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Impact of combining fungicide use with calcium fertiliser for managing maize downy mildew

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Abstract. Downy mildew is one of the most destructive diseases of maize worldwide. Disease prevention needs to be done at an early stage of plant growth. Maize is most susceptible to downy mildew between 10 and 45 days after sowing (DAS). This study aims to determine the effectiveness of applying fungicides in the early stages of plant growth combined with the addition of calcium nutrients. The research was conducted in Maros Regency, South Sulawesi, Indonesia, using a randomized block design two factor design. The first factor was five levels of fungicide application frequency, while the second included four fertiliser elements. The downy mildew inoculum source plants were planted and inoculated with *Peronosclerospora spp.* one month before planting the test genotypes. The results showed that repeated fungicide sprays in the early stages of plant growth effectively suppressed the downy mildew infection by 19.30%. Meanwhile, the first fertilisation at 10 DAS with nitrogen, phosphorus, and calcium (NPCa) was the most effective in suppressing downy mildew compared to other fertilisation packages. The treatment combination of three repeated fungicide applications (10, 15, and 20 DAS) with NPCa fertilisation can reduce the incidence of downy mildew by up to 33.23%. However, this combination resulted in lower yields compared to NPK fertilisation. Consequently, further research is required to explore the most effective method for applying potassium and calcium fertilisers.

Key words: downy mildew, preventive, fungicide, calcium fertilizer.

INTRODUCTION

Maize is one of the world's most important food crops, besides rice and wheat. Corn is cultivated globally across diverse agro-climatic zones, such as Bangladesh (Biswas et al., 2023), Mexico (Kennett et al., 2020), and Indonesia (Herawati et al., 2023). In Indonesia, increased maize production continues to fulfil food and animal feed needs. In continuous maize production, biotic and abiotic environmental factors can be yield-limiting. One of the biotic factors frequently reported as a significant constraint on maize productivity globally is downy mildew disease, caused by *Peronosclerospora* spp.

Peronosclerospora species found in Indonesia are *P. phillippinensis, P. maydis*, *P. sorghi*, and *P. neglecta* sp. nov., which spread in different regions (Muis et al., 2023). The pathogens are obligate parasites, and the primary vector for their spore dispersal is wind. Spore germination and the infection process of *Peronosclerospora* spp. require high humidity of 80–100% and temperatures of 18–30 °C (Lukman et al., 2016; Kalqutny & Pakki, 2020). Symptoms of downy mildew on maize are chlorotic streaks on the leaves running parallel to the leaf blade. The lower and upper surfaces of the leaves are covered with white clusters of spores. Systemic infections stunt plants and cause them to fail to produce (Lukman et al., 2016; Janruang & Unartngam, 2018). Disease incidence varies between maize cultivars (Widiantini et al., 2015). Susceptible cultivars are infected early in their growth. The disease is usually visible in plants 10 to 14 days after sowing (Mirsam et al., 2021). Pakki (2017) reported that planting susceptible cultivars without fungicide application can cause plant damage up to 100%. In addition to plant genetic factors, the availability of inoculum through continuous cultivation of maize in an area causes disease outbreaks (Prasetyo et al., 2021).

Maize downy mildew can be controlled by preventive treatments. Seed treatment with systemic fungicides, such as metalaxyl, effectively controls downy mildew. However, genetic mutations of pathogens to resist metalaxyl fungicides have rendered the method ineffective. In 2019, Pakki & Djaenuddin reported that seed treatment with metalaxyl fungicide up to a dose of 7 g kg^{-1} seed was ineffective in controlling downy mildew. Therefore, additional preventive measures, such as using fungicides during the initial phases of plant development, are necessary (Gupta et al., 2014). Ganesh et al. (2024) reported that spraying fungicides on ridge gourd before and immediately after the appearance of downy mildew symptoms can reduce disease severity by up to 74.35%. Similarly, downy mildew in basil decreased by 80% after two to four applications of calcium oxide, followed by azoxystrobin and reduced plant density (Gilardi et al., 2020). These findings are supported by Dudchenko et al. (2025), who found that repeated applications of the fungicide Acanto Plus SC significantly reduced downy mildew in sunflowers in Ukraine. Disease incidence in fungicide-treated plants was limited to 3.2%, compared with 28.9% in untreated control plants.

Control of maize downy mildew needs to be carried out through integrated management. Crop resistance to downy mildew can be increased by combining fungicide with the addition of certain nutrients, such as calcium. The application of calcium (Ca^{2+}) in crops contributes to maintaining tissue firmness and cell turgor, thereby reducing the incidence of diseases and preventing physiological disorders (Jain et al., 2019). Spraying propineb-based fungicides at 2,100 g ha^{-1} is more effective in suppressing downy mildew than several other fungicides on onions (Araújo & Resende, 2020). The effect of calcium fertiliser combined with fungicide in suppressing plant diseases has been reported by Percival & Graham (2021), who demonstrated that the application of the fungicide Penconazole together with calcium fertiliser reduced the severity of guignardia leaf blotch. Spraying fungicide penconazole combined with calcium chloride three times yielded a disease incidence level that did not differ significantly from that obtained with six applications of the fungicide alone.

Bezvikonnyi et al. (2020) reported other research about demonstrating that combining fungicide with micronutrients can suppress plant disease. They conducted a study from 2015 to 2017 at the Podillya State Agricultural and Technical University Training Center experimental field in Ukraine. They found that fertilising beet with micronutrient fertilisers with the Impact fungicides reduces the prevalence of leaf disease. The effectiveness of this treatment was evidenced by an increase in leaf area and photosynthetic capacity compared with the other treatments, with the photosynthetic potential reaching 2.37 million m^2 days per ha.

In this study, we conducted an experiment focused on the control of downy mildew in maize. The foliar application of systemic fungicides at the early stages of plant growth was combined with a calcium-nutrient fertiliser.

MATERIALS AND METHODS

Planting and inoculation of inoculum source plants

The research was conducted at the Indonesian Cereals Research Institute, Ministry of Agriculture, Maros District, South Sulawesi. The planting of inoculum sources followed the method of Mirsam et al. (2021). Maize seeds of the Anoman variety were planted in three rows around the perimeter of the test plots with a spacing of 50×20 cm. The preparation of the pathogen suspension was done by taking downy mildew-infected plants at 17.00 PM by cutting the leaves starting from the base and then putting them in a plastic bag. Once collected, the leaves to be used as inoculum sources were washed under running water to remove old conidia and dirt on the leaf surface. The bases of the leaves were then soaked in sugar water at a concentration of 2% in a container under dark conditions to stimulate spore formation (sporulation). At 03:00 AM, spores were harvested by rinsing the leaves covered with white spores in a container of water. This suspension is sprayed onto the inoculum source plants.

Planting the test material

Maize variety Nasa 29 was used in this study. The test material was planted when the inoculum source plants exhibited 60–70% downy mildew infection. Maize seeds were planted in test blocks with a length of 5 m and spacing of 70×20 cm. One seed was planted per hole.

Fungicide and fertiliser application

This study was conducted using a Factorial Randomised Block Design (RBD) with two factors. The first factor involved repeated applications of a synthetic fungicide containing the active ingredients fluopicolide (6%) and propineb (66.7%) at a rate of 2 kg ha^{-1}. The five applications were F1-No fungicide spraying; F2-Fungicide spraying when the plants were 10 DAS; F3-Fungicide spraying when the plants were 10 and 15 DAS; F4-Fungicide spraying when the plants were 10, 15 and 20 DAS; and F5-Fungicide spraying when the plants were 10, 15, 20 and 25 DAS. The second factor was the application of fertiliser elements, which consists of 4 levels, namely P1-Fertilisation with N + P + K + Ca; P2-Fertilisation with N + P + K; P3-Fertilisation with N + P + Ca; and P4-Fertilisation with N + P. Overall, there are 20 treatment combinations, each of which is repeated 3 times. The first fertilisation was done when the plants were 10 DAS using the type of fertiliser elements (N from urea; P_2O_5 from SP36; K_2O from KCL; Ca) based on each treatment. The doses used in the first fertiliser were urea at 200 kg ha^{-1}, SP36 250 kg ha^{-1}, KCL 166.7 kg ha^{-1} and Calcium 6.2 kg ha^{-1}. Furthermore, the second fertilisation at the age of 30 DAS entirely used urea 200 kg ha^{-1}. Fertilisation is done by digging the soil at a distance of 5–10 cm from the corn stalks, then the fertiliser is inserted and covered again with soil.

Effect of repeated fungicide applications combined with several types of fertiliser elements on the downy mildew incidence

Observation of downy mildew intensity

Downy mildew incidence was observed at 14, 21, 28, and 35 DAS using the following formula (Formula 1):

$$\mathrm{DI} = \frac{\mathrm{A}}{\mathrm{B}} \cdot 100\% \tag{1}$$

where DI, Disease Incidence (%); A, Number of plants infected with downy mildew; and B, number of plants observed in each treatment.

Analysis of the area under the disease development curve (AUDPC*)*

The AUDPC is calculated from the downy mildew disease intensity recorded over a defined observation period. Development. The AUDPC can describe the level of disease development in a certain time span. The AUDPC is calculated using the following equation (Mehmood & Khan, 2016):

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2}\right)(t_{i+1} - t_i) \tag{2}$$

where n, number of observations; x, downy mildew incidence; and $(t_{i+1} - t_i)$, time interval between observations.

Effect of repeated fungicide applications combined with several types of fertiliser elements on the plant growth and production aspects

At 60 days after sowing (DAS), plant height was measured from the soil surface to the base of the panicle using a measuring tape. Observations of yield and 1,000-seed weight were made at harvest by collecting the entire plant in each treatment. Seed production (t/ha) was calculated using the following equation:

$$\text{Yield (ton.ha}^{-1}) = \frac{10{,}000}{\text{plot size}} \cdot \frac{100 - Mo}{100 - 15} \cdot \frac{EW}{1{,}000} \cdot SP \tag{3}$$

where *Mo*, harvest moisture content (%); Plot size, harvest area (m^2); *EW*, ear weight per plot (kg); and *SP*, shelling percentage (Azrai et al., 2023).

Data analysis

Observed data on downy mildew incidence, plant height, yield, and 1,000-seed weight were statistically analysed using ANOVA. The analysis was followed by the Least Significant Difference (*LSD*) test at the 5% level (α 0.05).

RESULTS AND DISCUSSION

Effect of repeated fungicide applications combined with several types of fertiliser elements on the downy mildew incidence

Downy mildew symptoms were not found during the first observation at 14 days after sowing (DAS). However, in the subsequent observation, disease symptoms were detected with varying levels of incidence across treatments. In the second observation at 21 DAS, we detected the onset of disease infection, with varying incidence levels among the treatments. The number of plants infected with downy mildew continued to increase until the plants were 35 DAS. Plants without fungicide treatment were infected by 53.27%, significantly higher than plants applied with fungicides with 4 different application frequency levels (Fig. 1, a).

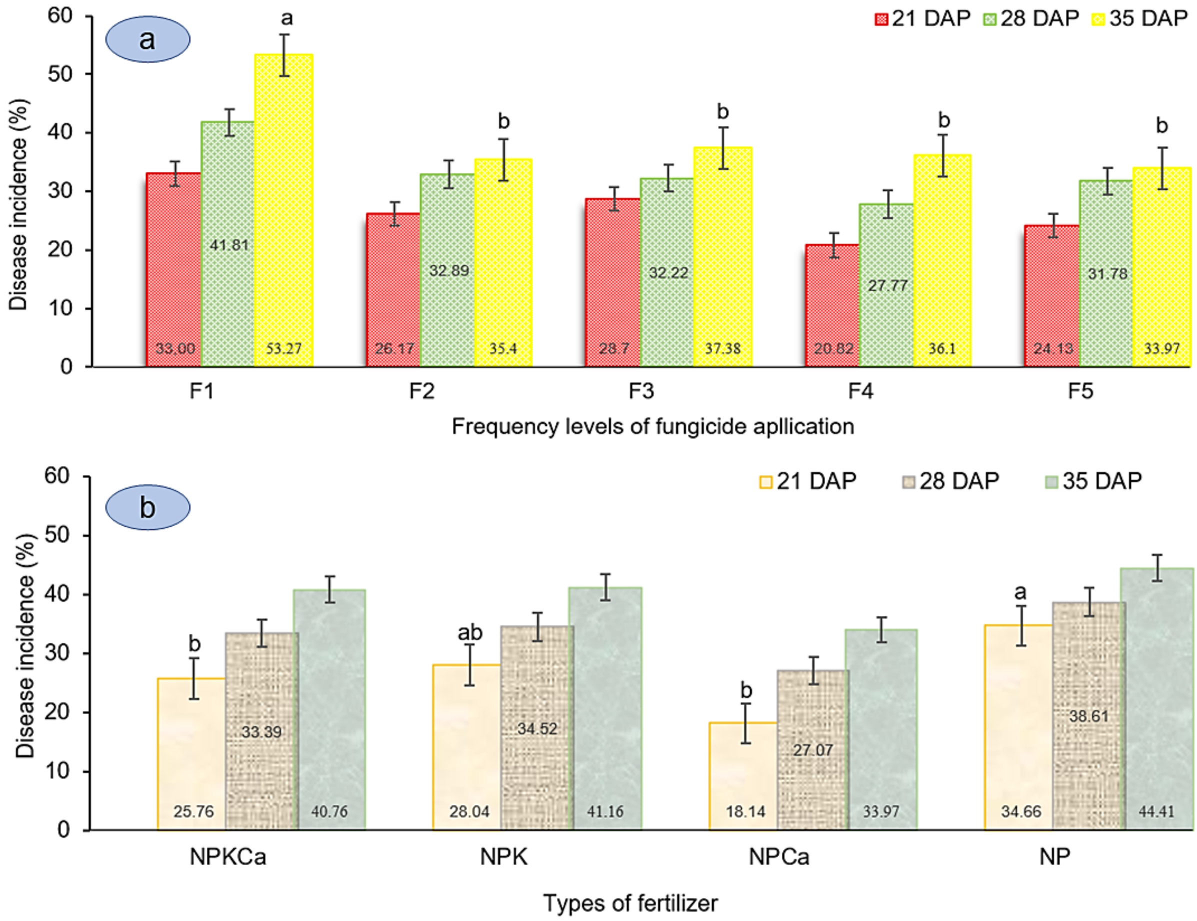

Figure 1. The incidence of maize downy mildew for fungicide treatment applied at five frequencies of application (a) and four types of fertilisation (b), observed at 21, 28, and 35 days after sowing (DAS). F1: no fungicide application; F2: Fungicide application at 10 DAS; F3: Fungicide application at 10 and 15 DAS; F4: Fungicide application at 10, 15, and 20 DAS; F5: Fungicide application at 10, 15, 20, and 25 DAS. Bar charts showing one observation time followed by the same letter are not significantly different based on the 5% *LSD* test.

The fertiliser element treatment significantly affected downy mildew incidence at 21 DAS (Fig. 1, b). Plants treated with calcium-containing fertilisers (P1 and P3) showed lower disease incidence than those receiving only nitrogen and phosphorus. However, at 28 and 35 DAS, downy mildew incidence did not differ significantly among treatments. The lowest disease incidence occurred in treatment P3 (NPCa fertilisation) at 33.97%. Fig. 2 illustrates the symptoms and signs of downy mildew disease.

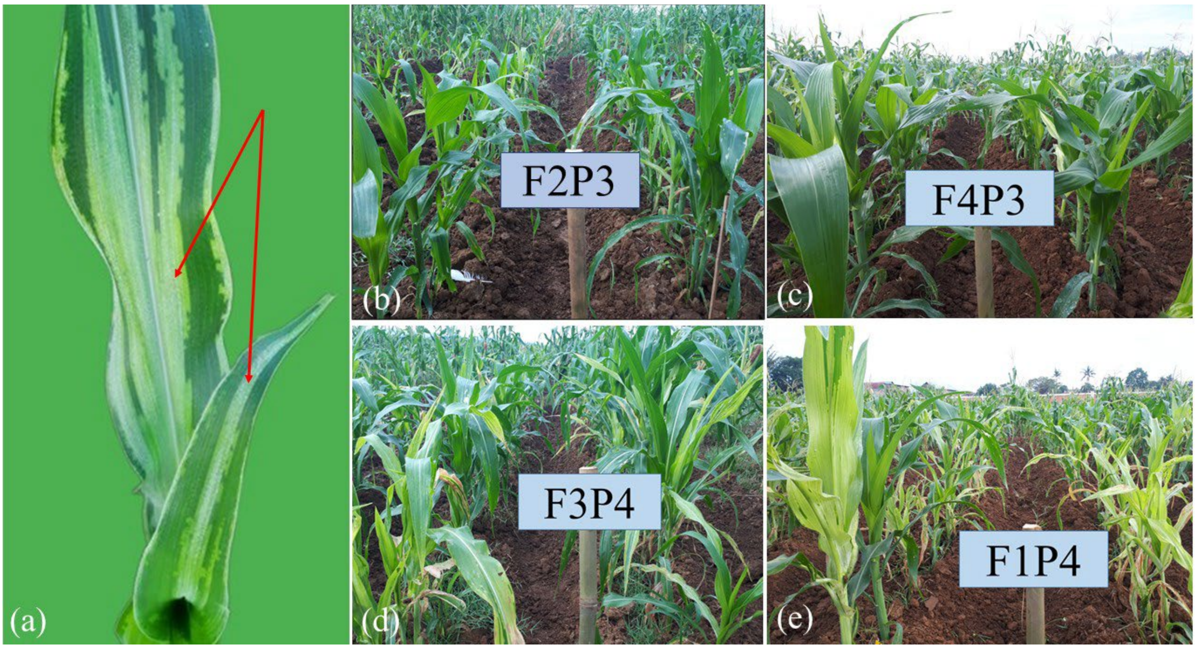

Figure 2. Symptoms of maize downy mildew appear as leaf chlorosis and white conidial growth on the leaf surface (a); Downy mildew infections under various treatments were the fungicide spraying treatment at 10 DAS combined with elemental NPKCa fertilisation (b), the fungicide spraying treatment at 10, 15, and 20 DAS combined with elemental NPKCa fertilisation (c), the fungicide spraying treatment at 10 and 15 DAS combined with elemental NP fertilisation (d), and no fungicide spraying treatment combined with elemental NP fertilisation (e).

The factor of fungicide application through the leaves showed a significant effect on reducing downy mildew disease incidence. At 35 DAS, plants that received four fungicide applications showed a 19.30% reduction in disease incidence relative to the untreated control. Preventive pesticide applications need to be done to prevent the development of *Peronosclerospora* spp. McGrath (2020) reported that fungicide applications initiated one day after sowing and repeated at seven-day intervals were highly effective, reducing downy mildew infection in basil by 87%. The applied fungicide is certain to adhere to the leaf surface, especially the lower leaf surface, so that it can inhibit or damage conidia (Kreuser & Broderick, 2017; Ons et al., 2020). While the factor of the type of fertiliser used shows the application of NPCa fertiliser has a downy mildew incidence of 10.44% lower than the disease incidence that occurs in plants fertilised with nitrogen and phosphorus elements. This shows that calcium in plants plays an important role in suppressing pathogen infection. Calcium is a structural component of the cell wall and other plant membranes that maintain the integrity and function of the cell wall (El Habbasha & Ibrahim, 2015; Lorenzetti et al., 2021). Calcium contributes to disease resistance by strengthening plant cell membrane stability and activating antioxidant enzyme systems (Yan et al., 2024).

The effect of 20 combinations of fungicide and calcium fertiliser on downy mildew incidence did not show significant differences between treatments at 21 and 28 DAS. The F4P3 combination consistently had the lowest disease incidence at both observation times, at 8.89% and 22.24%, respectively. The difference in disease incidence was significant when the plants were 35 DAS. The combination of F5P3, F4P3, F2P3 showed the lowest incidence, at 28.83, 29.11, and 30.73%, respectively (Table 1).

Table 1. Downy mildew incidence in maize as affected by different pesticide spraying frequencies and types of elemental fertilisers

Treatment	Disease incidence (%)				AUDPC	Resistance criteria
	14 DAS	21 DAS	28 DAS	35 DAS		
F1P1	0.00	37.60	42.54	58.55 ab	765.92	Susceptible
F1P2	0.00	29.14	40.33	54.31 abc	676.36	Susceptible
F1P3	0.00	25.46	36.12	49.27 abcd	603.48	Susceptible
F1P4	0.00	39.79	43.24	62.06 a	798.41	Very Susceptible
F2P1	0.00	21.21	28.20	35.86 cdef	471.38	Moderately resistant
F2P2	0.00	25.39	33.43	35.19 def	534.89	Moderately resistant
F2P3	0.00	17.76	24.87	30.73 ef	405.94	Moderately resistant
F2P4	0.00	40.33	45.06	39.82 cdef	737.09	Moderately resistant
F3P1	0.00	25.98	30.81	37.53 cdef	603.48	Moderately resistant
F3P2	0.00	30.61	33.71	37.84 cdef	582.66	Moderately resistant
F3P3	0.00	22.46	24.24	31.90 def	438.53	Moderately resistant
F3P4	0.00	37.50	42.26	48.13 abcde	726.79	Susceptible
F4P1	0.00	19.87	25.76	33.81 def	437.74	Moderately resistant
F4P2	0.00	28.88	32.40	42.91 bcdef	579.15	Susceptible
F4P3	0.00	8.89	22.44	29.11 f	321.22	Moderately resistant
F4P4	0.00	25.63	30.46	38.58 cdef	527.65	Moderately resistant
F5P1	0.00	24.15	34.66	38.05 cdef	544.84	Moderately resistant
F5P2	0.00	26.18	32.75	35.54 def	536.93	Moderately resistant
F5P3	0.00	16.12	27.68	28.83 f	407.45	Moderately resistant
F5P4	0.00	30.06	32.02	33.46 def	551.67	Moderately resistant
LSD 5%		tn	tn	1.46		

Notes: Cell numbers followed by the same letter are not significantly different at the 5% level of the Least Significant Difference test (*LSD*) 5%.

The treatment combination that most effectively suppressed downy mildew infection was found in the treatment combination of F5P3, F4P3, F2P3. These three combinations are the use of NPCa fertiliser. While the NPKCa fertiliser treatment showed higher infection than the NPCa fertiliser treatment. It is known that potassium and calcium are elements that can increase plant resistance to pathogen infection. The combination of these two elements is expected to provide higher plant resistance, but the research found different things. There was an interaction between potassium and calcium nutrients that influenced each other so that the absorption of both elements was higher when applied alone than in combination. The relationship between two or more nutrients in plant tissues can be antagonistic or synergistic depending on the plant species, cell type, leaf age, and source organ. Therefore, determining the optimal dose of nutrients is necessary to maintain proper nutritional status in plants to support increased production (Xie et al., 2021). AUDPC analysis found that the F4P3 treatment had the lowest value of 321.22 (Fig. 3). A lower AUDPC value indicates a slower rate of disease progression and lower overall disease incidence. Therefore, the findings suggest that fungicide application in the early growth stage up to 20 DAS, in combination with calcium fertilisation, effectively reduces the incidence of downy mildew.

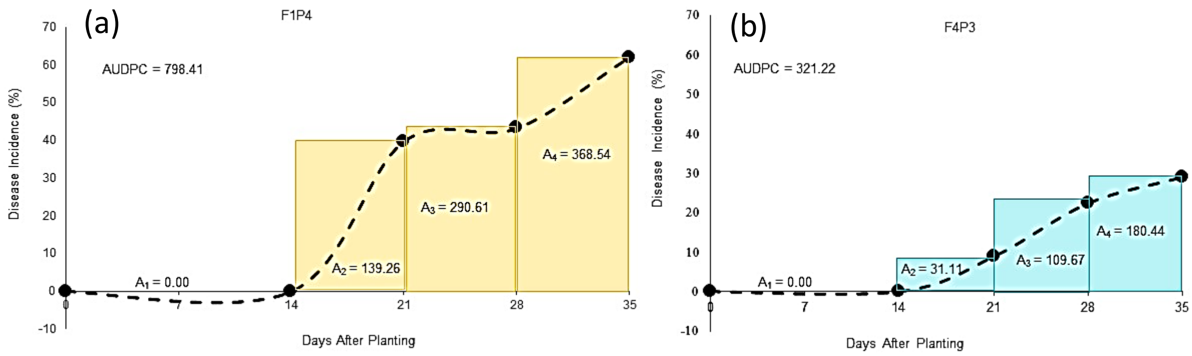

Figure 3. Maize downy mildew AUDPC graph for (a) F1P4 (no fungicide application + NP); (b) F4P3 (foliar application of fungicide at 10, 15, and 20 DAS + NPCa). A1–A4 indicate AUDPC for the first to fourth observation intervals.

Effect of repeated fungicide applications combined with several types of fertiliser elements on the plant growth and production aspects

The effect of treatments on the overall plant height of maize was not statistically significant. The highest plant height was observed in the F2P3 treatment (148.6 cm), followed by F4P1 (147.5 cm) and F4P3 (147.8 cm). In contrast, significant differences among treatments were observed in yield and the weight of 1,000 seeds (Table 2). The highest yield was recorded in treatment F5P1, reaching 6.88 ton ha^{-1}. This yield was significantly higher than those obtained from the four treatment combinations without pesticide application (F1) and from treatments involving only N and P fertilisation. The significant decrease in production, especially in the NP fertiliser treatment, was due to the low level of plant resistance to downy mildew so that the plant population was reduced. Therefore, plants require the application of macro- and micronutrient fertilisation to sustain cellular function stability and enhance their defence against pathogen infection. Rosyidah et al. (2017) reported that the use of potassium fertiliser can reduce the attack rate of bacterial wilt disease caused by *Rhizoctonia solanacearum* by 73.15%. In addition, the application of a balanced N-P-K fertiliser resulted in the highest maize yield compared to the application of NP, NK, or PK combinations (Otieno et al., 2019).

The findings of this study indicate that there are differences in effectiveness between treatments in suppressing the development of downy mildew disease and influencing plant productivity. The F4P3 treatment demonstrated the highest inhibition of downy mildew incidence. However, its crop yield was lower than the F5P1 treatment, although the difference was not statistically significant. Our findings show that calcium played an important role in lowering downy mildew incidence. As reported by previous researchers, calcium can directly affect the germination of pathogen spores (Percival & Graham, 2021). Calcium can trigger biochemical reactions that change leaf structure and function, helping the plant prevent pathogen entry (Tripathi et al., 2022). However, researchers, calcium can directly affect the germination of pathogen spores (Percival and treatments that combine potassium and calcium show lower pathogen inhibition compared to those without potassium. This reduced inhibition is likely due to the negative interaction that occurs between the two elements. Several researchers have reported that there is a cationic antagonism between calcium and potassium elements, so that high levels of one nutrient can result in reduced absorption of the other nutrient (Jēkabsone et al., 2024; He et al., 2025). Furthermore, Rhodes et al. (2018) found that potassium is more potent in reducing calcium absorption than vice versa. Although treatments with calcium elements without potassium showed the best effectiveness in suppressing corn downy mildew, production was lower. This lower yield occurs because the plants lack potassium, which plays an important role in plant physiology (Johnson et al., 2022). Therefore, future research should include additional treatment factors, such as varying calcium fertiliser application methods or other relevant agronomic practices.

Table 2. Growth and production of maize plants in repeated fungicide applications combined with several types of fertiliser elements

Treatments	Plant height (cm)	Yield (ton ha^{-1})	Seeds weight 1,000 (gr)
F1P1	126.9	4.92 bcdef	0.31 ab
F1P2	137.1	4.93 bcdef	0.33 ab
F1P3	143.3	4.44 cdef	0.30 ab
F1P4	145.1	3.89 ef	0.28 b
F2P1	144.3	5.32 abcde	0.35 ab
F2P2	128.4	5.79 abcd	0.34 ab
F2P3	148.6	5.59 abcd	0.31 ab
F2P4	127.6	4.15 def	0.28 b
F3P1	143.4	5.85 abc	0.31 ab
F3P2	143.0	5.65 abcd	0.29 b
F3P3	130.4	4.84 bcdef	0.28 b
F3P4	135.9	4.55 cdef	0.28 b
F4P1	147.5	6.47 ab	0.30 ab
F4P2	147.8	5.50 abcde	0.38 a
F4P3	147.7	6.46 ab	0.35 ab
F4P4	144.7	3.63 f	0.31 ab
F5P1	142.3	6.88 a	0.33 ab
F5P2	145.7	5.53 abcde	0.30 ab
F5P3	124.9	4.81 cdef	0.31 ab
F5P4	133.6	3.93 ef	0.29 b
LSD 5%		1.64	0.08

Notes: Cell numbers followed by the same letter are not significantly different at the 5% level of the Least Significant Difference test (*LSD*).

CONCLUSIONS

The results of the research showed that repeated fungicide spraying at the beginning of plant growth effectively reduced the incidence of downy mildew by up to 19.30%. Fungicide spraying was carried out when the plants were 10, 15, and 20 days after sowing. The treatment combination of three repeated fungicide applications (10, 15, and 20 DAS) with NPCa fertilisation can reduce the incidence of downy mildew by up to 33.23%. However, this combination resulted in lower yields compared to NPK fertilisation. Consequently, further research is required to explore the most effective method for applying potassium and calcium fertilisers.

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