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Water deficit stress alleviation by bio-formulated native mycorrhizal species for wheat grown in a saline calcareous soil

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Abstract. Arbuscular mycorrhizal fungi (AMF) are a genus of obligatory root biotrophs that can develop mutualistic symbioses with most terrestrial plants. This study aimed to investigate the impact of three different isolates of AMF (*Acaulospora spinosa* [M1], *Glomus ambisporum* [M2], and *Scutellospora heterogama* [M3]) isolated from native environments and three carriers (biochar, alginate, and polyacrylate) on wheat (*Triticum aestivum* L.) grown in a saline calcareous soil in conditions of water deficit. In a pot experiment, reduced amounts of water were applied at intervals of 4, 8, and 12 days, while in a field experiment, the intervals were 1, 2, and 3 weeks (W1, W2, and W3). By analyzing the chlorophyll index and dry weight data from the pot experiment, it was revealed that two AMF isolates (M1 and M2), along with two carriers (biochar and alginate), showed promising results in stimulating wheat growth. Based on these findings, a field validation experiment was conducted to further evaluate the effects of these isolates and carriers. The wheat plants subjected to water deficit stress exhibited improved vegetation characteristics, grain yield, nutrient uptake, and colonization percentage when treated with the AMF isolate M2 formulated on biochar. For instance, under W2 conditions without any mycorrhiza or carrier, the grain yield was recorded at 6,600 kg ha^{-1}. However, with the inoculation of M2-biochar at the same W2 level, the yield significantly increased to 9110 kg ha^{-1}. The study concluded that AMF formulated on biochar outperformed other carriers, leading to enhanced wheat growth under water stress conditions.

Key words: drought stress, arbuscular mycorrhizal fungi, biocompatible carriers, biochar, alginate, wheat yield.

INTRODUCTION

Wheat (*Triticum aestivum* L.) serves as a staple food worldwide, providing sustenance for approximately one-third of the global population (Acevedo et al., 2018; Grote et al., 2021). However, the occurrence of drought has a significant impact on wheat production, leading to severe consequences for both global food security and economic stability (Daryanto et al., 2016; Leng & Hall, 2019; Pequeno et al., 2021). The growth and development of wheat plants are intricately linked to the availability of water, as it plays a crucial role in various physiological processes such as photosynthesis, nutrient uptake, and protein synthesis (Torrion & Stougaard 2017; Plett et al., 2020). When wheat

plants are exposed to prolonged drought conditions, they experience a range of detrimental effects, including reduced leaf area, impaired stomatal conductance, diminished photosynthetic activity, and altered carbohydrate metabolism (Lonbani & Arzani, 2011; Ahmad et al., 2018; Zhang et al., 2018; Pour-Aboughadareh et al., 2020). Consequently, these stress-induced changes lead to decreased yield and compromised grain quality, posing substantial challenges for farmers and food systems worldwide.

Microbial interactions with plants play a crucial role in our ecosystem. These interactions, as highlighted by Barea et al. (2005) and acknowledged by numerous scientists, can provide valuable support to crop plants during periods of acute stress. One promising approach to enhance plant adaptation strategies involves increasing the presence of arbuscular mycorrhizal fungi (AMF) in soils (Plouznikoff et al., 2016; Diagne et al., 2020). AMF, when established in root cortical cells, actively participate in nutrient exchange, leading to improved plant growth and productivity (Park et al., 2015; Heydarian et al., 2018; Bhantana et al., 2021). Moreover, AMF can mitigate the negative effects of drought by reducing water and nutrient loss, thereby sustaining plant growth and restoring leaf moisture levels (Borriello et al., 2012; Bahadur et al., 2019). The most important mechanisms by which symbiosis can alleviate drought stress in host plants are direct water uptake through the fungal hyphae, changes in soil water holding properties, better osmotic adjustment of plants, enhancement of water-use efficiency, and protection against oxidative damage caused by severe water deficiency (Barea et al., 2011; Ruiz-Lozano et al., 2011; Borde et al., 2017).

Due to environmental limitations and the challenges posed by the short shelf life of inoculum, the adoption of bio-fertilizers by farmers has been relatively low compared to other methods. Consequently, the utilization of specific materials, known as carriers, becomes crucial in facilitating microbial growth and ensuring effective delivery to the rhizosphere (Zafar-ul-Hye et al., 2019). These carriers can be derived from either organic sources such as compost, crushed corn, peat biochar, or inorganic sources like lignite (Zafar-ul-Hye et al., 2019). However, it is imperative that these carrier materials possess certain characteristics. They should be readily available, cost-effective, physically and chemically stable, non-toxic to plant growth-promoting microbes, biodegradable, free from pollutants, and easy to process (Pacheco-Aguirre et al., 2017; Moghadam et al., 2018; Sohaib et al., 2020). To maintain the efficacy of AMF in the field, gel encapsulation techniques have been employed to preserve the isolated vesicles, internal hyphae, or spores of AMF. These techniques have proven effective in enabling AMF to renew hyphae even under adverse conditions (Herrmann & Lesueur, 2013; Pitaktamrong et al., 2018).

Only a limited number of studies have conducted pairwise comparisons between various encapsulation methods. Among these studies, the effects of AMF inoculation in soil, both with and without Na-alginate encapsulated AMF, have been extensively surveyed (Schütz et al., 2018; Basiru et al., 2020). However, there is currently a dearth of information regarding the impact of biochar on soil microbial communities, despite its known role in enhancing soil fertility (Lehmann et al., 2011). The objectives of this study were to investigate the effects of three native AMF isolated from abiotic environments on the growth rate and production of wheat in saline calcareous soils under water deficit conditions. This was accomplished by manipulating irrigation intervals and reducing water amounts. Furthermore, the study aimed to assess the influence of three different carriers (biochar, alginate, and polyacrylate) on the performance of drought-

stressed wheat plants. These carriers were chosen based on their potential to improve water retention and nutrient availability in the soil.

MATERIALS AND METHODS

Isolation and purification of mycorrhizal spores

Soil samples were gathered from the root zones of indigenous plants that thrive in the harshest conditions found across various locations in the West Delta of Egypt. These plants predominantly consisted of wild species that have flourished without any human intervention. These locations, namely El-Khatatba, South El-Tahrir, El-Bostan, North El-Tahrir, and El-Nobaria, are characterized by arid climates, where visible signs of salinization and degradation are evident. At each location, a total of five soil samples were carefully collected from a depth of 15–30 cm. These samples predominantly consisted of calcareous sandy soils with elevated salinity levels. To facilitate further analyses, the collected samples, along with the corresponding plant roots, were promptly transferred to the laboratory.

In the laboratory, sieving in wet condition and decanting technique, as described by Gerdemann & Nicolson (1963), was employed to separate the coarse particles of the soil while retaining mycorrhiza spores and organic particles on sieves of different sizes. Under a dissecting microscope (model SZX9, Olympus, Tokyo, Japan), the spores were carefully selected using a pipette. Morphological identification of the spores was conducted, including measurements of their size and thickness of wall layers, following the methodology outlined by Trappe (1982). To maintain AMF propagules (spores/hyphae/colonized root pieces), the samples taken from cultures that are in the late or stationary phase of growth have been used. These samples were mixed with alginate or biochar, and then dried out before cryopreservation for the utilization in the pot and field experiments.

Treatments and experimental design

A pot experiment consisting of three replicates was conducted in the greenhouse of the Desert Research Center in Cairo during the 2019/2020 season. The experiment followed a completely randomized design. The identified isolates were *Acaulospora spinosa* (M1), *Glomus ambisporum* (M2), and *Scutellospora heterogama* (M3). The three AMF species (M1, M2, M3) in addition to control (M4) were formulated on three different carriers, namely alginate (C1), polyacrylate (C2), biochar (C3), and soil (C4). Treatments also included three levels of irrigation (every 4, 8, and 12 days; W1, W2, and W3, respectively) in low amounts of water to induce further drought stress. For the pot experiment, large plastic polyethylene pots measuring 30 cm in diameter and 35 cm in depth were used. These pots were chosen specifically for their suitability in conducting the research. To ensure consistency, each pot was filled with 10 kg of air-dried soil obtained from the surface layer of the Mariout Research Station in the West Delta region of Egypt. This soil was carefully selected as the field experiment will be implemented in this location in the subsequent season. To sow the seeds, 15 grains of wheat from the Giza 171 variety were distributed into each pot. After a period of three weeks, the plants had grown sufficiently, and it was necessary to thin them out. Eight plants were retained in each pot, ensuring that they had the best chance of thriving and producing accurate results.

The validation field experiment was conducted at the Mariout Research Station in the West Delta region of Egypt during the 2020/2021 season. The experiment location being at altitude of 15 metres above sea level and is located in latitude 31° 0' 12.2" N and longitude 29° 47' 3.0" E. According to the FAO classification, the soil in this area is categorized as Haplic Xerosols (FAO, 1998). The average rainfall in this area during the growing season was around 140 mm $year^{-1}$, mostly in the months of December-February. The average temperature during the growing season ranged between 10 and 20 °C. The main plots were divided into three irrigation intervals: 1, 2, and 3 weeks, with reduced amounts of water. Normally, a hectare requires 5,200 m^3 of water (W1), but for the purpose of this experiment, it was limited to 3,800 m^3 for W2 and 2,800 for W3. The sub-plots were assigned to three different carriers: biochar, alginate, and a control group. The sub-sub-plots were assigned to three AMF as M1, M2, and M3 (control).

Before sowing wheat, the soil was ploughed twice, levelled, before dividing into 3×5 m plots (15 m^2 for sub-sub plots, 45 m^2 for subplots, and 135 m^2 for main plots). Wheat (Giza 171, the most often used variety in the study area) was sown in early November at a seeding rate of 155 kg ha^{-1} (15.5 gm m^{-1}) and harvested in mid-April. A total of 500 gm of beads, consisting of various carriers such as alginates, polyacrylate, and biochar, were utilized for field inoculation. Each plot was enriched with 180 entrapped propagules through the application of these beads during sowing. It is important to note that this particular wheat belongs to the spring type. In accordance with the general recommendations, P fertilizer (60 kg P_2O_5 ha^{-1} as single superphosphate) and K fertilizer (50 kg K_2O ha^{-1} as potassium sulphate) were applied. Standard techniques were applied to control weeds, insects, and diseases.

Optical sensor and chlorophyll meter measurements

The atLEAF chlorophyll meter (FT Green LLC, Wilmington, DE, USA) and the GreenSeeker optical sensor (Trimble, Sunnyvale, CA, USA) were used to measure the spectral properties of wheat leaves at the Feekes 6^{th} growth stage (stem elongation growth stage). This growth stage, which corresponds to 30 on the BBCH scale and occurs approximately 50 days after sowing, was selected based on the research conducted by Ali (2020) and Ali et al. (2021). These studies have identified this particular stage as the optimal time for gathering crucial information and making informed management decisions for wheat crops. Chlorophyll meter readings were obtained from the pot experiment, while both sensors were utilized in the field experiment to gather data. This approach was adopted because the GreenSeeker optical sensor requires canopy volume to collect data, unlike the atLEAF meter which measures readings from a specific spot on the leaf.

The atLEAF chlorophyll meter was used to assess the chlorophyll index, the meter measures light transmittance through the leaf at two different wavelengths of 660 and 940 nm to give a single indicative index. The highest fully expanded leaf was utilized to gather the atLEAF readings by inserting the middle section of the leaf into the meter's slit. Measurements were documented from 10 plants in each plot, and average values were computed. Spectral reflectance of the canopy was measured using the GreenSeeker active optical sensor and expressed as a normalized difference vegetation index (NDVI), the sensor has a red (656 nm) and near infrared (774 nm) self-illumination system. The GreenSeeker measurements were conducted by traversing the plot at an approximate

speed of 0.5 m s^{-1} while maintaining a sensor height of approximately 1 m above the canopy.

Assessment of mycorrhizal characteristics in the field experiment

At harvest, rhizosphere soil samples were collected to determine the characteristics of mycorrhizal spores. Mycorrhizal colonization was assessed using a composite root sample from three plants in the centre of each plot. Mycorrhizal colonization in roots was evaluated by washing and staining of 1-cm root segment cut from the plant. Phillips & Hayman's (1970) staining procedure was used to prepare root samples for microscopic examination.

The gridlines intersect approach developed by Giovannetti & Mosse (1980) was also used to calculate the percentage of mycorrhizal colonization. The formula used for calculating mycorrhizal colonization percentage is:

$$Colonization\ percentage = \frac{Number\ \ of\ positive\ intersect\ points}{Total\ number\ of\ observed\ intersect\ points} \times 100 \quad (1)$$

Soil and plant sampling in the field experiment

Samples from the surface layer of the experimental soil (0–30 cm depth) were collected, mixed, air dried, ground, sieved through a 2 mm sieve, and analysed for several physical and chemical properties prior to sowing, as shown in Table 1. Particle size distribution in soil samples was determined using the pipette technique (Page et al., 1982). Soil pH and electrical conductivity (EC) were measured according to Page et al., 1982. The Walkley & Black (1934) method was used to determine soil organic matter.

Soil cation exchange capacity (CEC) was determined using the ammonium acetate-saturation method (Page et al., 1982). A calcimeter was used to determine the total calcium carbonate content. Soil available N was extracted using a 2 m KCl solution, and then determined using the micro-Kjeldahl method as described by Bremner (1965). Available P and K were extracted by 1 m NH_4HCO_3 in 0.005 m DTPA adjusted to a pH of 7.6. In a spectrophotometer (Pye unican SP1900), P was colorimetrically determined using ascorbic acid and ammonium molybdate, while K was measured using a flame photometer (CL 387).

Table 1. Some physical and chemical properties of the topsoil layer (0–30 cm depth) of the experimental site

Soil characteristics	Values
Sand, %	57.2
Silt, %	26.5
Clay, %	16.3
Texture class	Sandy loam
Saturation percentage, %	35.4
Cation exchange capacity, $cmol_{(+)}$ kg^{-1}	11.5
pH*	8.43
EC**, dS m^{-1}	8.91
$CaCO_3$, %	22.4
Organic matter, %	0.94
Available N, mg kg^{-1}	62.1
Available P, mg kg^{-1}	8.9
Available K, mg kg^{-1}	218

* pH in saturated soil paste. ** Electrical conductivity in saturated soil paste extract.

Aggregate stability was determined as described by Kemper & Rosenau (1986). The soil samples were collected from each plot after harvest and air-dried before determining aggregate stability. The aggregate stability was then determined using the sieving and re-sieving in dry and wet conditions by the following equation:

$$Stable\ aggregate\ (Soil\ >\ 0.25\ mm) = \frac{Weight\ of\ stable\ soil\ aggregates}{Total\ weight\ of\ soil\ samples} \times 100 \quad (2)$$

In mid–April, when the wheat crop reached maturity, it was manually harvested from each plot's net area of 4 m^2. To separate the grains from the straw, a small thresher was used, and the resulting grains were weighed. Additionally, samples were taken for further analysis. To ensure consistency, the collected samples were dried in a hot air oven at 70 °C until they reached a consistent weight. The grain yield was adjusted to 14% moisture content per hectare for reporting. Subsequently, the samples were ground and prepared for analysis. The samples were digested in an H_2SO_4–H_2O_2 mixture, and the total N, P, and K contents were determined according to Kalra (1998). The nutrient uptake was calculated by multiplying the nutrient concentration with dry matter and dividing by 100.

Data processing

Microsoft Excel was utilized for conducting mathematical calculations. According to Gomez & Gomez (1984), the effects of treatments on the collected data were examined using analysis of variance (ANOVA). To assess the disparities between means, the Least Significant Difference (*LSD*) test was employed at a significance level of $P \leq 0.05$. The software utilized for statistical analysis in this study is Statistix 9.0.

RESULTS AND DISCUSSION

Effects of AMF and drought stress on plants in the potted plant experiment

The duration of irrigation intervals had a significant (P-value ≤ 0.05) effect on all measurements as shown in Table 2. For example, the chlorophyll index value in the control treatment (M4C4) in W1 (irrigation every 4 days) was 34.1, but dropped to 20.25 in W3 (irrigated every 12 days). A similar pattern was also observed in wheat dry weight. Irrigation intervals of W1, W2, and W3 resulted in dry weights of 12.2, 8.76, and 7.83 g pot^{-1}, respectively.

When different treatments (either alone or in combination) were used, significant effects on wheat parameters were recorded (Table 2). In M1, the highest chlorophyll index was observed in biochar carrier, followed by alginate, polyacrylate, then the control in W1 irrigation regime. In M2, the highest chlorophyll index was observed in alginate, followed by biochar, control and polyacrylate. The difference between biochar, control, and polyacrylate, on the other hand, is not statistically significant. In M3, the highest chlorophyll index was observed in polyacrylate, followed by biochar, alginate, and control. The differences between these carriers are not statistically significant. In M4, the highest chlorophyll index was observed in biochar, followed by control, alginate, and polyacrylate. The differences in these values are not statistically significant, with the exception of biochar and polyacrylate that indicated a significant difference.

Table 2. Effect of different AMF species bio-formulated on different carriers on chlorophyll index and dry weight of wheat in the pot experiment under different water regimes

Mycorrhizae (M)	Carrier (C)	Chlorophyll index			Dry weight (g pot^{-1})		
		Water regime*			Water regime		
		W1	W2	W3	W1	W2	W3
M1 (*Acaulospora* sp.)	Alginate (C1)	60.54	36.08	29.82	26.30	21.92	17.04
	Polyacrylate (C2)	54.52	29.97	33.93	26.97	21.52	16.12
	Biochar (C3)	69.54	33.39	27.73	29.36	25.37	19.71
	Control (C4)	34.39	34.66	29.52	18.23	17.32	14.82
M2 (*Glomus* sp.)	Alginate (C1)	50.38	37.15	31.60	26.39	21.60	14.71
	Polyacrylate (C2)	38.21	34.79	23.88	25.48	23.54	15.65
	Biochar (C3)	43.18	42.18	28.02	27.39	19.35	18.73
	Control (C4)	40.63	39.24	23.86	17.60	18.65	15.70
M3 (*Scutellospora* sp.)	Alginate (C1)	36.60	37.11	31.98	19.44	15.86	11.99
	Polyacrylate (C2)	39.86	37.28	26.11	18.47	14.97	12.16
	Biochar (C3)	38.72	41.60	36.09	21.73	16.01	14.16
	Control (C4)	36.2	41.05	35.1	15.64	13.90	9.65
M4 (No-mycorrhizae)	Alginate (C1)	33.47	33.43	23.03	15.68	13.59	10.77
	Polyacrylate (C2)	30.04	30.92	20.51	16.69	11.41	9.90
	Biochar (C3)	35.81	35.51	21.14	18.36	14.24	10.87
	Control (C4)	34.10	34.83	20.25	12.20	8.76	7.83
LSD W (*P*-value < 0.05)		1.68			1.04		
LSD m (*P*-value < 0.05)		1.21			0.65		
LSD C (*P*-value < 0.05)		1.33			0.62		
LSD W×M (*P*-value < 0.05)		2.45			1.41		
LSD W×C (*P*-value < 0.05)		2.5846			1.3759		
LSD M×C (*P*-value < 0.05)		2.6094			1.2558		
LSD M×W×C (*P*-value < 0.05)		5.0419			2.3808		

* The water regimes in the pot experiment were irrigation every 4 (W1), 8 (W2), and 12 (W3) days.

Pertaining to wheat dry matter, the highest values were observed in biochar carrier, followed by polyacrylate, alginate, and control in W1 irrigation regime under AMF isolate M1. For AMF isolate M2, the highest dry matter was the one observed in biochar, followed by alginate, polyacrylate and the control.

The increasing in irrigation intervals, or the wheat plants exposed to drought stress, have a significant detrimental influence on wheat growth and productivity. In the pot experiment, the performance of both biochar and alginate (carriers) was better than polyacrylate for wheat dry weight. Findings also suggest that AMF (M1 and M2) isolates were superior than M3 in the tested parameters. Data suggest that AMF isolates (M1 and M2) when using biochar and alginate as carriers gave better data in chlorophyll index values. These are in consistent with those recorded by Nirmala & Selvaraj (2011) and Pitaktamrong et al. (2018), who reported that alginate can be employed as a possible carrier of AMF, and promotes colonization, provides resilience to drought stress. Encapsulation of living cells in polymeric gels, such as alginate, is a well-established technology with a wide and expanding variety of uses (Park & Change, 2000). However, biochar can provide a safe refuge for colonizing fungal and bacterial communities, with a protection from natural soil predators (Warnock et al., 2007) and improvement of both chemical and biological soil properties (Pandian et al., 2016). Biochar with AMF is reported to improve the overall growth and plant yields (Aggangan et al., 2019).

Effect of the bio-formulated AMF on the vegetation growth and grain yield of wheat in the field experiment

Table 3 illustrates the influence of AMF, carrier, and water regime on chlorophyll index, NDVI, and grain yield of wheat in the field experiment. As expected, increase in irrigation intervals (from one week to three weeks) had a significant impact on all measured parameters. The chlorophyll index and NDVI values in the absence of AMF (control) were 34.66 and 0.41 in W1 (every week), significantly decreased to 31.65 and 0.34 in W2 (every two weeks) and 20.92 and 0.16 in W3 (every three weeks), respectively. This resulted in a significant reduction in the grain yield of wheat as well, the grain yield free of AMF was 8243 kg ha^{-1} in W1, decreased to 6,600 kg ha^{-1} in W2 and dropped to 3,390 kg ha^{-1} in W3.

The use of the two isolates of AMF along with the two types of carriers have a significant effect (P-value ≤ 0.05) on vegetation growth and grain yield of wheat (Table 3). In M1W1, the highest chlorophyll index and NDVI was observed using biochar as a carrier, followed by alginate, but they were statistically different. Grain yield took the same trend, as biochar and alginate were the statistically highest values, followed by the control. In W1M2, the chlorophyll index and NDVI were statistically higher in biochar treatment, followed by alginate, and the control.

In W2M1, the chlorophyll index and NDVI were statistically similar in biochar experiment and alginate, but higher than control. Grain yields in biochar and alginate were also statistically similar, but higher than control. In W2M2, the chlorophyll index and NDVI were statistically highest in biochar, followed by alginate, then control.

In W3M1, the chlorophyll index and NDVI in biochar and alginate were statistically similar, but higher than control. However, grain yield in all treatments were statistically similar. In W3M2, biochar gave the statistically highest chlorophyll index and NDVI, followed by alginate, and then control. However, grain yields in all treatments were statistically similar, ranging from 4,906 to 4,500 kg ha^{-1}. In W3M3, biochar and alginate gave statistically similar chlorophyll index and NDVI values, but higher than control. However, grain yields in all treatments were statistically similar, ranging from 3,686 to 3,390 kg ha^{-1}.

In the field experiment, the single effect of AMF/carrier types were examined, the average chlorophyll index and NDVI for AMF M1 were higher than others. The grain yield for M1 and M2 were nearly similar, and higher than the control. For the tested carriers, the overall average of biochar gave the statistical highest values of chlorophyll index and NDVI, followed by alginate, then the control. A similar pattern also observed on grain yield, as biochar gave the highest productivity, followed by alginate. These data emphatically reflect that AMF isolate (M2) and the carrier (biochar) may interact with better results expected.

An accurate analysis of the data revealed that the M2-biochar combination could reduce drought stress caused by irrigation intervals (1–3 weeks) on wheat grain yield. Under W2 the control (free of AMF and carrier), the grain yield was 6,600 kg ha^{-1}. Inoculation with M2-biochar at the same conditions, the value raised to 9,110 kg ha^{-1}. Following the inoculation with M2-biochar increased grain yield of 4,906 kg ha^{-1} compared with 3,390 kg ha^{-1} for the control by inoculation with M2-biochar.

Table 3. Effect of different AMF species bio-formulated on different carriers on chlorophyll index, normalized difference vegetation index (NDVI) and grain yield of wheat in the field experiment under different water regimes

Water regime (W)*	Mycorrhizae (M)	Carrier (C)	Chlorophyll index	NDVI	Grain yield (kg ha^{-1})
W1	M1 (*Acaulospora* sp.)	Alginate (C1)	56.62	0.72	9,303
		Biochar (C2)	57.58	0.79	9,600
		Control (C3)	40.48	0.46	7,623
	M2 (*Glomus* sp.)	Alginate (C1)	51.99	0.75	9,500
		Biochar (C2)	59.98	0.80	9,780
		Control (C3)	45.25	0.47	7,496
	M3 (control)	Alginate (C1)	51.52	0.50	7,956
		Biochar (C2)	52.96	0.51	8,243
		Control (C3)	34.66	0.41	7,050
W2	M1 (*Acaulospora* sp.)	Alginate (C1)	37.66	0.65	9,398
		Biochar (C2)	39.22	0.65	9,120
		Control (C3)	28.72	0.33	6,966
	M2 (*Glomus* sp.)	Alginate (C1)	37.68	0.65	8,950
		Biochar (C2)	42.94	0.70	9,110
		Control (C3)	27.65	0.29	6,776
	M3 (control)	Alginate (C1)	30.63	0.32	6,880
		Biochar (C2)	31.65	0.34	7,586
		Control (C3)	23.05	0.27	6,600
W3	M1 (*Acaulospora* sp.)	Alginate (C1)	24.31	0.23	4,956
		Biochar (C2)	25.65	0.26	4,890
		Control (C3)	21.26	0.21	4,600
	M2 (*Glomus* sp.)	Alginate (C1)	25.71	0.23	4,793
		Biochar (C2)	28.14	0.29	4,906
		Control (C3)	22.48	0.20	4,500
	M3 (control)	Alginate (C1)	22.34	0.19	3,686
		Biochar (C2)	23.98	0.20	3,540
		Control (C3)	19.92	0.16	3390
LSD W (*P*-value < 0.05)			2.61	0.0122	495.4
LSD m (*P*-value < 0.05)			0.71	0.0068	383.2
LSD C (*P*-value < 0.05)			0.47	0.0076	374.6
LSD W×M (*P*-value < 0.05)			2.79	0.0154	729.1
LSD W×C (*P*-value < 0.05)			2.69	0.0161	716.9
LSD M×C (*P*-value < 0.05)			0.98	0.0128	653.5
LSD M×W×C (*P*-value < 0.05)			2.97	0.0226	1,301.2

* The water regimes in the field experiment were irrigation every 1 (W1), 2 (W2), and 3 (W3) weeks in reduced amounts of water.

The soil in this study is a highly saline calcareous soil, which may create unfavourable conditions for AMF growth and development, in addition to the effect of drought stress. Changes in climate, complex soil composition, predation by soil microfauna, and competition amongst better-adapted native microflora, all pose serious challenges to the inoculated microorganisms' survival and viability (Bashan et al., 2014; Tao et al., 2018). Thus, immobilising these cells in an appropriate carrier, such as biochar, may improve tolerance to such challenges and protect cells from indigenous soil microorganisms (Chuaphasuk & Prapagdee, 2019).

Sajedi et al. (2010) previously supported the idea that AMF improves water efficiency in drought-stressed plants, this was later explained by Zou et al. (2015), who owed such improvement to active extraradical AMF mycelium in the soil. The composition of growth-promoting rhizobacteria with biochar found to have beneficial effects on the experimental crops, particularly in agriculturally hard environments such as water scarcity (Nadeem et al., 2017), seasonal differences (Ijaz et al., 2019), and drought (Egamberdieva et al., 2017). Yooyongwech et al. (2019) reported that for maize (*Zea mays* L.) plants, the alginate-AMF type of encapsulation may perform better than the agar-mycorrhiza type, and resulted in superior development under water-limited conditions.

Effect of the bio-formulated AMF on nutrient uptake in the field experiment

Table 4 lists the influence of AMF, carrier, and water regime on N, P, and K uptake by wheat. For the single effect of AMF isolate, the overall N, P, and K uptake in M1 were 215.4, 54.1, and 123.6 kg ha^{-1}, respectively. In M2, these nutrients were 239.2, 56.2, and 151.7 kg ha^{-1}, whereas in M3 (without AMF) were 149.8, 32.7, and 78.8 kg ha^{-1}, respectively.

Regarding the single effect of carrier types, the overall N, P, and K uptake in biochar was the highest even if at par with alginate for N and K uptake. In alginate, these nutrients were 219.1, 50.3, and 129.1 kg ha^{-1}, whereas in control were 165.9, 37.2, and 96.8 kg ha^{-1}, respectively. Biochar is statistically higher in N uptake, but at par with alginate in N and K uptake. The control exhibited statistically lowest values of N, P, and K uptake.

Data revealed that the M2-biochar interaction could reduce drought stress caused by varying irrigation intervals on nutrient uptake. Under W2 in M3-control (without AMF and carrier), N, P, and K uptake were 129.7, 28.4, and 73.6 kg ha^{-1}, respectively. However, with the inoculation with M2-biochar at the same W2 conditions, these values turned to be 177.6, 39.4, and 89.2 kg ha^{-1}, respectively. Under W3 conditions, which can be regarded as severe drought, the M3-control gave N, P, and K values of 57.7, 12.3, 34.4 kg ha^{-1}, respectively. However, the inoculation with M2-biochar at the same W3 conditions increased the N, P, and K uptake values to be 123.5, 17.0, and 63.6 kg ha^{-1}, respectively. This shows the positive impact of the treatments of alleviating the drought negative effects of nutrient uptake.

Ameloot et al. (2015) and Solaiman et al. (2019) showed that biochar application boosted AMF activity. Since the biochar amendment improves nutrient availability and retention in a variety of soils (Yadav et al., 2019), as well as contributing to the enhancement of other physical and biological soil parameters (Igalavithana et al., 2016; Novák et al., 2020; Zhang et al., 2020) and in metal retention (Xing et al., 2020). Positive biochar-AMF interacting effects on beans (*Phaseolus vulgaris* L.) P uptake were found by Vanek & Lehmann (2015) when sparsely soluble Fe-P was paired with biochar. In this regard, Solaiman et al. (2019) linked AMF increases to inadequate soil nutrient availability.

Table 4. Effect of different AMF species bio-formulated on different carriers on N, P, and K uptake by wheat at harvest in the field experiment under different water regimes

Water regime (W)*	Mycorrhizae (M)**	Carrier (C)	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})
W1	M1 (*Acaulospora* sp.)	Alginate (C1)	309.5	85.3	157.6
		Biochar (C2)	300.5	98.9	151.7
		Control (C3)	226.0	66.7	128.3
	M2 (*Glomus* sp.)	Alginate (C1)	326.8	90.0	228.6
		Biochar (C2)	341.7	92.6	207.9
		Control (C3)	213.0	64.1	126.3
	M3 (control)	Alginate (C1)	228.5	48.8	114.4
		Biochar (C2)	244.4	56.2	122.9
		Control (C3)	214.1	42.3	116.2
W2	M1 (*Acaulospora* sp.)	Alginate (C1)	291.1	64.4	205.5
		Biochar (C2)	263.7	64.2	173.6
		Control (C3)	180.8	37.3	116.6
	M2 (*Glomus* sp.)	Alginate (C1)	299.1	63.3	197.4
		Biochar (C2)	329.5	77.5	248.2
		Control (C3)	249.5	51.1	170.9
	M3 (control)	Alginate (C1)	172.4	36.2	96.5
		Biochar (C2)	177.6	39.4	89.2
		Control (C3)	129.7	28.4	73.6
W3	M1 (*Acaulospora* sp.)	Alginate (C1)	131.0	25.4	66.6
		Biochar (C2)	136.8	29.5	71.1
		Control (C3)	99.5	15.2	41.6
	M2 (*Glomus* sp.)	Alginate (C1)	133.5	23.4	59.7
		Biochar (C2)	135.8	27.0	63.2
		Control (C3)	123.5	17.0	63.6
	M3 (control)	Alginate (C1)	65.3	16.3	35.3
		Biochar (C2)	58.8	14.1	26.6
		Control (C3)	57.7	12.3	34.4
LSD W (*P*-value < 0.05)			15.7	10.7	12.3
LSD m (*P*-value < 0.05)			6.3	7.6	5.8
LSD C (*P*-value < 0.05)			5.2	3.8	11.4
LSD W×M (*P*-value < 0.05)			18.0	15.1	13.6
LSD W×C (*P*-value < 0.05)			17.2	11.9	12.4
LSD M×C (*P*-value < 0.05)			9.7	9.3	12.7
LSD M×W×C (*P*-value < 0.05)			21.8	17.1	19.4

* The water regimes in the field experiment were irrigation every 1 (W1), 2 (W2), and 3 (W3) weeks in reduced amounts of water.

Effect of the bio-formulated AMF on the soil aggregation and mycorrhizal colonization in the field experiment

In Table 5, influence of AMF, carrier, and water regime on each of spores count, colonization percentage, and soil aggregates are recorded. For the single effect of AMF isolate: overall spores count, colonization percentage, and soil aggregates in M1 were 52.7 spores 100 g^{-1}, 58.7%, and 24.1%, respectively. For isolate M2, the spores count was higher, but values were at par with M1 in colonization percentage and soil aggregates. For isolate M3 (control) all recorded values were lower.

Table 5. Effect of different AMF species bio-formulated on different carriers on spores count, colonization percentage, and soil aggregates in the wheat field experiment under different water regimes

Water regime (W)*	Mycorrhizae (M)**	Carrier (C)	Spore count (spores 100 g^{-1})	Colonization percentage (%)	Soil aggregates (%)
W1	M1 (*Acaulospora* sp.)	Alginate (C1)	77.1	77.3	35.1
		Biochar (C2)	79.8	79.3	32.4
		Control (C3)	43.4	66.2	23.7
	M2 (*Glomus* sp.)	Alginate (C1)	78.9	80.9	25.5
		Biochar (C2)	100.2	81.5	34.2
		Control (C3)	66.8	72.5	32.1
	M3 (control)	Alginate (C1)	45.9	50.2	23.6
		Biochar (C2)	45.8	36.4	21.3
		Control (C3)	41.7	30.7	21.1
W2	M1 (*Acaulospora* sp.)	Alginate (C1)	55.7	61.7	23.5
		Biochar (C2)	60.3	66.5	23.1
		Control (C3)	38.4	48.9	19.7
	M2 (*Glomus* sp.)	Alginate (C1)	60.5	64.2	23.0
		Biochar (C2)	66.4	65.5	22.4
		Control (C3)	39.0	51.7	19.8
	M3 (control)	Alginate (C1)	61.7	32.1	20.1
		Biochar (C2)	65.2	33.8	20.4
		Control (C3)	39.9	22.0	19.9
W3	M1 (*Acaulospora* sp.)	Alginate (C1)	41.8	45.8	21.4
		Biochar (C2)	46.0	46.1	19.4
		Control (C3)	32.1	36.2	18.5
	M2 (*Glomus* sp.)	Alginate (C1)	45.0	40.0	15.4
		Biochar (C2)	43.9	43.4	14.9
		Control (C3)	35.9	35.0	14.1
	M3 (control)	Alginate (C1)	30.0	25.2	14.2
		Biochar (C2)	29.6	23.1	12.3
		Control (C3)	25.7	19.1	12.9
LSD W (*P*-value < 0.05)			16.2	18.4	2.8
LSD m (*P*-value < 0.05)			10.3	11.7	2.8
LSD C (*P*-value < 0.05)			10.7	15.0	3.4
LSD W×M (*P*-value < 0.05)			21.7	22.4	4.9
LSD W×C (*P*-value < 0.05)			21.9	15.6	5.6
LSD M×C (*P*-value < 0.05)			18.3	21.2	5.6
LSD M×W×C (*P*-value < 0.05)			35.9	39.4	9.5

* The water regimes in the field experiment were irrigation every 1 (W1), 2 (W2), and 3 (W3) weeks in reduced amounts of water.

The AMF isolate M2-biochar changed the most spores, counting 100.2 spores 100 g^{-1} under W1 conditions. The highest colonization percentage was that recorded also for AMF isolate M2-biochar which statistically little higher than the one recorded under W1 conditions for AMF isolate M2-alginate. The highest soil aggregates were in AMF isolate M1-alginate, which statistically at par with those recorded under W1 conditions in treatments: AMF isolate M2-biochar and AMF isolate M2-control. The AMF isolate

M2-biochar interaction, generally, resulted in overall improved values for drought stress relief.

Regarding the root colonization, the findings seem to be in line with those recorded by Videgain-Marco et al. (2021) who found that biochar addition boosted AMF root colonization, number of spores, and the infective potential of indigenous AMF. Also, biochar addition is very recalcitrant and capable of improving soil characteristics through affecting both biochemical and biological processes (Arif et al., 2017; Song et al., 2019). However, few studies, on the other hand, have documented successful rhizospheric colonization of immobilized growth-promoting rhizobacteria strains when biochar was utilised as the inoculant carrier, which also encouraged root colonization by native AMF (Saxena et al., 2013).

CONCLUSIONS

In the pot experiment, the three tested isolates of AMF and the three proposed carriers had diverse effects on wheat performance under water deficit stress. The treatment effects were refined using the chlorophyll index and dry weight of wheat for two AMF isolates (*Acaulospora spinosa*, and *Glomus ambisporum*) and two carriers (biochar and alginate), both of which performed better. When applied in the field experiment, wheat plants exposed to water deficit stress performed better (grain yield, nutrient uptake, and colonization percentage) for AMF isolate *Glomus ambisporum* formulated on biochar and alginate, with better performance of biochar over alginate. For example, *Glomus*-biochar treatment could save 2,510 kg ha^{-1} grain yield lost when irrigation was shifted from W1 (irrigation every week) to W2 (irrigation every two weeks). This indicates that *Glomus*-biochar was effective in relieving water deficit stress in wheat. The results of this study hold significant implications for enhancing wheat growth under drought conditions.

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