliskan, O. 2015. Mediterranean Figs (*Ficus carica* L .) Functional Food Properties. In The Mediterranean Diet. **2011**. Elsevier Inc. https://doi.org/10.1016/B978-0-12-407849-9.00056-7

Çalişkan, O. & Aytekin Polat, A. 2011. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. *Scientia Horticulturae* **128**(4), 473–478. https://doi.org/10.1016/j.scienta.2011.02.023

dos Santos, T.B., Ribas, A.F., de Souza, S.G.H., Budzinski, I.G.F. & Domingues, D.S. 2022. Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. *Stresses* **2**(1), 113–135. https://doi.org/10.3390/stresses2010009

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* **28**(3), 350–356. doi.org/10.1021/ac60111a017

FAOSTAT. 2020. Statistical Database. Food and Agriculture Organization of the United Nations, Rome. Rev. May 18, 2023.

Engineer, C., Hashimoto-Sugimoto, M., Neri, J., Israelsson-Nordstrom, M., Azoulay-Shemer, T., Rappel, W.-J., Iba, K. & Schroeder, J. 2016. CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. *Physiology & Behavior* **21**(1), 1–23. https://doi.org/10.1016/j.tplants.2015.08.014.CO

Evans, J.R. & Santiago, L.S. 2014. Prometheus Gold Leaf Protocol: gas exchange using LI-COR 6400. *Functional Plant Biology*, **6**(41), 223–226. https://doi.org/http://dx.doi.org/10.1071/FP10900

Flexas, J., Bota, J., Galmés, J., Medrano, H. & Ribas-Carbó, M. 2006. Keeping a positive carbon balance under adverse conditions: Responses of photosynthesis and respiration to water stress. *Physiologia Plantarum* **127**(3), 343–352. https://doi.org/10.1111/j.1399-3054.2006.00621.x

Fontanetti-Rodrigues, M.G., Arruda-Ferreira, A.F., Honorato-Monteiro, L.N., Paes dos Santos, T., Manzani-Lisboa, L.A. & Monteiro de Figueiredo, P.A. 2019. Initial growth and physiological indexes of fig accessions in active germplasm bank. *Revista Brasileira de Fruticultura* **41**(4), 1–10. https://doi.org/10.1590/0100-29452019154

Gao, X., Zhao, X., Li, H., Guo, L., Lv, T. & Wu, P. 2018. Exotic shrub species (*Caragana korshinskii*) is more resistant to extreme natural drought than native species (Artemisia gmelinii) in a semiarid revegetated ecosystem. *Agricultural and Forest Meteorology* **263**, 207–216. https://doi.org/10.1016/j.agrformet.2018.08.029

Gavrilescu, M. 2021. Water, soil, and plants interactions in a threatened environment. *Water* (Switzerland), **13**(19). https://doi.org/10.3390/w13192746

Gholami, M., Rahemi, M., Kholdebarin, B. & Rastegar, S. 2012. Biochemical responses in leaves of four fig cultivars subjected to water stress and recovery. *Scientia Horticulturae* **148**, 109–117. https://doi.org/10.1016/j.scienta.2012.09.005

Gul, M.U., Paul, A., Manimurugan, S. & Chehri, A. 2023. Hydrotropism: Understanding the Impact of Water on Plant Movement and Adaptation. *Water*, **15**(3), 1–16. https://doi.org/10.3390/w15030567

Hayat, S., Hayat, Q., Nasser Alyemeni, M., Shafi Wani, A., Pichtel, J. & Ahmad, A. 2012. Role of proline under changing environments A review. *Plant Signaling and Behavior* **7**(11), 1456–1466.

Hernandez, J.O. & Bae Park, B. 2022. The Leaf Trichome, Venation, and Mesophyll Structural Traits Play Important Roles in the Physiological Responses of Oak Seedlings to Water-Deficit Stress. *International Journal of Molecular Sciences* **23**(15). https://doi.org/10.3390/ijms23158640

Hosseinifard, M., Stefaniak, S., Javid, M.G., Soltani, E., Wojtyla, Ł. & Garnczarska, M. 2022. Contribution of Exogenous Proline to Abiotic Stresses Tolerance in Plants: A Review. *International Journal of Molecular Sciences*, **23**(9), 1–20. https://doi.org/10.3390/ijms23095186

Kartal, S., Değirmenci, H. & Arslan, F. 2019. Ranking irrigation schemes based on principle component analysis in the arid regions of Turkey. *Agronomy Research* **17**(2), 456–465. https://doi.org/10.15159/AR.19.053

Köppen, W. 1948. Climatology: Com a study of the climates of the earth. Economic Culture

Kunjet, S., Thaler, P., Gay, F., Chuntuma, P., Sangkhasila, K. & Kasemsap, P. 2013. Effects of drought and tapping for latex production on water relations of *Hevea brasiliensis* trees. *Kasetsart Journal - Natural Science* **47**(4), 506–515.

Lawson, T. & Blatt, M.R. 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology* **164**(4), 1556–1570. https://doi.org/10.1104/pp.114.237107

Laxa, M., Liebthal, M., Telman, W., Chibani, K. & Dietz, K.J. 2019. The role of the plant antioxidant system in drought tolerance. *Antioxidants* **8**(4). https://doi.org/10.3390/antiox8040094

López-Galiano, M.J., García-Robles, I., González-Hernández, A.I., Camañes, G., Vicedo, B., Real, M.D. & Rausell, C. 2019. Expression of miR159 Is Altered in Tomato Plants Undergoing Drought Stress. *Plants* **8**(201), 1–11.

Mareri, L., Parrotta, L. & Cai, G. 2022. Environmental Stress and Plants. *International Journal of Molecular Sciences* **23**(10), 1–9. https://doi.org/10.3390/ijms23105416

Meena, M., Divyanshu, K., Kumar, S., Swapnil, P., Zehra, A., Shukla, V., Yadav, M. & Upadhyay, R.S. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **5**(e02952), 1–20. https://doi.org/10.1016/j.heliyon.2019.e02952

Moradi, P. 2016. Key plant products and common mechanisms utilized by plants in water deficit stress responses. *In Botanical Sciences* **94**, 4, 657–671. Sociedad Botanica de Mexico, A.C. https://doi.org/10.17129/botsci.709

Nikolaou, G., Neocleous, D., Christou, A., Kitta, E. & Katsoulas, N. 2020. Implementing sustainable irrigation in water-scarce regions under the impact of climate change. *Agronomy* **10**(8), 1–33. https://doi.org/10.3390/agronomy10081120

Parkash, V. & Singh, S. 2020. A review on potential plant-basedwater stress indicators for vegetable crops. *In Sustainability* (Switzerland) **12**, 10.MDPI. https://doi.org/10.3390/SU12103945

Paulus, D., Zorzzi, I.C., Becker, D., Nava, G.A., de Paula, A.L. & de Paula, F.M. 2020. Physiological indicators and yield of the Chinese cabbage cultivated at different soil water tensions. *Agronomy Research* **18**(1), 214–255. https://doi.org/10.15159/AR.20.013

Rabei Metwali, E.M., Ahemaidan Soliman, H., Mohammed Howladar, S., Fuller, M.P. & Saeed Al-Zahrani, H. 2016. Appraisal of in vitro drought stress among three different cultivars of fig (*Ficus carica* L.) using RAPD and ISSR markers. *Plant Omics Journal* **9**(1), 1–11.

Rostami, A.A. & Rahemi, M. 2013. Responses of Caprifig Genotypes to Water Stress and Recovery. *Biol.Environ.Sci*. **7**(21), 131–139.

Sachdev, S., Ansari, S.A., Ansari, M.I., Fujita, M. & Hasanuzzaman, M. 2021. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, **10**(2), 1–37. https://doi.org/10.3390/antiox10020277

Sallam, A., Alqudah, A.M., Dawood, M.F.A., Baenziger, P.S. & Börner, A. 2019. Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. *International Journal of Molecular Sciences* **20**, 13. MDPI AG. https://doi.org/10.3390/ijms20133137

Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H. & Battaglia, M.L. 2021. Drought stress impacts plants, and different approaches to alleviate its adverse effects. *Plants,* **10**(2), 1–25. https://doi.org/10.3390/plants10020259

Sherin, G., Aswathi, K.P.R. & Puthur, J.T. 2022. Photosynthetic functions in plants subjected to stresses are positively influenced by priming. *Plant Stress* **4**(March), 100079. https://doi.org/10.1016/j.stress.2022.100079

Soberanes-Pérez, A., Calderón-Zavala, G., López-Jiménez, A. & Alvarado-Raya, H.E. 2020. Biorreguladores Para La Producción De Higo Bajo Condiciones De Invernadero Bioregulators for Fig Production Under Greenhouse Conditions. *Rev. Fitotec. Mex*, **43**(1), 61–69.

Soni, N., Mehta, S., Satpathy, G. & Gupta, R.K. 2014. Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*). *Journal of Pharmacognosy and Phytochemistry* **3**(2), 158–165.

Taiz, L. & Zeiger, E. 2002. Plant Physiology. *In Sinauer Associates* **3**(132), 1. https://doi.org/10.1104/pp.900074

Tan, S., Yang, Y., Liu, T., Zhang, S. & Huang, W. 2020. Responses of photosystem I compared with photosystem II to combination of heat stress and fluctuating light in tobacco leaves. *Plant Science* **292**(110371), 1–15. https://doi.org/10.1016/j.plantsci.2019.110371

Tan, X. & Zwiazek, J.J. 2019. Stable expression of aquaporins and hypoxia-responsive genes in adventitious roots are linked to maintaining hydraulic conductance in tobacco (*Nicotiana tabacum*) exposed to root hypoxia. *PLoS ONE*, **14**(2), 1–13. https://doi.org/10.1371/journal.pone.0212059

Tzanakakis, V.A., Paranychianakis, N.V. & Angelakis, A.N. 2020. Water supply and water scarcity. *Water* (Switzerland), **12**(9), 1–16. https://doi.org/10.3390/w12092347

Valliyodan, B. & Nguyen, H.T. 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current Opinion in Plant Biology* **9**(2), 189–195. https://doi.org/10.1016/j.pbi.2006.01.019

Vincent, C., Rowland, D., Schaffer, B., Bassil, E., Racette, K. & Zurweller, B. 2020. Primed acclimation: A physiological process offers a strategy for more resilient and irrigation-efficient crop production. *Plant Science* **295**(June), 1–10. https://doi.org/10.1016/j.plantsci.2019.110240

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z. & Chen, S. 2021. Response mechanism of plants to drought stress. *Horticulturae* **7**(3), 1–36. https://doi.org/10.3390/horticulturae7030050

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How do rhizobacteria species influence the growth and yield of soybean in a tropical environment?

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Abstract. The application of rhizobacteria has gained space in agricultural production, given the demand for more sustainable systems. However, most of the results obtained are related to soil or seed application, leaving a gap in relation to the foliar application of these microorganisms. The objective of this work was to evaluate the impact of foliar application of different types of growth-promoting rhizobacteria on morphological aspects and production components of soybean. For this, the foliar application of four rhizobacteria (*Serratia* sp.; *Bacillus subtilis*; *Bacillus* sp.; *Pseudomonas fluorescens*) was used, as well as a control without inoculation. Morphological variables of growth and production components were analyzed. The yield ratio of the treatments with rhizobacteria, concerning the control was also calculated. The foliar application with different rhizobacteria in soybean did not affect the vegetative parameters of plant height, stem diameter and dry weight of the canopy. For the number of pods per plant, number of grains per plant and grain yield the use of *Bacillus* sp. was superior to the other treatments, providing an average increase of 27.65%, 20.32% and 28.59%, respectively. Also, the *Serratia* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* application increased the grain yield by 8.49%, 10.73% and 5.71%, respectively. In conclusion, for the condition of the tropical region where this study was conducted, the foliar application with different growth-promoting rhizobacteria in soybean did not interfere in the vegetative development of soybean plants. In addition, considering the factors related to the increase of production in cultivated areas, all rhizobacteria have the potential to improve yield gains when applied as foliar treatment, especially the *Bacillus* sp.

Key words: *Bacillus* sp., biostimulants, biological inputs, *Glycine max*, growth promotion, regenerative agriculture.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is the most economically and nutritionally important legume in the world. Because its grains contain 40% protein and 20% lipids (Saito et al., 2021), being employed also in the animal feed sector and for the production of biofuel, currently, the world's production exceeds 365 million tons (USDA, 2021).

Due to the exponential increase in the number of people in the world, there is a need to increase the production of food and make the best use of technology in agricultural production. The overuse of inputs, such as fertilizers and pesticides, can cause severe damage to health and the environment, leading producers to adopt sustainable measures that decrease the impact of agriculture on the environment (Canellas et al., 2015).

As a result of the growing demand for food as well as the concern to preserve the environment, there is interest in the manipulation of endophytic microorganisms in agricultural practices. Endophytic microorganisms are considered sustainable technologies for agricultural management, reducing dependence on chemical inputs, promoting a decrease in the use of fertilizers and insecticides, and consequently reducing the impact on the environment (Mahanty et al., 2017). In addition, microorganisms can be used in both plant growth promotion and biological control of plant pests and diseases (Ait-El-Mokhtar et al., 2019; Ben-Laouane et al., 2019; Abdel Latef et al., 2020).

Rhizobacteria are a select group of microorganisms that live closely associated with plants, colonizing their roots and providing for the development of their host (Adoko et al., 2021; Vendruscolo & Lima, 2021). This plant development is linked to the ability of rhizobacteria to contribute to the availability of nutrients that are in short supply in the soil, such as iron (Fe) and phosphorus (P), but also contributes by helping the plant in the defense against harmful microorganisms (Raklami et al., 2019; Wu et al., 2020).

Plant growth-promoting bacteria (PGPBs) are a group of microorganisms that are beneficial to plants and can colonize the root surface, rhizosphere, phyllosphere, and internal plant tissues. They can provide a biological alternative of great benefit, increasing crop productivity and reducing the need for fertilizers in agroecosystems (Marques et al., 2010). Thus, the purpose of raising the production of agricultural areas with the use of PGPBs has become an alternative and PGBPs have been interesting research molds to obtain formulation for manufacturing commercial products (Walia et al., 2014; Abdel Latef et al., 2020).

The rhizobacteria promote growth in soybeans, and the stimulation of growth influences other factors such as greater germination in the field, grain production, nutrient absorption, dry weight and height of the cultivars (Adoko et al., 2021; Kalenska et al., 2022). Growth promotion is attributed to the better absorption of nutrients by the roots, which increases the concentration of nutrients translocated to the leaves (Vendruscolo & Lima, 2021). According to Andy et al. (2020), rhizobacteria favor the development of the root zone of different legumes acting as biofertilizers and bioinoculants, because they aim to partially or even completely supply the demand for some development factors, such as nitrogen.

The use of PGPBs in inoculation and co-inoculation is an alternative for sustainable production (Rosas et al., 2009), being used as a technology with the potential to improve the productivity of agricultural systems in the long term and not attack in a harmful way the environment (Naiman et al., 2009; Silva et al., 2020). Thus, Korber et al., 2021; Silva et al., 2023, suggest that the production of inoculants in a sustainable manner with rhizobacteria is an alternative to decrease the environmental risks caused by the inappropriate, and sometimes excessive, use of inputs and pesticides.

In addition to the benefits found with the inoculation of PGPBs during seed treatment or via direct application to the soil, it appears that their use in the form of foliar application also allows gains in terms of the vegetative development of plants and increased productivity. These results are mainly related to the increase in leaf health, as observed for *Bacillus* sp. (Ortiz et al., 2022) and *Serratia* sp. (Nagrale et al., 2023), which can be used as bioproducts for biological control and for ameliorating abiotic stress effects. But also, the effects may be related to metabolic gains and increased nutrient absorption (Mahmood et al., 2022), increased gas exchange activity (Silva et al., 2020) and accumulation of energetic reserves (Asghari et al., 2020).

Thus the use of these rhizobacteria is of fundamental environmental, ecological, and economic importance, as it results in savings for farmers and an improvement in soil fertility. Therefore, due to the use of pesticides, these rhizobacteria can be affected. In light of the above, this work aimed to evaluate the impact of foliar application of different types of growth-promoting rhizobacteria on morphological aspects and production components of soybean.

MATERIALS AND METHODS

The trial was conducted at Fazenda Sozinha, in Leopoldo de Bulhões, belonging to the State of Goiás, with a South latitude of 16° 32'02.4", West longitude of 48° 57'49.3" and an altitude of 1,008 m. The region's climate, according to Köppen, is classified as Aw (tropical with dry season), with a minimum of 18 °C and maximum of 28 °C, with an average temperature of 22 °C, and average annual rainfall of 1,450 mm (Cardoso et al., 2014).

The soil of the property is classified as LATOSSOLO VERMELHO (Oxisol) with a sandy loamy texture. As planting fertilization, 150 kg ha^{-1} of K_2O and 250 kg ha^{-1} of P_2O_5 were applied, where the K_2O was applied before planting, in total area, and the P_2O_5 was applied directly in the planting furrow.

The cultivar used was the ‘CZ 26B77 IPRO’, which has a transgenic related to tolerance to glyphosate herbicide (RR technology), being super-early with excellent yield potential (Cultivar Catalog, 2020). This cultivar belongs to the relative maturity group of 6.7, with indeterminate growth habits, and medium to high fertility requirements, its population recommendation is 300–320 thousand plants ha^{-1}, and its cycle time varies from 100 to 105 days.

The seeds obtained industrial seed treatment (TSI) with Standak®Top at a dosage of 200 mL 100 kg^{-1} of seed. Inoculation was performed with *Bradyrhizobium elkanii,* with the commercial name Gelfix 5® at a dosage of 200 mL ha^{-1}.

Sowing was performed on November 4, 2020, with a sower-tractor set, where an area of 400 m² was planted, divided into 40 rows spaced at 0.50 m, and a length of 20 m.

The experimental design was a randomized block with five treatments, one control and all four repetitions. Each repetition was a 4.0 m wide by 4.0 m long plot, and the six central lines were evaluated, totaling a usable area of 9.0 m². The spacing between blocks was 0.5 m and between plots 0.5 m, totaling 300,000 seeds ha^{-1}.

The treatments used were with biological products, being the bacterial strains *Serratia* sp. and *Bacillus subtillis* from the Collection of Microbial Isolates of the Agrolab Laboratory, and the strains of *Pseudomonas fluorescens* from the Collection of Multifunctional Microorganisms of Embrapa Rice and Beans. The initial bacterial concentration of all biological products used was 1×10^9 CFU mL^{-1}, being: T1 - *Serratia* sp.; T2 - *Bacillus subtilis*; T3 - *Bacillus* sp.; T4 *Pseudomonas fluorescens*; T5 - fresh water (control). The treatments were applied using a knapsack sprayer, with an application speed of 1.0 m s^{-1} and a syrup volume of 140 L ha^{-1}, at the V2 and V5 stages in the vegetative phase, and R1 and R5.1 in the reproductive phase. The biological materials were concentrated in an aqueous solution and were subsequently diluted in fresh water for application in the aforementioned concentrations.

For the evaluations, 10 random plants were collected from the four central rows of the plot and evaluated at the R1 stage: canopy fresh weight, aboveground height, and stem diameter. For height a tape measure graduated in centimeters was used, for the diameter of the stem a digital caliper was used and the measurement in millimeters. In the canopy fresh weight of ten plants was used, then only the aerial part was selected, and the fresh weight was estimated using a precision balance.

At the R9 stage, 10 plants per plot were collected and the following parameters were evaluated: number of pods per plant, number of grains, average number of grains per pod, and grain yield per hectare of each plot. The percentage yield gain of the treatments over the control treatment was also calculated.

The mean data of vegetative development and those related to grain production were submitted to analysis of variance and the means were compared using the Tukey test at 5% probability, except for the percentage gain in grain yield, which was expressed as a percentage. The software SISVAR 5.6 (Ferreira, 2014) was used for the statistical analyses.

RESULTS AND DISCUSSION

It was found that for plant height, stem diameter, and aboveground fresh weight there were no significant effects of the treatments with the rhizobacteria (Fig. 1).

The results related to the vegetative growth (Fig. 1), found in the present experiment, do not corroborate with those observed by Paula et al., (2021), who state that inoculation with rhizobacteria is viable for stimulating the development of soybean plants. But these authors also showed that different bacterial isolates can act differently concerning soybean plants, influencing the production of phytohormones, nutrient uptake, or even interacting antagonistically with possible pathogens that may attack the crop. In addition, there is also the possibility that the genetic material of the chosen cultivar does not respond to the stimulation during vegetative growth. Environmental conditions may also have masked the initial effects of the rhizobacteria's action, given that the plants developed properly and without health problems.

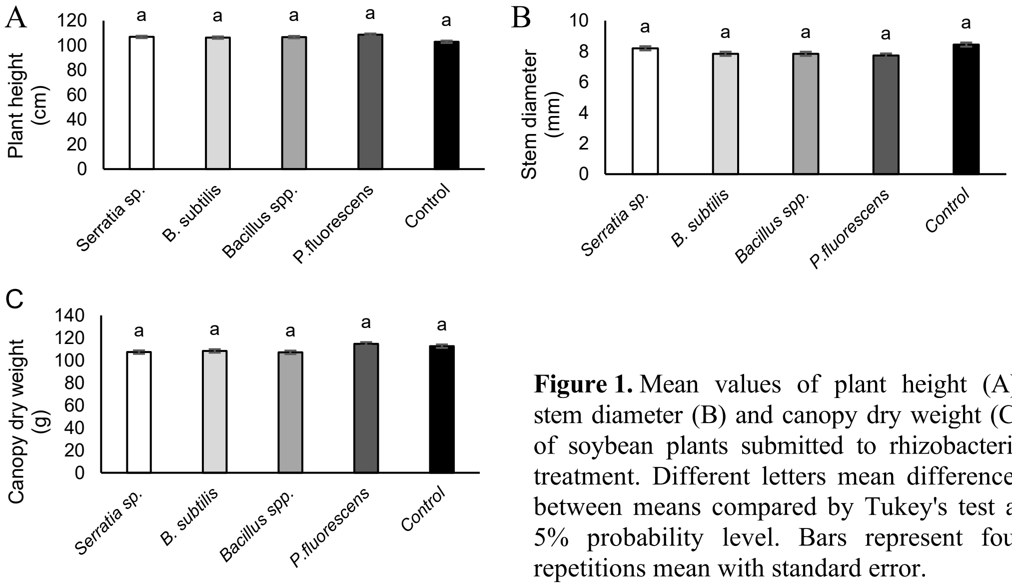

Figure 1. Mean values of plant height (A), stem diameter (B) and canopy dry weight (C) of soybean plants submitted to rhizobacteria treatment. Different letters mean differences between means compared by Tukey's test at 5% probability level. Bars represent four repetitions mean with standard error.

For the number of pods per plant (Fig. 2, A), the use of *Bacillus* sp. bacteria was superior to the other treatments, providing an average increase of 27.65%. The other treatments showed no difference among themselves or concerning the control treatment.

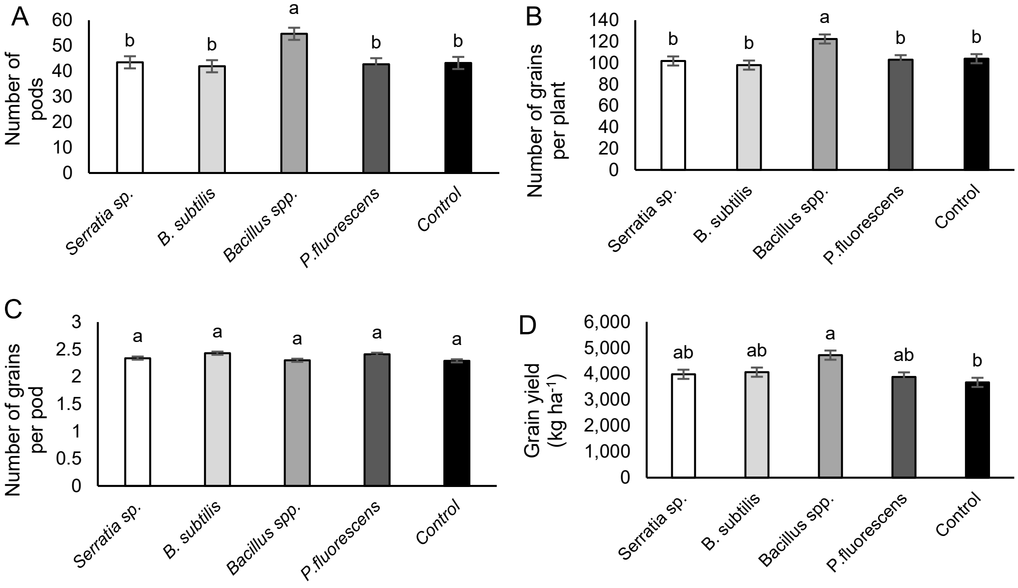

Figure 2. Mean values of number of pods (A), number of grains per plant (B), number of grains per pod (C) and grain yield (D) of soybean plants submitted to rhizobacteria treatment. Different letters mean differences between means compared by Tukey's test at 5% probability level. Bars represent four repetitions mean with standard error.

Also, for the parameter of the number of grains per plant (Fig. 2, B), the treatment with *Bacillus* sp. showed a significant difference from the other treatments, with average superiority of 20.32%, equivalent to 20.67 grains per plant.

There was no difference between treatments concerning the number of grains per pod (Fig. 2C). However, it was found that there was an increase in grain yield when the treatment with *Bacillus* sp. was performed, but without a significant difference to the other treatments composed by the application of rhizobacteria (Fig. 2D).

Compared to the control treatment, inoculation with *Bacillus* sp. promoted the greatest gain in grain yield, about 28.59%, equivalent to more than 1,048 kg ha^{-1}. In sequence, the treatments with *Serratia* sp*.*, *Bacillus subtillis* and *Pseudomonas fluorescens* also resulted in yield gains of 8.49%, 10.73% and 5.71%, respectively (Fig. 3).

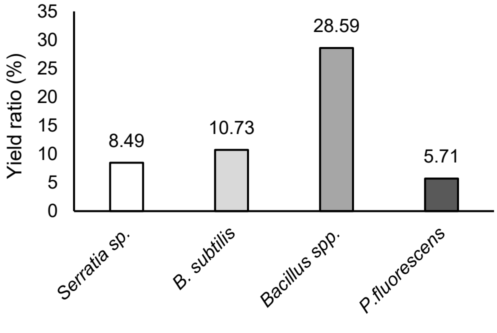

Figure 3. Yield ratio of soybean plants subjected to rhizobacteria treatment.

The beneficial effect of applying *Bacillus* sp. is largely related to its ability to act protectively against the attack of diseases common to soybean cultivation, such as Asian rust (*Puccinia pachyrhizi*), downy mildew (*Peronospora manshurica*), target spot (*Corynespora cassiicola*), brown spot (*Septoria glycines*) and Anthracnose (*Colletotrichum truncatum*). By helping to control these diseases, the application of *Bacillus* reduces the loss of leaf area of soybean plants, which is directly linked to the plant's productive capacity, while it appears that when managing the application of the bacteria together with fungicides chemicals, it is possible to obtain superior results (Santos et al., 2022).

Inoculation via foliar application with *Bacillus* sp., alone or in combination with other microorganisms, also results in a higher photosynthetic rate of plants, accompanied by greater root development and greater accumulation of nutrients in plant organs (Silva et al., 2020; Solanki et al., 2023), which is positively related to the productive capacity of soybean plants (Moretti et al., 2020). However, as observed in the present study, it appears that these effects have little interference in relation to vegetative growth (Solanki et al., 2023).

The positive effects of applying Bacillus sp. on reproductive characteristics are verified for other cultivated plant species, such as tomato in an organic cultivation system, for which the application of *B. megaterium* increased the productive capacity, in addition to improving the quality of the harvested fruits (Yagmur & Gunes, 2021). In addition, the application of *B. Amyloliquefaciens* was efficient for the control of Botrytis in cucumber plants grown in a protected environment, also contributing to the increase in fruit productivity of this species (Nakkeeran et al., 2020).

Despite the inferior results obtained with other microorganisms, in relation to *Bacillus* sp., it appears that there is potential for its use (Fig. 3). In this sense, new studies should explore different application management, verifying better environmental conditions and also taking into account the genetic material used. Other studies have shown that the application of *Serratia* sp. has a positive effect on alleviating abiotic

(Moon et al., 2023) and biotic (Ortiz & Sansinenea, 2023) stresses. In addition, similar effects were observed for *Pseudomonas fluorescente*, verifying its ability to act against pathogens of cultivated species (Vicentini et al., 2022) and increasing leaf nutrient content, as well as lentil grain productivity (Erdemci, 2020).

Considering the results, the present study brings a new perspective on the use of rhizobacteria in soybean production, showing that foliar application can have a positive effect on the characteristics linked to grain production. There is also a need for future studies that point to better management of this application, taking into account the possibility of combinations between the studied materials and other inputs, such as nutrients, beneficial elements, bio-inputs, hormones and chemical products conventionally used in the cultivation of soybean.

CONCLUSIONS

For the condition of the tropical region where this study was conducted, the foliar application with different growth-promoting rhizobacteria in soybean did not interfere in the vegetative development of soybean plants. In addition, considering the factors related to the increase of production in cultivated area, all rhizobacteria have the potential to improve yield gains when applied as foliar treatment, especially the *Bacillus* spp.

REFERENCES

Abdel Latef, A.A.H., Abu Alhmad, M.F., Kordrostami, M., Abo–Baker, A.B.A.E. & Zakir, A. 2020. Inoculation with Azospirillum lipoferum or Azotobacter chroococcum reinforces maize growth by improving physiological activities under saline conditions. *Journal of Plant Growth Regulation* **39**, 1293–1306. doi: 10.1007/s00344-020-10065-9

Adoko, M.Y.E., Agbodjato, N.A., Noumavo, A.P., Amogou, O.E., Adjanohoun, A. & Baba-Moussa, L. 2021. Bioformulations based on plant growth promoting rhizobacteria for sustainable agriculture: Biofertilizer or Biostimulant?. *African Journal of Agricultural Research* **17**(9), 1256–1260. doi: 10.5897/AJAR2021.15756

Ait-El-Mokhtar, M., Laouane, R.B., Anli, M., Boutasknit, A., Wahbi, S. & Meddich, A. 2019. Use of mycorrhizal fungi in improving tolerance of the date palm (Phoenix dactylifera L.) seedlings to salt stress. *Scientia Horticulturae* **253**, 429–438. doi: 10.1016/j.scienta.2019.04.066

Andy, A.K., Masih, S.A. & Gour, V.S. 2020. Isolation, screening and characterization of plant growth promoting rhizobacteria from rhizospheric soils of selected pulses. *Biocatalysis and Agricultural Biotechnology* **27**, 101685. doi: 10.1016/j.bcab.2020.101685

Asghari, B., Khademian, R. & Sedaghati, B. 2020. Plant growth promoting rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. *Scientia Horticulturae* **263**, 109132.

Ben-Laouane, B., Meddich, A., Bechtaoui, N., Oufdou, K. & Wahbi, S. 2019. Effects of arbuscular mycorrhizal fungi and rhizobia symbiosis on the tolerance of Medicago sativa to salt stress. *Gesunde Pflanzen* **71**(2), 135–146. doi: 10.1007/s10343-019-00461-x

Canellas, L.P., da Silva, S.F., Olk, D.C. & Olivares, F.L. 2015. Foliar application of plant growth-promoting bacteria and humic acid increase maize yields. *Journal of Food, Agriculture & Environment* **13**(1), 131–138.

Cardoso, M.R.D., Marcuzzo, F.F.N. & Barros, J.R. 2014. Climatic classification of Köppen-Geiger for the State of Goiás and the Federal District. *Acta Geográfica* **8**(16), 40–55. doi: 10.5654/actageo2014.0004.0016

Erdemci, İ. 2020. Effect of Pseudomonas fluorescent rhizobacteria on growth and seed quality in lentil (*Lens culinaris* Medik.). *Communications in Soil Science and Plant Analysis* **51**(14), 1852–1858.

Ferreira, D.F. 2014. Sisvar: A Guide for Its Bootstrap Procedures in Multiple Comparisons. *Ciência e Agrotecnologia* **38**, 109–112. doi: 10.1590/S1413-70542014000200001

Kalenska, S., Novytska, N., Kalenskii, V., Garbar, L., Stolyarchuk, T., Doktor, N., Kormosh, S. & Martunov, A. 2022. The efficiency of combined application of mineral fertilizers, inoculants in soybean growing technology, and functioning of nitrogen-fixing symbiosis under increasing nitrogen rates. *Agronomy Research* **20**(4), 730–750. doi: 10.15159/AR.22.075

Korber, L.P.P., Korber, Â.H.C., Grange, L. & Klahold, C.A. 2021. Eficiência de produtos biológicos na coinoculação de sementes de soja. *South American Sciences* **2**(2), e21109. doi: 10.52755/sas. v2i2.109

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A. & Tribedi, P. 2017. Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research* **24**, 3315–3335. doi: 10.1007/s11356-016-8104-0

Mahmood, S., Daur, I., Yasir, M., Waqas, M. & Hirt, H. 2022. Synergistic practicing of rhizobacteria and silicon improve salt tolerance: implications from boosted oxidative metabolism, nutrient uptake, growth and grain yield in mung bean. *Plants* **11**(15), 1980. doi: 10.3390/plants11151980

Marques, A.P., Pires, C., Moreira, H., Rangel, A.O. & Castro, P.M. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. *Soil Biology and Biochemistry* **42**(8), 1229-1235. doi: 10.1016/j.soilbio.2010.04.014

Moon, Y.S., Khan, M., Khan, M.A. & Ali, S. 2023. Ameliorative symbiosis of *Serratia fonticola* (S1T1) under salt stress condition enhance growth-promoting attributes of *Cucumis sativus* L. *Symbiosis* **89**, 1–15. doi: 10.1007/s13199-023-00897-w

Moretti, L.G., Crusciol, C.A., Kuramae, E.E., Bossolani, J.W., Moreira, A., Costa, N.R. & Hungria, M. 2020. Effects of growth-promoting bacteria on soybean root activity, plant development, and yield. *Agronomy Journal* **112**(1), 418–428. doi: 10.1002/agj2.20010

Nagrale, D.T., Chaurasia, A., Kumar, S., Gawande, S.P., Hiremani, N.S., Shankar, R., Gokte-Narkhedkar, N. & Prasad, Y.G. 2023. PGPR: the treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. *World Journal of Microbiology and Biotechnology* **39**(4), 100. doi: 10.1007/s11274-023-03536-0

Naiman, A.D., Latrónico, A. & de Salamone, I.E.G. 2009. Inoculation of wheat with Azospirillum brasilense and Pseudomonas fluorescens: impact on the production and culturable rhizosphere microflora. *European Journal of Soil Biology* **45**(1), 44–51. doi: 10.1016/j.ejsobi.2008.11.001

Nakkeeran, S., Priyanka, R., Rajamanickam, S. & Sivakumar, U. 2020. *Bacillus amyloliquefaciens* alters the diversity of volatile and non-volatile metabolites and induces the expression of defence genes for the management of *Botrytis* leaf blight of Lilium under protected conditions. *Journal of Plant Pathology* **102**, 1179–1189. doi: 10.1007/s42161-020-00602-6

Ortiz, A. & Sansinenea, E. 2023. The possibility of using *Serratia* isolates for the production of biopreparations in the protection of plants against diseases and pests. *Archives of Microbiology* **205**(8), 288.

Ortiz, A., Sansinenea, E., Ilyas, N. & Sayyed, R.Z. 2022. Commercial and Technological Aspects of *Bacillus* spp. PGPR. In *Secondary Metabolites and Volatiles of PGPR in Plant-Growth Promotion* (pp. 277–288). Cham: Springer International Publishing. doi: 10.1007/978-3-031-07559-9_13

Paula, G.F., Demétrio, G.B. & Matsumoto, L.S. 2021. Biotechnological potential of soybean plant growth-promoting rhizobacteria. *Revista Caatinga* **34**(2), 328–338. doi: 10.1590/1983-21252021v34n209rc

Raklami, A., Bechtaoui, N., Tahiri, A.I., Anli, M., Meddich, A. & Oufdou, K. 2019. Use of rhizobacteria and mycorrhizae consortium in the open field as a strategy for improving crop nutrition, productivity and soil fertility. *Frontiers in Microbiology* **10**, 1106. doi: 10.3389/fmicb.2019.01106

Rosas, S.B., Avanzini, G., Carlier, E., Pasluosta, C., Pastor, N. & Rovera, M. 2009. Root colonization and growth promotion of wheat and maize by *Pseudomonas aurantiaca* SR1. *Soil Biology and Biochemistry* **41**(9), 1802–1806. doi: 10.1016/j.soilbio.2008.10.009

Saito, Y., Itakura, K., Kuramoto, M., Kaho, T., Ohtake, N., Hasegawa, H., Suzuki, T. & Kondo, N. 2021. Prediction of protein and oil contents in soybeans using fluorescence excitation emission matrix. *Food Chemistry* **365**, 130403. doi: 10.1016/j.foodchem.2021.130403

Santos, F.M., Viera, L.S., Camargo, D.P., Muniz, M.F., Costa, I.F., Guedes, J. V. & Silva, J.C. 2022. Integrating a *Bacillus*-based product with fungicides by foliar application to protect soybean: a sustainable approach to avoid exclusive use of chemicals. *Pest Management Science* **78**(11), 4832–4840. doi: 10.1002/ps.7104

Silva, M.A., Nascente, A.S., Filippi, M.C.C.D., Lanna, A.C., Silva, G.B.D. & Silva, J.F.A.E. 2020. Individual and combined growth-promoting microorganisms affect biomass production, gas exchange and nutrient content in soybean plants. *Revista Caatinga* **33**, 619–632. doi: 10.1590/1983-21252020v33n305rc

Silva, M.A., Nascente, A.S., Cruz, D.R.C., Frasca, L.L.De, M., Silva, J.F.A.E., Ferreira, A.L., Ferreira, E.P.De, B., Lanna, A.C., Bezerra, G.De, A. & Filippi, M.C.C. 2023. Initial development of upland rice inoculated and co-inoculated with multifunctional rhizobacteria. *Semina: Ciências Agrárias* **44**(1), 273–284. doi: 10.5433/1679-0359.2023v44n1p273.

Solanki, A.C., Gurjar, N.S. & Sharma, S. 2023. Co-inoculation of non-symbiotic bacteria bacillus and paraburkholderia can improve the soybean yield, nutrient uptake, and soil parameters. *Molecular Biotechnology*, **2023**, 1–13.

USDA. WASDE. World Soybean Supply and use. August, 2018. Available at: https://www.ers.usda.gov/publications/pub-details/?pubid=44093.

Vendruscolo, E.P. & de Lima, S.F. 2021. The Azospirillum genus and the cultivation of vegetables. A review. *Biotechnol. Agron. Soc. Environ* **25**(4), 236–246. doi: 10.25518/1780-4507.19175

Vicentini, S.N.C., de Carvalho, G., Krug, L.D., Nunes, T.C., da Silva, A.G., Moreira, S.I. & Ceresini, P.C. 2022. Bioprospecting fluorescent Pseudomonas from the Brazilian Amazon for the biocontrol of signal grass Foliar blight. *Agronomy* **12**(6), 1395. doi: 10.3390/agronomy12061395

Walia, A., Mehta, P., Chauhan, A. & Shirkot, C.K. 2014. Effect of Bacillus subtilis strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **84**, 145–155. doi: doi.org/10.1007/s40011-013-0189-3

Wu, H., Xiang, W., Chen, L., Ouyang, S., Xiao, W., Li, S. & Kuzyakov, Y. 2020. Soil phosphorus bioavailability and recycling increased with stand age in Chinese fir plantations. *Ecosystems* **23**, 973–988. doi: 10.1007/s10021-019-00450-1

Yagmur, B. & Gunes, A. 2021. Evaluation of the Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Quality Parameters of Tomato Plants in Organic Agriculture by Principal Component Analysis (PCA). *Gesunde Pflanzen* **73**(2). doi: 10.1007/s10343-021-00543-9

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The implementation of sustainable urban agriculture: response of mustard (*Brassica juncea* L.) towards planting media composition of top soil, biochar and manure at vertical farming

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Abstract. The study investigates the growth and yield response of mustard to different planting media of soil, biochar, and manure as planting media, within a vertical cultivation technique. Three-month research was carried out at the Screen House of Agroclimatology Laboratory, Faculty of Agriculture, Papua University, employing a Completely Randomized Design (CRD) with four treatments and triplicates. The results of the analysis of variance revealed that at 2 weeks after planting (WAP) period demonstrated a statistically significant effect of growing media composition on mustard height. Notably, Soil:Biochar (M1) treatment exhibited the highest plant height, surpassing Soil:Biochar:Manure (M3) treatment. Although biochar addition had an insignificant effect compared to control (M0) and Soil:Manure (M2) treatments, Soil: Biochar still achieved the greatest height. Further significance tests revealed that Soil:Biochar treatment recorded the longest leaf length, a measure not significantly different from Soil:Manure and Soil:Biochar:Manure treatments, but notably different from control which produced the shortest leaf length. Moreover, the Soil:Manure treatment presented the highest yield in terms of plant fresh weight compared to other treatments. While Soil:Biochar and Soil:Biochar:Manure treatments did not show statistical differences, their results were higher than control. In conclusion, the Soil:Manure treatment displayed the highest yield for plant dry weight and total plant weight per pot compared to other treatments, where control consistently performed the lowest. These findings underscore the efficacy of the Soil: Manure treatment in optimizing mustard growth and yield within a vertical cultivation system.

Key words: biochar, manure, mustard, planting media, urban agriculture, verticulture.

INTRODUCTION

Mustard (Brassica juncea L.) is a highly commercial horticultural crop (Tanwar & Goyal, 2021) with promising applications in the industrial sector (Fan et al., 2017). The growing population of Indonesia, coupled with an increasing awareness of nutritional needs, has led to a rise in demand for mustard. It is primarily consumed for its leaves or flowers, which contain significant nutritional value. According to Thomas et al. (2012), 100 g of mustard wet weight includes vitamin A (0.09 mg), vitamin B10 (2 mg), vitamin C (not specified), calcium (Ca) (220 mg), phosphorus (P) (38 g), iron (Fe) (2.9 g), protein (2.3 g), fat (0.3 g), and carbohydrates (4.0 g).

The rapid increase in Indonesia's annual population, as mentioned earlier, has exerted pressure on limited agricultural areas. Some agricultural lands have been transformed into industrial zones, residential areas, and offices, creating an opportunity for the intensive development of vertical cultivation. Verticulture represents a noteworthy example of urban farming, characterized by a vertical plant cultivation technique that arranges plants vertically to optimize land usage (Eigenbrod & Gruda, 2015; Beacham et al., 2019). In simpler terms, it involves farming by utilizing columns arranged vertically (Al-Chalabi, 2015; Beacham et al., 2019; Loman Jan Luciano, 2018). When verticulture is implemented, the number of plant populations in a given area can increase by 3 to 10 times compared to conventional agriculture, depending on the model or design of the planting media container. This vertical system is particularly well-suited for adoption by farmers or individuals with limited land who wish to cultivate numerous plants more sustainably (Barthel & Isendahl, 2013; Oh & Lu, 2023), contributing to future food security (Eigenbrod & Gruda, 2015).

One crucial factor significantly influencing the growth and production of plants in a vertical cultivation system is the composition of the growing media. The ideal planting media composition should effectively provide nutrients and ensure water availability to support the growth and production of plants (Landis et al., 1990). In simpler terms, the soil structure in the planting media must be sufficiently loose to allow flexibility for plant root growth. Typically, the composition of the planting media mixture can include various materials, although the common practice involves creating a mixture comprising loose topsoil, rice husk ash, and organic fertilizer.

In a vertical farming system, fertilization is a crucial factor to be considered for enhancing plant growth and production (De Bon et al., 2009; Orsini et al., 2013). Among the organic fertilizers, manure stands out. Manure encompasses the collective excrement of various animal species, urine, plant materials, straw, as well as residues from livestock feed and human household waste (Gross & Glaser, 2021). Notably, chicken manure boasts a relatively high nutrient composition compared to other types of manure. On average, chicken manure contains 55% H_2O, 1.00% N, 0.8% P_2O_5, and 0.42% K_2O (Gross & Glaser, 2021).

In addition to fertilization, in sustainable soil management (Kaur et al., 2020; Koul et al., 2022; Kumar & Bhattacharya, 2021), biochar is charcoal that is formed through the combustion process of organic matter without oxygen (pyrolysis) at a temperature of 250 °C–500 °C (Fang et al., 2015; Brassard et al., 2019). The use of biochar is being taken into account as it is capable of stimulating the activity of soil micro-organisms (Ladygina & Rineau, 2013; Rutigliano et al., 2014) and can increase soil aggregates (Kookana et al., 2011). Biochar is not consumed by microbes directly like other organic materials (Cross et al., 2016) and it does not disturb the carbon-nitrogen balance in the long run (Clough & Condron, 2010), and is even able to hold and allow more water and nutrients available to plants (Lin et al., 2022).

The availability and uptake of plant nutrients are strongly related to soil pH, with the organic matter and ash content in biochar playing a role in enhancing soil pH and quality, thereby improving nutrient utilization (Tsai & Chang, 2019). Moreover, depending on the nature of biochar production or feedstock, the organic matter component of biochar contains nitrogen, while the ash component contains phosphorus and potassium (Jindo, 2020). Additionally, biochar has a notable impact as negative ions result from oxidation and reduction reactions between biochar particles and oxygen in the soil. Consequently, biochar can effectively retain nutrients for plant uptake (Bolan et al., 2022; Joseph et al., 2010).

The presence of biochar has been shown to elevate microbial activity around plant roots (Ayaz et al., 2021; Murtaza et al., 2021). The fermentation process in biochar contributes to an increased surface area, acting as an attractant for microbes and extending their lifespan in organic matter. This, in turn, has additive effects on soil fertility and plant growth (Zhang et al., 2023). Through microbial activity and biochar absorption, biologically rich nutrients undergo breakdown (Kocsis et al., 2022) and are converted into readily available forms for plants (Rondon et al., 2007). Biochar can function as a nutrient source and serve as a fertilizer (Ding, 2016). Additionally, biochar has the potential to reduce leaching of nitrogen and other nutrients, thereby enhancing crop yields. It may also enhance microbial C-use efficiency and support the stability of active soil organic carbon fractions, promoting long-term carbon sequestration. Studies have demonstrated that the combined application of biochar and manure increases crop yields, as seen in the case of turnips (Nouar et al., 2019). Therefore, the addition of other inputs such as manure is a recommended approach to enhance the concurrent efficiency of using biochar. However, there is a lack of research on media composition under vertical farming specifically for mustard.

This study aims to determine the growth and yield of mustard (*Brassica juncea* L.) due to different composition of growing media on vertical cultivation techniques. While the benefit of the research is to provide information material those who are dealing with limited cultivated areas to increase crop production especially on mustard cultivation.

MATERIALS AND METHODS

Experimental site description

A 3-month experiment was conducted in 2022 at the Screen House of the Agroclimatology Laboratory, Faculty of Agriculture, Papua University, located in Manokwari, Indonesia. The research site is characterized by lowland topography, which is relatively uniform, and the soil exhibits characteristics of deep, well-drained, and calcareous alluvium soil, predominantly alkaline in reaction. The environmental conditions during the experiment included a mean temperature of 27.5 °C, humidity at 81.3%, and a total rainfall of 255.3 mm (Central Statistics Agency Manokwari Regency, 2023).

Experimental design and treatments

The materials utilized in the experiment included mustard seeds, chicken manure, biochar from coconut shells, top soil and sand. Mustard seeds, representing lowland varities, were obtained from commercial shops. The verticulture pots were arranged as a completely randomized design (CRD) comprising twelve experimental units with four treatments in three replicates. The treatments consisted of M0 : control (topsoil soil); and combination of treatments as follows: M1 : soil : biochar (1:1); M2 : soil : manure (1:1) and M3 : soil : biochar : manure (1:1:1).

Experiment procedures

The research implementation covered three stages. In the initial stage, we prepared the planting media by combining soil and manure. The soil used in the planting media composition was sourced from the top layer of soil (10 cm-depth). After removing grass and dirt, we horizontally hoed the soil, adhering to the top layer criteria, and subsequently cleared it of any large boulders before shifting it. The prepared manure was obtained in the form of ready-to-use chicken manure. Prior to application, the chicken manure underwent a cleaning process to remove unnecessary objects such as stones, wood, and plastic. It was the ground and sieved to achieve a finer texture for optimal utilization in the planting media.

Figure 1. PVC verticulture pots.

The second stage involved the construction of verticulture using paralon pipes (Fig. 1), following the method outlined by Werdhany (2012). This process included preparing a 6-inch paralon, divided into three parts, each measuring 4 m in length, resulting in three paralon verticulture pots with a total length of 130 cm. For each pipe, 6 rows of alternating holes were measured and marked, with a distance of 20 cm between each hole, totaling 24 holes. The use of high-quality PVC pipe, characterized by thickness and hardness, was emphasized. To create holes in the PVC pipe, a drill was employed, utilizing a round drill attachment and a ‘heat gun’. To prevent the planting medium from falling through, the tops of the holes were intentionally left attached and unremoved. For the drilled paralon to be securely positioned, it was recommended to soften it with a ‘heat gun’ before pressing it down into place. The heating process was focused on the specific area intended for indentation, ensuring that it became soft enough to be molded before pressing it into the desired shape.

In the third stage, we employed the kontiki method (Nurida et al., 2015) to produce biochar. This involved creating a conical hole with an upper diameter and depth of 150 cm and 75 cm, respectively, with the flexibility to adjust the diameter as needed. A fire was initiated at the bottom center of the hole using flammable materials, such as wood leftovers. In the kontiki model, oxygen is restricted from falling below the fire, allowing it to burn solely at the designated point of combustion. Gradually, coconut shells were added to the lit kontiki when the fire burned steadily at temperatures between 400–450 °C. New raw materials were introduced when almost all previously added materials had been burned. From a practical standpoint, burning using kontiki cannot be completed all at once; instead, raw materials are added gradually, little by little, according to the volume of the kontiki. This process continued until all the coconut shells were burnt. Once all the raw materials turned black into charcoal and there were no more flames, all burning coals were extinguished with water to prevent further burning. In a single trial lasting less than 1 hour, approximately 6 kg of coconut shell raw material produced biochar. After allowing it to cool sufficiently, the biochar was dried and ground as needed before application.

Cultural Practices

The nursery phase involved sowing mustard seeds in plastic trays filled with a mixed medium of soil and sand in a 1:2 ratio, adequately watered. The seeds were evenly spread at a depth of 1 cm and lightly covered with soil. Regular watering was maintained by using a hand sprayer once a day until the seeds were fully hydrated, and the seedlings were ready for transplantation into verticulture pots. For the experimental treatments, the planting media prepared according to specific treatments were placed in verticulture pots, left for a day to reach field capacity before growing seedlings. Planting was conducted simultaneously to ensure uniform plant growth, either 15 days after sowing or when two young leaves had emerged. The vertical planting technique was adjusted based on the number of holes created, with one selected seedling planted in each vertical hole. Consequently, each verticulture pot served as an experimental unit, comprising 24 mustard plants.

Plant maintenance activities during the experiment encompassed various tasks, including replanting, regular watering, weeding, and pest and disease control. Harvesting was carried out using two methods: uprooting the entire plant along with its roots and cutting the base of the above-ground stem. The harvesting phase occurred 30–40 days after planting (DAP), and well-grown plants in each pot could yield approximately 175 grams. Consistent cultural practices, such as daily watering using a sprayer, were uniformly implemented throughout the experiment to ensure optimal plant growth and development.

Data collection and Statistical Analysis

Observational variables, primarily growth parameters such as plant height and the number of leaves, were recorded between 14 to 35 days after planting for six sample plants within each experimental unit or paralon. Additionally, observations of leaf length and width were conducted as the plants approached the harvesting stage. After harvesting, the components of fresh and dry weights of both plant shoots and roots were meticulously observed and measured.

The collected data were then analysed using analysis of variance (Anova) at the 95% confidence level according to Fisher (1938) to calculated the nature and magnitude of treatment effects revealed by 'F'-test. If the effect of treatments are statistically significant ($p < 0.05$), means of different sources of variation were compared using Fisher's least significant difference (*LSD*) test.

RESULTS AND DISCUSSION

The analysis of variance of observed growth and yield components showed variation in the effect of planting media composition treatments on observed variables. The summary of analysis of variance is given in Table 1.

Table 1. Recapitulation of analysis of variance of observed variables

No	Observed variable	F-statistics	Notation	Coefficient of Variance (%)
1	Plant Height 1 WAP (cm)	3.61	ns	20.68
2	Plant Height 2 WAP (cm)	6.26	*	11.23
3	Plant Height 3 WAP (cm)	1.69	ns	17.31
4	Plant Height 4 WAP (cm)	2.93	ns	11.94
5	Plant Height 5 WAP (cm)	3.06	ns	11.21
6	Leaf Number 1 WAP	16.22	**	4.48
7	Leaf Number 2 WAP	2.79	ns	5.44
8	Leaf Number 3 WAP	15.71	**	8.82
9	Leaf Number 4 WAP	5	*	9.67
10	Leaf Number 5 WAP	6.57	*	5.88
11	Leaf Length (cm)	9.35	**	8.96
12	Leaf Width (cm)	2.44	ns	13.83
13	Leaf Area (cm^2)	6.99	*	15.99
14	Plant Fresh Weight (g)	22.34	**	17.70
15	Plant Dry Weight (g)	2.55	ns	25.45
16	Plant Total Weight per Verticulture Pot (g)	2.68	ns	35.44

Notes: ns – Treatments had no significant effect ($p > 0.05$); * – Treatments had significant effect ($p \leq 0.05$); ** – Treatments had significant effect ($p \leq 0.01$); WAP – Weeks After Planting.

Growth Components

Plant growth is the process in plant life that leads to changes in size and influences plant yields. The expansion of plant organ systems is attributed to the growth of plant organs through development and an increase in cellular tissue (Hamant & Traas, 2010).

Fig. 2 illustrates that the planting media composition had no effect during the 1st, 3rd, 4th, and 5th WAP, but a significant effect was observed at 2 WAP. During the early growth stages at 1 and 3 WAP, the control treatment (M0) exhibited the highest plant height, surpassing other treatments and indicating superior initial plant growth. The Soil:Biochar (M1) treatment also demonstrated a more favorable contribution than the other two treatments. However, by 4 and 5 WAP, the Soil:Manure (M2) treatment exhibited a tendency to achieve greater plant height.

The composition of planting media had a significant effect on the height of mustard at 2 WAP. Soil:Biochar gave the highest plant height compared to Soil:Biochar:Manure (M3), although it was not statistically different as compared to control and Soil:Manure treatments. This is presumably because biochar is able to retain and store water as a supporting factor on the initial growth of plants. In addition, biochar also plays a role in providing essential nutrients.

The planting media composition exerted a significant influence on mustard's height at 2 WAP. While Soil:Biochar treatment resulted in the highest plant height compared to Soil:Biochar:Manure, the difference was not statistically significant when compared to control and Soil:Manure treatments. This outcome is likely attributed to the water retention capabilities of biochar (Razzaghi et al., 2020), providing crucial support for the initial growth of plants. Additionally, biochar plays a dual role by supplying essential nutrients and enhancing the physical, chemical, and biological properties of the soil, as indicated by previous studies (Brassard et al., 2019; Murtaza et al., 2021) and to improve and restore the quality of degraded soil as well (Barrow, 2012; Abhishek et al., 2022).

Supporting this, the addition of biochar to soil enhances nutrient availability (Cao et al., 2018), retention (Clough & Condron, 2010), and water retention (Ajayi et al., 2016). For instance, biochar has been noted to enhance the efficiency of nitrogen fertilizer use in plants (Chan et al., 2007).

Plant Height

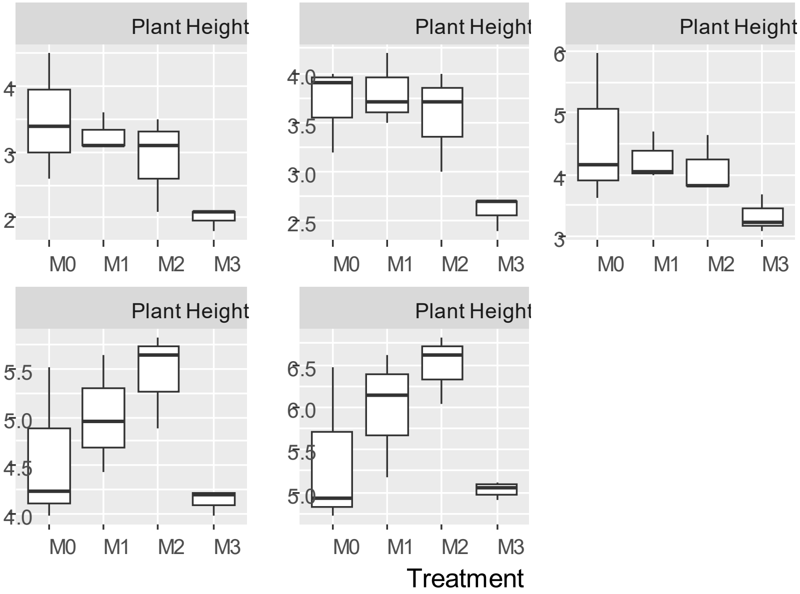

Figure 2. Means of Plant Height due to the Application of Different Planting Media Composition in the Verticulture Technique.

Furthermore, both control and Soil:Manure treatments demonstrated a positive contribution to plant height gain. This may be attributed to the application of the Soil:Manure treatment, which proved effective in providing the necessary nutrients for plant growth. Specifically, decomposed chicken manure in the Soil:Manure treatment contributed macro nutrients such as nitrogen and phosphorus, essential for cell division and the subsequent increase in plant height.

In relation to nutrient content, chicken manure is reported to contain three times more nitrogen than other manures, as highlighted by Aziz et al. (2020). Additionally, phosphorus plays a crucial role in synthesizing ATP as an energy source and serves as a precursor for DNA and RNA, essential nucleic acid compounds. ATP, functioning as an energy source, is vital for cell division and elongation, contributing to increased plant height. Moreover, phosphorus actively promotes cell division, particularly in root organs. The heightened cell division facilitated by the availability of phosphorus has a positive impact on shoot growth, as the development of plant shoots and roots is interconnected. This underscores the significance of phosphorus in fostering overall plant growth and height.

Our result of analysis of variance (Table 1) showed that the composition of planting media had a significant effect on number of plant leaves at 1, 3, 4 and 5 WAP and had no effect at 2 WAP. Based on further tests, Soil:Biochar:Manure treatment gave the best results statistically compared to other treatments at 1 and 3 WAP. At 4 and 5 WAP, Soil:Biochar:Manure treatment gave higher yields than control but did not significantly different from Soil:Biochar and Soil:Manure treatments. The combined treatment of Soil:Biochar:Manure gave the best results for number of leaves (Fig. 3). Nutrients contained in chicken manure are mainly macro nutrients, namely N, P and K where N is needed for vegetative growth of the upper part of plant. Element K to strengthen stem and P to stimulate root growth (Fageria & Moreira, 2011). Manure works synergistically with biochar in supporting the increase in size of mustard. Kookana et al. (2011) suggested that biochar is able to improve fertility by making fertilization more effective, where biochar can bind nutrients, therefore plants can avoid micro-nutrient poisoning and nutrient deficiencies. Another advantage is that biochar is more persistent in the soil (Abhishek et al., 2022), thus all the benefits related on improving soil fertility can work well when organic fertilizers decompose.

Leaf Number

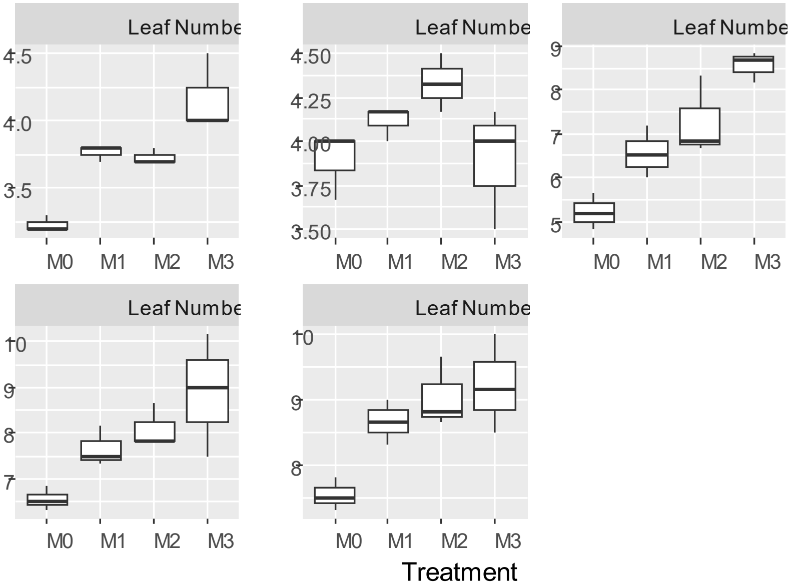

Figure 3. Means of Leaf Number due to the Application of Different Planting Media Composition in Verticulture Technique.

The treatment of planting media composition greatly influenced leaf length of mustard and affected leaf area, but did not affect leaf width (Fig. 4). Post hoc analysis showed that Soil:Biochar treatment gave the longest leaf length which was not different from Soil:Manure and Soil:Biochar:Manure treatments, whereas it was different from control which produced the shortest leaf length. Furthermore, for the leaf area variable, Soil:Manure treatment revealed the widest leaf area followed by Soil:Biochar:Manure treatment and the lowest was expressed by control treatment. In terms of leaf width, regardless the fact that planting media composition showed no statistical effect, there was a tendency that Soil:Biochar:Manure treatment recorded higher leaf width and control treatment contributed the lowest leaf area. Control treatment gave the lowest yield since top soil without being enriched with organic matter and biochar had a minimal role in providing nutrients and water.

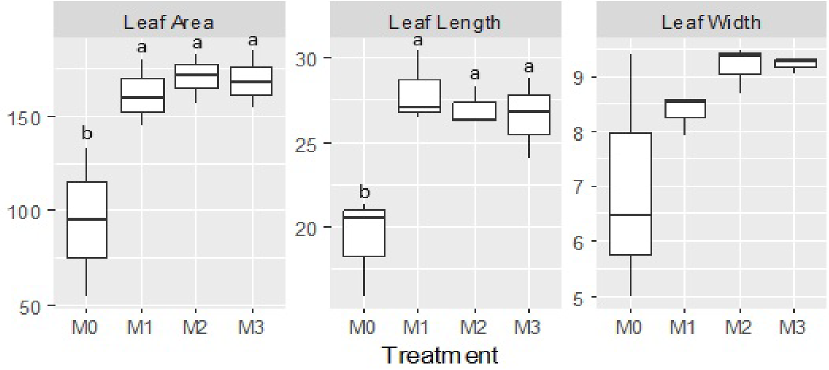

Figure 4. Means of Length, Width and Leaf Area of Mustard Due to Differences of Planting Media Composition in Verticulture Technique; different letters in a same letter indicate significant differences (*p* < 0.05) between treatments according to Fishers Protected; *LSD* test.

Yield Components

Yield component refers to weight of plants observed during harvest including fresh weight, dry weight and total weight per pot as presented in Fig. 5.

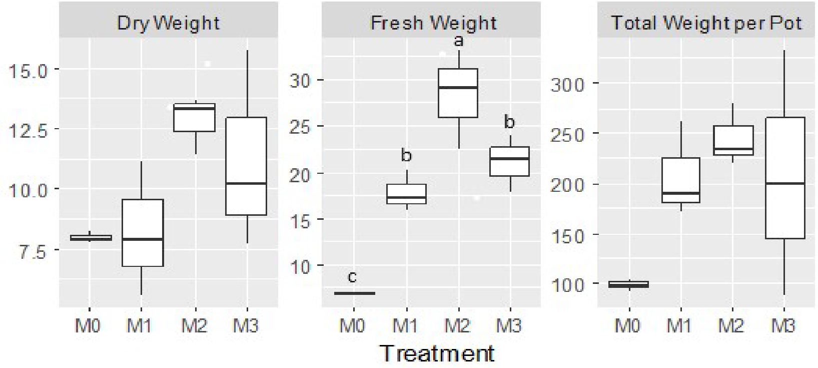

Figure 5. Means of Fresh, Dry and Total Weight per Pot of Mustard Due to Differences of Planting Media Composition in Verticulture Technique; different letters in a same letter indicate significant differences (*p* < 0.05) between treatments according to Fishers Protected; *LSD* test.

Among three variables of plant weight, different growing media composition showed a statistically significant on plant fresh weight (Table 1). From the results of significance test, Soil:Manure treatment gave the highest yield on plant fresh weight compared to other treatments, where Soil:Biochar and Soil:Biochar:Manure treatments were not statistically different, although the results were higher than control treatment. It can be assumed that Soil:Manure treatment independently gave the highest results for plant dry weight and total plant weight per pot compared to other treatments, where control treatment consistently performed the lowest.

Plant fresh weight was positively correlated to plant dry weight and total plant weight per pot. The greater the value of fresh weight, the greater dry weight and total weight per pot are. Soil:Biochar:Manure treatment can simultaneously improve soil fertility, triggered to the availability of nutrients, to promote growth and production because there are macro nutrients playing a vital role. Nutrient content of chicken manure is 1.5% N; 1.3%P; 0.8%K; 4% CaO, 9–11% C/N ratio and contains 57% moisture content (McCall, 1980).

In addition, fresh chicken manure composed of secondary and micronutrients in fresh chicken manure, where calcium and magnesium are in the largest proportion and molybdenum and zinc are the least (Wall & Plunkett, 2021), has higher nutrients than other types of livestock. This is because solid manure in livestock is mixed with liquid manure. Besides containing the nutrients needed by plants, based on the analysis found existing bacteria *lactobacillus archidophilus, lactobacillus mesenteroides* and *streptococcus thermophilus,* small portion of *actynomycetes* and fungi in chicken manure (Li et al., 2020). The presence of bacteria greatly assists decomposition process of organic matter perfectly with good quality, causing nutrients provision for plants as well as physical, chemical and biological properties improvement of the soil (Fries et al., 2005). The application of chicken manure can improve environmental conditions for plant growth (Nyakatawa et al., 2000; Adekiya et al., 2020) which in turn can increase yield (Pujiastuti et al., 2018; Zahanis et al., 2023).

The role of biochar when synergizing with other inputs is to maximize nutrient absorption in plant roots (Mate et al., 2015) which are translocated to shoots. In plant shoots, these nutrients are processed into growth compounds and transported to all parts of plant (Hall et al., 1993). In addition, humus acid from applied soil organic matter has a high cation exchange capacity, ranging from 150–300 m per 100 g and a surface area of 800–900 m^2 per g. The high cation exchange capacity and surface area of humus increase the availability of nutrients and water for plants and reduce soil acidity (Joseph et al., 2010). The availability of sufficient nutrients and water will lead to effective photosynthesis in the formation of carbohydrates, increasing plant growth rate, indicated by a heavier wet weight.

Biochar and chicken manure together act as organic matter which can improve the physical and chemical properties of soil and can also increase number and activity of soil microorganisms. One of the benefits of using biochar is to create a proper habitat for microbial development (Ladygina & Rineau, 2013; Saxena et al., 2013) and symbiotic microorganisms such as mycorrhizae because of their ability to hold water and air and create a neutral environment, especially in acid soils (Mohan et al., 2014; Jeffery et al., 2017). Neonbeni et al. (2020) proved that the application of biochar 5 tons ha^{-1} gave the best plant height, stem diameter, number of leaves, leaf area, flower diameter on cauliflower (*Brassica oleraceae* L.). The application of biochar 9 tons ha^{-1} alone was able to increase plant height, number of leaves, leaf area, fresh weight per plant, dry weight per plant, plant weight per plot. The application of biochar has real potential to improve several soil chemical properties such as soil pH, CEC (Li et al., 2020), and several compounds such as C-organic, N-total, and can reduce the activity of Fe and Al compounds which have an impact on increasing available P (Semita et al., 2017). Other studies confirmed that Rondon et al. (2007) the use of biochar can increase nitrogen fixation (Bolan et al., 2022), improve growth and increase plant yields. Moreover, the combination of biochar with plant residue and non-essential nutrient input enhanced Oryza sativa (L.) growth and yield (Widiasri et al., 2022).

Chicken manure is recognized as a rich source of both macro and micro nutrients, as indicated by Ahmad et al. (2009). Its application has been shown to enhance soil fertility and improve the physical characteristics of soil, as highlighted by Šařec & Žemličková (2016). Moreover, chicken manure serves as a substrate for soil microorganisms, promoting increased microbial activity and accelerating decomposition, as observed in studies by Ilodibia & Chukwuma (2015) and Li et al. (2020).

The high nitrogen (N) content in chicken manure contributes to vigorous vegetative plant growth, leading to substantial gains. This elevated N content also enhances protein levels in the soil, promoting the synthesis of amino acids and proteins in plants. Petit (2004) suggests that when organic fertilizers like chicken manure are applied to the soil, the organic matter breaks down into inorganic compounds through the activity of decomposing microorganisms. This breakdown improves soil structure, making it more porous and facilitating easier nutrient absorption by plants. Several studies support the positive impact of chicken manure on leaf vegetable plants. Anwar et al. (2017), for instance, reported that co-composted manure significantly improved the growth and nutrient availability of spinach. In this context, the application of chicken manure led to variations in dry biomass, phosphorus (P), and potassium (K) contents in spinach shoots. However, nitrogen (N), zinc (Zn), iron (Fe), copper (Cu), and cadmium (Cd) contents in spinach shoots decreased with increasing amounts of leaf litter in the manure amendment. These findings underscore the complex and multifaceted effects of chicken manure on plant growth and nutrient dynamics.

CONCLUSIONS

Based on the findings from the study on the response of mustard growth and yield to various planting media compositions using verticulture techniques, it can be concluded that the treatments did not have a significant effect on plant height, leaf width, plant dry weight, and total weight per pot across all observation times. However, there were effects on the number of leaves, leaf length, leaf area, and fresh weight based on the planting media composition.

Notably, the Soil:Biochar:Manure treatment exhibited the highest number of leaves, while the Soil:Manure treatment showed the best results in terms of leaf area and plant fresh weight. As a recommendation for future research, further experiment into different compositions of biochar and other biosolids in verticulture techniques is suggested. This could provide valuable insights into optimizing plant growth and yield in verticulture techniques.

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REFERENCES

Abhishek, K., Shrivastava, A., Vimal, V., Gupta, A.K., Bhujbal, S.K., Biswas, J.K., Singh, L., Ghosh, P., Pandey, A., Sharma, P. & Kumar, M. 2022. Biochar application for greenhouse gas mitigation, contaminants immobilization and soil fertility enhancement: A state-of-the-art review. *Science of the Total Environment* **853**(June), 158562. https://doi.org/10.1016/j.scitotenv.2022.158562

Adekiya, A.O., Ejue, W.S., Olayanju, A., Dunsin, O., Aboyeji, C.M., Aremu, C., Adegbite, K. & Akinpelu, O. 2020. Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of okra. *Scientific Reports* **10**(1), 1–9. https://doi.org/10.1038/s41598-020-73291-x

Ahmad, A.A., Fares, A., Abbas, F. & Deenik, J.L. 2009. Nutrient concentrations within and below root zones from applied chicken manure in selected Hawaiian soils. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes* **44**(8), 828–843. https://doi.org/10.1080/03601230903238723

Ajayi, A.E., Holthusen, D. & Horn, R. 2016. Changes in microstructural behaviour and hydraulic functions of biochar amended soils. *Soil and Tillage Research* **155**, 166–175. https://doi.org/10.1016/j.still.2015.08.007

Al-Chalabi, M. 2015. Vertical farming: Skyscraper sustainability? *Sustainable Cities and Society*, **18**, 74–77. https://doi.org/10.1016/j.scs.2015.06.003

Anwar, Z., Irshad, M., Mahmood, Q., Hafeez, F. & Bilal, M. 2017. Nutrient uptake and growth of spinach as affected by cow manure co-composted with poplar leaf litter. *International Journal of Recycling of Organic Waste in Agriculture* **6**(1), 79–88. https://doi.org/10.1007/s40093-017-0154-x

Ayaz, M., Feizienė, D., Tilvikienė, V., Akhtar, K., Stulpinaitė, U. & Iqbal, R. 2021. Biochar role in the sustainability of agriculture and environment. *Sustainability (Switzerland)* **13**(3), 1–22. https://doi.org/10.3390/su13031330

Aziz, A., Khan, B.A., Tahir, M.A., Nadeem, M.A., Amin, M.M., Qura-Tul-Ain, Adnan, M., Munawar, N., Hussain, A., Khisham, M., Toor, M.D. & Sultan, M. 2020. Effect of poultry manure on growth and yield of forage sorghum (Sorghum bicolor L.) International Journal of Botany Studies Effect of poultry manure on growth and yield of forage sorghum (Sorghum bicolor L.). *International Journal of Botany Studies* **5**(June), 401–406. https://www.researchgate.net/publication/342231552

Barrow, C.J. 2012. Biochar: Potential for countering land degradation and for improving agriculture. *Applied Geography* **34**, 21–28. https://doi.org/10.1016/j.apgeog.2011.09.008

Barthel, S. & Isendahl, C. 2013. Urban gardens, Agriculture, And water management: Sources of resilience for long-term food security in cities. *Ecological Economics* **86**, 224–234. https://doi.org/10.1016/j.ecolecon.2012.06.018

Beacham, A.M., Vickers, L.H. & Monaghan, J.M. 2019. Vertical farming: a summary of approaches to growing skywards. *Journal of Horticultural Science and Biotechnology* **94**(3), 277–283. https://doi.org/10.1080/14620316.2019.1574214

Bolan, N., Hoang, S.A., Beiyuan, J., Gupta, S., Hou, D., Karakoti, A., Joseph, S., Jung, S., Kim, K.H., Kirkham, M.B., Kua, H.W., Kumar, M., Kwon, E.E., Ok, Y.S., Perera, V., Rinklebe, J., Shaheen, S.M., Sarkar, B., Sarmah, A.K., … Van Zwieten, L. 2022. Multifunctional applications of biochar beyond carbon storage. *International Materials Reviews* **67**(2), 150–200. https://doi.org/10.1080/09506608.2021.1922047

Brassard, P., Godbout, S., Lévesque, V., Palacios, J.H., Raghavan, V., Ahmed, A., Hogue, R., Jeanne, T. & Verma, M. 2019. Biochar for soil amendment. In *Char and Carbon Materials Derived from Biomass: Production, Characterization and Applications*. https://doi.org/10.1016/B978-0-12-814893-8.00004-3

Cao, Y., Gao, Y., Qi, Y. & Li, J. 2018. Biochar-enhanced composts reduce the potential leaching of nutrients and heavy metals and suppress plant-parasitic nematodes in excessively fertilized cucumber soils. *Environmental Science and Pollution Research* **25**(8), 7589–7599. https://doi.org/10.1007/s11356-017-1061-4

Central Statistics Agency Manokwari Regency. 2023. *Weather Condition*. Weather Report. https://manokwarikab.bps.go.id/subject/153/iklim.html#subjekViewTab3

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A. & Joseph, S. 2007. Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research* **45**(8), 629–634. https://doi.org/10.1071/SR07109

Clough, T.J. & Condron, L.M. 2010. Biochar and the Nitrogen Cycle: Introduction. *Journal of Environmental Quality* **39**(4), 1218–1223. https://doi.org/10.2134/jeq2010.0204

Cross, A., Zwart, K., Shackley, S. & Ruysschaert, G. 2016. The role of biochar in agricultural soils. *Biochar in European Soils and Agriculture: Science and Practice*, pp.73–98. https://doi.org/10.4324/9781315884462

De Bon, H., Parrot, L. & Moustier, P. 2009. Sustainable urban agriculture in developing countries: A review. *Sustainable Agriculture* **30**, 619–633. https://doi.org/10.1007/978-90-481-2666-8_38

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L. & Zheng, B. 2016. Biochar to improve soil fertility. A review. *Agronomy for Sustainable Development* **36**(2). https://doi.org/10.1007/s13593-016-0372-z

Eigenbrod, C. & Gruda, N. 2015. Urban vegetable for food security in cities. A review. *Agronomy for Sustainable Development* **35**(2), 483–498. https://doi.org/10.1007/s13593-014-0273-y

Fageria, N.K. & Moreira, A. 2011. The Role of Mineral Nutrition on Root Growth of Crop Plants. In *Advances in Agronomy* (1st ed., Vol. 110, Issue C). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385531-2.00004-9

Fang, Y., Singh, B. & Singh, B.P. 2015. Effect of temperature on biochar priming effects and its stability in soils. *Soil Biology and Biochemistry* **80**, 136–145. https://doi.org/10.1016/j.soilbio.2014.10.006

Fisher, R. 1938. *The Statistical Utilization of Multiple Measurements*. Annals of Eugenics.

Fries, R., Akcan, M., Bandick, N. & Kobe, A. 2005. Microflora of two different types of poultry litter. *British Poultry Science* **46**(6), 668–672. https://doi.org/10.1080/00071660500395483

Gross, A. & Glaser, B. 2021. Meta - analysis on how manure application changes soil organic carbon storage. *Scientific Reports* **11**(5516), 1–13. https://doi.org/10.1038/s41598-021-82739-7

Hall, D.O., Scurlock, J.M.O., Bolhar-Nordenkampf, H.R., Leegood, R.C. & Long, S.P. 1993. *Photosynthesis and Production in A Changing Environment: A field an laboratory manual*. Springer Science + Business Media, 152 pp.

Hamant, O. & Traas, J. 2010. The mechanics behind plant development. *New Phytologist* **185**(2), 369–385. https://doi.org/10.1111/j.1469-8137.2009.03100.x

Ilodibia, C. V. & Chukwuma, M. U. 2015. Effects of application of different rates of poultry manure on the growth and yield of tomato (Lycopersicum esculentum Mill.). *Journal of Agronomy* **14**(4), 251–253. https://doi.org/10.3923/ja.2015.251.253

Jeffery, S., Memelink, I., Hodgson, E., Jones, S., van de Voorde, T.F.J., Martijn Bezemer, T., Mommer, L. & van Groenigen, J.W. 2017. Initial biochar effects on plant productivity derive from N fertilization. *Plant and Soil* **415**(1–2), 435–448. https://doi.org/10.1007/s11104-016-3171-z

Jindo, K., Audette, Y., Higashikawa, F.S., Silva, C.A., Akashi, K., Mastrolonardo, G., Sánchez-Monedero, M.A. & Mondini, C. 2020. Role of biochar in promoting circular economy in the agriculture sector. Part 1: A review of the biochar roles in soil N, P and K cycles. *Chemical and Biological Technologies in Agriculture* **7**(1), 1–12. https://doi.org/10.1186/s40538-020-00182-8

Joseph, S.D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., Van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P., Lehmann, J., Foidl, N., Smernik, R.J. & Amonette, J.E. 2010. An investigation into the reactions of biochar in soil. *Australian Journal of Soil Research* **48**(6–7), 501–515. https://doi.org/10.1071/SR10009

Kaur, H., Kumar, A. & Choudhary, A. 2020. *Biochar : A Sustainable Approach for Improving Soil Fertility* **1**(4), 68–70.

Kookana, R.S., Sarmah, A.K., Van Zwieten, L., Krull, E. & Singh, B. 2011. Biochar application to soil : agronomic and environmental benefits and unintended consequences. In *Advances in Agronomy* (1st ed., Vol. **112**). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385538-1.00003-2

Koul, B., Yakoob, M. & Shah, M.P. 2022. Agricultural waste management strategies for environmental sustainability. *Environmental Research* **206**(May 2021), 112285. https://doi.org/10.1016/j.envres.2021.112285

Kumar, A. & Bhattacharya, T. 2021. Biochar: a sustainable solution. *Environment, Development and Sustainability* **23**(5), 6642–6680. https://doi.org/10.1007/s10668-020-00970-0

Ladygina, N. & Rineau, F. 2013. *Biochar and soil biota*. In CRC Press Taylor & Francis Group. https://doi.org/10.1201/b14585

Landis, T.D., Jacobs, D.F., Wilkinson, K.M. & Tara Luna. 1990. *Tropical Nursery Manual-Chapter 6 Growing Media*.

Li, X., Zhao, X., Yang, J., Li, S., Bai, S. & Zhao, X. 2020. Recognition of core microbial communities contributing to complex organic components degradation during dry anaerobic digestion of chicken manure. *Bioresource Technology* **314**(June), 123765. https://doi.org/10.1016/j.biortech.2020.123765

Lin, S., Wang, W., Sardans, J., Lan, X., Fang, Y., Singh, B.P., Xu, X., Wiesmeier, M., Tariq, A., Zeng, F., Alrefaei, A.F. & Peñuelas, J. 2022. Effects of slag and biochar amendments on microorganisms and fractions of soil organic carbon during flooding in a paddy field after two years in southeastern China. *Science of the Total Environment* **824**. https://doi.org/10.1016/j.scitotenv.2022.153783

Loman Jan Luciano. 2018. Vertical Farming Can it change the global food production landscape? *TIAA-CREF Global Agriculture* **1601**.

Mate, C.J., Mukherjee, I. & Das, S.K. 2015. Persistence of spiromesifen in soil: influence of moisture, light, pH and organic amendment. *Environmental Monitoring and Assessment* **187**(2). https://doi.org/10.1007/s10661-014-4207-6

McCall, W.W. 1980. Chicken Manure. HAWAII COOPERATIVE EXTENSION SERVICE. College of Tropical Agriculture and HUman Resources University of Hawaii. (Issue General Home Garden Series No. **2**, pp. 1–2).

Mohan, D., Sarswat, A., Ok, Y.S. & Pittman, C.U. 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent - A critical review. *Bioresource Technology* **160**, 191–202. https://doi.org/10.1016/j.biortech.2014.01.120

Murtaza, G., Ahmed, Z., Usman, M., Tariq, W., Ullah, Z., Shareef, M., Iqbal, H., Waqas, M., Tariq, A., Wu, Y., Zhang, Z. & Ditta, A. 2021. Biochar induced modifications in soil properties and its impacts on crop growth and production. *Journal of Plant Nutrition* **44**(11), 1677–1691. https://doi.org/10.1080/01904167.2021.1871746

Neonbeni, E.Y., Ceunfin, S. & Mau, T.T. 2020. The Effect of Rice Husk Biochar and The Composition of Chicken Film on Growth and Results of Flower Cubes (Brassica oleraceae, L.). *Dryland Conservation Agriculture Journal* **5**(4). https://doi.org/10.32938/sc.v5i04.640

Nouar, S., Baha, M., Latati, M., Djebbar, R. & Reguieg, L. 2019. Short-term effect of sawdust biochar and bovine manure on the physiological behavior of turnip (Brassica rapa L.) grown in open fields in the algiers region. *Agronomy Research* **17**(6), 2373–2385. https://doi.org/10.15159/AR.19.210

Nyakatawa, E.Z., Reddy, K.C. & Mays, D.A. 2000. Tillage, cover cropping, and poultry litter effects on cotton: II. Growth and yield parameters. *Agronomy Journal* **92**(5), 1000–1007. https://doi.org/10.2134/agronj2000.9251000x

Oh, S. & Lu, C. 2023. Vertical farming - smart urban agriculture for enhancing resilience and sustainability in food security. *Journal of Horticultural Science and Biotechnology* **98**(2), 133–140. https://doi.org/10.1080/14620316.2022.2141666

Orsini, F., Kahane, R., Nono-Womdim, R. & Gianquinto, G. 2013. Urban agriculture in the developing world: A review. *Agronomy for Sustainable Development* **33**(4), 695–720. https://doi.org/10.1007/s13593-013-0143-z

Pujiastuti, E.S., Tarigan, J.R., Sianturi, E. & Ginting, B.B. 2018. The effect of chicken manure and beneficial microorganisms of EM-4 on growth and yield of kale (Brassica oleraceae acephala) grown on Andisol. *IOP Conference Series: Earth and Environmental Science* **205**(1). https://doi.org/10.1088/1755-1315/205/1/012020

Razzaghi, F., Obour, P.B. & Arthur, E. 2020. Does biochar improve soil water retention? A systematic review and meta-analysis. *Geoderma* **361**(November 2019), 114055. https://doi.org/10.1016/j.geoderma.2019.114055

Rondon, M.A., Lehmann, J., Ramírez, J. & Hurtado, M. 2007. Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. *Biology and Fertility of Soils* **43**(6), 699–708. https://doi.org/10.1007/s00374-006-0152-z

Rutigliano, F.A., Romano, M., Marzaioli, R., Baglivo, I., Baronti, S., Miglietta, F. & Castaldi, S. 2014. Effect of biochar addition on soil microbial community in a wheat crop. *European Journal of Soil Biology* **60**, 9–15. https://doi.org/10.1016/j.ejsobi.2013.10.007

Šařec, P. & Žemličková, N. 2016. Soil physical characteristics and soil-tillage implement draft assessment for different variants of soil amendments. *Agronomy Research* **14**(3), 948–958.

Saxena, J., Rana, G. & Pandey, M. 2013. Impact of addition of biochar along with bacillus sp. on growth and yield of french beans. *Scientia Horticulturae* **162**, 351–356. https://doi.org/10.1016/j.scienta.2013.08.002

Semita, I.K., Sujana, I.P. & Suryana, I.M. 2017. Brassica Juncea L.) Pada Lahan Yang Tercemar Limbah Cair. *Agrimeta*, **7**(14), 26–30.

Tanwar, B. & Goyal, A. 2021. Oilseeds: Health Attributes and Food Applications. In *Oilseeds: Health Attributes and Food Applications*. https://doi.org/10.1007/978-981-15-4194-0

Thomas, J., Kuruvilla, K.M. & Hrideek, T.K. 2012. Mustard. *Handbook of Herbs and Spices: Second Edition* **1**, 388–398. https://doi.org/10.1533/9780857095671.388

Tsai, C.C. & Chang, Y.F. 2019. Carbon dynamics and fertility in biochar-amended soils with excessive compost application. *Agronomy* **9**(9). https://doi.org/10.3390/agronomy9090511

Wall, D. & Plunkett, M. 2021. *Major and micro nutrient advice for productive agricultural crops*. **5**, 51–53. http://hdl.handle.net/11019/2475

Widiasri, E., Maulani, R.R., Nofitasari, D., Lambangsari, K., Manurung, R. & Abduh, M.Y. 2022. Enrichment of growing media using biochar, compost, and nanosilica for the cultivation of Oryza sativa L. *Agronomy Research* **20**(Special Issue I), 1175–1186. https://doi.org/10.15159/AR.22.061

Zahanis, Siska & Ermawati. 2023. The Effect of Chicken Cage Fertilizer with Dosage of Waste Shrimp on Growth and Production of Sweet Corn (Zea mays saccharata Sturt). *Eksakta Berkala Ilmiah MIPA Universitas Taman Siswa Padang Indonesia* **24**(01), 56–66.

Zhang, L., Tsui, T.H., Wah Tong, Y., Sharon, S., Shoseyov, O. & Liu, R. 2023. Biochar applications in microbial fermentation processes for producing non-methane products: Current status and future prospects. *Bioresource Technology* **386**(June), 129478. https://doi.org/10.1016/j.biortech.2023.129478

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Behera, K.B. & Varma, A. 2019. *Bioenergy for Sustainability and Security*. Springer International Publishing, Cham, pp. 1–377.

- **For articles in a journal**

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Doddapaneni, T.R.K.C., Praveenkumar, R., Tolvanen, H., Rintala, J. & Konttinen, J. 2018. Techno-economic evaluation of integrating torrefaction with anaerobic digestion. *Applied Energy* **213**, 272–284. doi: 10.1016/j.apenergy.2018.01.045

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