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Effects of mycorrhizal inoculum, organic inputs, and carbofuran on maize growth in Alfisol

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Abstract. *Fataya AD, Dewi WS, Hadiwiyono H, Cahyani VR. 2026. Effects of mycorrhizal inoculum, organic inputs, and carbofuran on maize growth in Alfisol. Intl J Trop Drylands 10 (1): t100101. https://doi.org/10.13057/tropdrylands/t100101.* Mycorrhiza played a crucial role in enhancing nutrient absorption in plants and increasing their tolerance to pests and diseases. Its existence and diversity were affected by several factors, including planting patterns. This research aimed to assess the effectiveness of mycorrhiza sourced from maize fields under intercropping and monoculture systems and of a mixture of rock phosphate, rice straw compost, and carbofuran on Alfisol soil. The greenhouse experiment used a two-factor Completely Randomized Design (CRD), significance level ($p<0.05$), and had three replications. The first factor involved the source of mycorrhizal spores (M), categorized into four levels: no spores (M0), monoculture (M1), intercropping (M2), and mixed spores (M3) comprising 50% from each system. The second factor was treatment input, which included four levels: no input (P0), rock phosphate and straw compost (P1), carbofuran (P2), and a combination of compost, carbofuran, and rock phosphate (P3). The finding is the treatment M3P3, which combined Arbuscular Mycorrhizal Fungi (AMF) spores from the maize rhizosphere with rock phosphate, rice straw compost, and carbofuran, significantly enhanced plant growth. It resulted in increases of 58.7% in fresh weight, 52.8% in dry weight, and 69.9% in phosphorus uptake compared to the control. This improvement was linked to a high percentage of AMF infectivity (68.67%) and spore density (369 spores/100 g soil), along with increased soil fertility. Conversely, treatment M0P2, which lacked AMF spore inoculation and used high doses of carbofuran, led to poor plant growth. The findings demonstrate a significant synergistic effect of the mixed AMF derived from monoculture and intercropping systems (M3), showing significantly higher effectiveness than the separate application of AMF spores from each system, and enhancing the chemical characteristics and productivity of Alfisol soil.

Keywords: Insecticide effect, mycorrhizal infectivity, rice straw compost, rock phosphate, soil fertility

INTRODUCTION

Maize (*Zea mays*) is a globally important staple crop that supports food security for billions and serves as a key source of animal feed and industrial products (Burlakoti et al. 2024). However, modern agricultural practices-such as intensive tilling, monocropping, and heavy use of synthetic fertilizers-have degraded soil health and diminished the effectiveness of native beneficial microorganisms, including Arbuscular Mycorrhizal Fungi (AMF) (Chaudhary et al. 2025). AMF form a vital mutualistic symbiosis with the roots of over 80% of terrestrial plants, including maize (Vargas-Espinoza et al. 2023), where the fungus receives carbohydrates from the plant and, in return, its extensive hyphal network enhances the plant's uptake of water and nutrients, especially those with low soil mobility like phosphorus (P), zinc (Zn), and nitrogen (N) (Cavagnaro 2015; Thirkell et al. 2016; Billah et al. 2020). Importantly, mycorrhiza has been shown to work more effectively in providing phosphorus nutrients under critical soil conditions, such as Alfisol soil (Nahar et al. 2021).

Alfisol soil was often characterized by low productivity due to a combination of physical and chemical constraints. A key chemical limitation is its low Soil Organic Carbon (SOC) content, typically less than 5 g/kg^{-1}, which resulted in poor fertility (Ajiboye et al. 2019). This issue is further exacerbated by the rapid oxidation of organic matter caused by high temperatures and frequent tillage. The lack of organic matter also led to poor water-holding capacity and reduced nutrient retention, making Alfisols highly vulnerable to nutrient leaching (Ogunjinmi et al. 2017; Anantha et al. 2023). To overcome this low productivity, organic fertilization was employed, and farmers often used carbofuran as a pesticide to enhance the soil's productivity value.

Carbofuran is a powerful insecticide and nematicide used to protect crops from pests (Afify et al. 2023). However, its extensive and long-term use has raised significant environmental and human health concerns due to its high toxicity, persistence, and mobility in soil, which can lead to groundwater contamination (Mustapha et al. 2020). Research by Baćmaga et al. (2024) revealed that carbofuran negatively impacts soil microbiota, causing a loss of microbial diversity and essential soil functions. In contrast, organic fertilizers derived from compost and crop residues can decrease carbofuran's movement in soil and enhance its breakdown while improving soil health (Omokaro et al. 2024). The combination of carbofuran and organic fertilizers is complex, as organic matter can help reduce pesticide mobility and support beneficial microbial activities that break down toxins (Carpio et al. 2021). Additionally, AMF function as biological control agents protecting host plants from soil-borne pathogens (Veresoglou and Rillig 2012), and the diversity of mycorrhizae was believed to be higher in intercropping systems compared to monocropping systems.

Zhang et al. (2020) reported that the diversity and composition of AMF in the rhizosphere could be influenced by various plants in an intercropping system due to interactions between plant species. Furthermore, a study by Meng and Humphreys (2015) found that maize in an intercropping system with soybeans had a much higher AMF colonization rate compared to maize planted as a monocrop. This increased colonization of mycorrhiza in the intercropping system enhanced plant growth and nutrition uptake (such as N, P, and K) by AMF hyphae (Zhang et al. 2020)

Given the potential of isolates from the rhizosphere of different cropping patterns, further study was warranted. While research had extensively compared mycorrhiza in intercropping and monocropping systems, as well as with rock phosphate or compost, limited information existed on the combination of mycorrhiza isolates from maize rhizosphere with different planting patterns, straw compost, rock phosphate, and carbofuran. Therefore, this study aimed to examine the effects of applying mycorrhiza isolated from the maize rhizosphere under intercropping and monocropping patterns, combined with rice straw compost, rock phosphate, carbofuran treatment (at twice the recommended dose), and a combination of these factors on maize growth in pot experiments using Alfisol soil.

MATERIALS AND METHODS

Rhizosphere soil sampling for the source of arbuscular mycorrhizal spores

Soil samples for the source of AMF spore isolates were collected from the rhizosphere of maize plants in two types of fields: maize monocropping and maize-peanut intercropping fields in Sukosari Village, Jumantono, Karanganyar, Central Java, Indonesia (7.63010°S, 110.94830°E) during March-April 2023. From each field, rhizosphere soil of maize at an amount of 500 g per plant with three replications was collected as the source of AM spores. The isolation of AM spores was conducted using the wet sieving and pouring method (Gerdemann and Nicolson 1963), followed by centrifugation with a 60% sugar solution (Ianson and Allen 1986). The density of AM spores in 100 g of rhizosphere soil from each field was determined using a binocular microscope at 400x magnification. The AM spores were prepared as AM inoculums for the greenhouse experiment.

Inoculum preparation for the greenhouse experiment

To prepare the mycorrhizal inoculum for the greenhouse experiment, spore sources were collected from rhizosphere soil samples of maize plants grown on an upland field at their maximum vegetative age (55 days after planting (DAP)). For the single-source treatment, 50 spores, either from the maize rhizosphere in a monocropping system or from the maize rhizosphere in an intercropping system, were prepared separately to be applied to each pot (Cahyani et al. 2019), and for the mixed mycorrhizal spores treatment, mycorrhizal spores in a 50:50 ratio for each pot (25 spores from the maize rhizosphere in a monocropping system, and 25 spores from the maize rhizosphere in an intercropping system).

Greenhouse experiment

Soil samples for the pot experiment were taken from the non-rhizosphere area of maize plants in the same village. This soil was classified as an Alfisol. The soil samples were air-dried, sieved to 2 mm, and prepared in 1 kg portions per polybag. The soil characteristics were determined as follows: pH 5.63, organic C 0.9%, total N 0.4%, and 6.2 ppm available P.

The pot experiment was conducted in the greenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, from May to September 2023. The experiment used a Completely Randomized Design (CRD) with two factors: AM inoculation (M) and fertilizer application, carbofuran (P). The AMF inoculation levels were as follows: M0 (without inoculation), M1 (50 spores from the maize rhizosphere in a monocropping system), M2 (50 spores from the maize rhizosphere in an intercropping system) (Cahyani et al. 2019), and M3 (a mixture of AM spores from the maize rhizosphere in monocropping and intercropping systems, 50% each). The combination input of fertilizer and carbofuran levels were: P0 (without fertilizer and carbofuran), P1 (rock phosphate and rice straw compost), P2 (carbofuran), and P3 (a combination of rock phosphate, rice straw compost, and carbofuran). There were 16 treatments with 3 replications. Rock phosphate (75 kg/ha) (Ditta et al. 2018) was incubated for 1 month, while rice straw compost (10 tons/ha) (Ginting 2019) and carbofuran at twice the recommended dosage (10 g/plant) were added 7 days before planting. Maize seeds of the Pertiwi 2 variety were planted (3 seeds per polybag) and weeded until one plant had germinated. AM spores (50 spores per polybag) were applied 7 days after planting (DAP) to the rhizosphere area. Plants were harvested at the maximum vegetative phase (55 DAP).

Arbuscular Mycorrhizal (AM) parameters

Soil and maize root samples were analyzed for spore density using the wet sieving and pouring method (Gerdemann and Nicolson 1963), followed by centrifugation with a 60% sugar solution (Ianson and Allen 1986). AM infection in plant roots was determined using the method described by Phillips and Hayman (1970), with the formula:

$$\%\ \text{Mycorrhizal infection} = \frac{\text{number of infected roots}}{\text{number of roots observed}} \text{ x } 100$$

Maize plant and soil parameters analysis

Plant and soil samples were collected at harvest to measure maize plant parameters, including plant height, fresh weight, dry weight, plant tissue P content, and in-plant P uptake, as well as soil parameters such as soil pH, organic C content, total soil N content, available soil P, and total soil P content.

Statistic analysis

Data analysis was performed using Analysis of Variance (ANOVA), Duncan's Multiple Range Test (DMRT) at the 5% ($p<0.05$) significance level), and Pearson’s correlation analysis to determine the linear plant fresh and dry weights and AMF infectivity and spore density, as well as between plant weights and soil chemical properties (organic C, total N, available P, and total P).

RESULTS AND DISCUSSION

Effects of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on soil chemical characteristics

The results of the interaction between mycorrhizal spores from monoccropping and intercropping systems with rock phosphate, rice straw compost, and carbofuran had a significant effect ($p<0.01$) on all measured soil chemical characteristics: soil pH, organic C, total N, available P, and total P. The main effects of M (mycorrhizal inoculum) and P (combination input of fertilizer and carbofuran) were also highly significant ($p<0.01$) for all of these parameters.

As detailed in Table 1, the M3P3 treatment, which combined mixed AM spores with rock phosphate, rice straw compost, and carbofuran, resulted in the highest values for all soil chemical parameters. Specifically, M3P3 showed a soil pH of 6.87±0.02, 2.86±0.01% organic C, 0.28±0.00% total N, 7.87±0.01 ppm available P, and 4.22±0.01 mg/100 g soil of total P. These values were all significantly different from the control treatment (M0P0), which had values of 5.68±0.01 for pH, 1.51±0.00% for organic C, 0.16±0.00% for total N, 6.22±0.03 ppm for available P, and 2.39±0.00 mg/100 g soil for total P. The M3P1 (mixed spores with rock phosphate and compost) and M3P2 (mixed spores with carbofuran) treatments showed the second- and third-highest improvements, respectively, for soil pH and nutrients compared to other treatments, indicating that the M3 treatment effectively enhanced soil chemical properties in combination with various inputs.

Effects of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on density and infectivity of mycorrhiza

The results of the interaction between mycorrhizal spores from monoccropping and intercropping systems with rock phosphate, rice straw compost, and carbofuran had a significant effect ($p<0.01$) on both AM infection and spore density. The main effects of M (mycorrhizal inoculum) and P (fertilizer mixed with carbofuran inputs) were also highly significant ($p<0.01$) for both parameters.

Table 1. Effect AM inoculum, rock phosphate, compost and carbofuran on soil chemical characteristics in the pot experiment

Treatment	pH (H_2O)	Organic C (%)	Total N (%)	Available P (ppm)	Total P (mg/100 g soil)
F Values					
M	0.01 **	0.01 **	0.01 **	0.01 **	0.01 **
P	0.01 **	0.01 **	0.01 **	0.01 **	0.01 **
M×P	0.01 **	0.01 **	0.01 **	0.01 **	0.01 **
Interaction (P×M)					
M0P0	5.68± 0.01 l	1.51±0.00 m	0.16±0.00 i	6.22±0.03 m	2.39±0.00 o
M1P0	5.72±0.01 k	1.63±0.00 k	0.17±0.01 hi	6.32±0.01 l	2.46±0.00 n
M2P0	6.51±0.01 i	1.62±0.00 k	0.17±0.00 fgh	6.88±0.00 de	2.49±0.00 m
M3P0	6.52±0.01 i	1.58±0.00 l	0.18±0.00 ef	6.84±0.00 f	2.58±0.01 l
M0P1	6.46±0.01 j	1.74±0.01 j	0.17±0.00 fgh	6.63±0.01 j	2.67±0.01 k
M1P1	6.57±0.01 h	1.92±0.01 i	0.17±0.01 ghi	6.80±0.00 g	2.82±0.01 j
M2P1	6.66±0.01 g	2.67±0.00 d	0.18±0.01 fg	6.88±0.00 d	3.44±0.01 e
M3P1	6.80±0.01 b	2.75±0.00 b	0.25±0.01 b	7.37±0.02 b	4.19±0.00 b
M0P2	5.55±0.05 m	1.34±0.01 n	0.15±0.01 j	6.14±0.00 n	2.35±0.01 p
M1P2	6.52±0.02 i	2.13±0.01 h	0.17±0.01 ghi	6.48±0.01 k	3.23±0.00 g
M2P2	6.70±0.01 e	2.44±0.00 f	0.19±0.01 e	6.76±0.01 h	3.21±0.00 h
M3P2	6.78±0.01 c	2.73±0.00 c	0.23±0.00 c	6.94±0.01 c	3.82±0.01 c
M0P3	6.73±0.01 d	2.64±0.00 e	0.18±0.01 ef	6.72±0.00 i	3.15±0.01 i
M1P3	6.68±0.01 f	2.44±0.00 f	0.21±0.00 d	6.86±0.01 e	3.25±0.01 f
M2P3	6.69±0.01 ef	2.42±0.01 g	0.19±0.00 e	6.82±0.01 fg	3.57±0.01 d
M3P3	6.87±0.02 a	2.86±0.01 a	0.28±0.00 a	7.87±0.01 a	4.22±0.01 a

Note: **: $p<0.01$, *: $p<0.05$, and ns: Not significant. Means followed by the same letter are not significantly different at a significance level of 5% by Duncan’s Multiple Range Test (DMRT)

The result in Table 2 showed that the highest mycorrhizal infectivity and spore density were observed in the M3P3 treatment, with a mean infectivity of 68.67±0.58% and a spore density of 369.00±1.00 spores/100 g soil. This was followed by the M3P2 treatment, which had an infectivity of 57.33±0.58% and a spore density of 356.67±0.58 spores/100 g soil, and the M3P1 treatment with an infectivity of 60.33±0.58% and a spore density of 363.67±1.53 spores/100 g soil. These values were significantly higher than the control (M0P0), which had an infectivity of 36.33%±0.58 and a spore density of 224.67±0.58 spores/100 g soil. This trend shows that the mixed mycorrhizal treatment (M3) consistently yielded higher spore density and infection rates than the single-source treatments (M1 and M2).

Effects of the application of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on the plant growth of maize crops

The results of the interaction between mycorrhizal spores from monoccropping and intercropping systems with rock phosphate, rice straw compost, and carbofuran inputs showed a significant effect ($p<0.01$) on plant fresh weight, dry weight, tissue P content, and P uptake. However, no significant interaction effect was found for plant height ($p>0.05$). The main effects of M (mycorrhizal inoculum) and P (fertilizer mixed with carbofuran inputs) were also highly significant for all parameters except for plant height, where the P main effect was not significant ($p>0.05$).

As shown in Table 3, the M3P3 treatment demonstrated the highest plant growth and P uptake, with a mean fresh weight of 67.45±0.82 g, dry weight of 35.09±0.88 g, P concentration of 0.19±0.00%, and P uptake of 2.77±0.01 mg/plant. These values were significantly higher than the control (M0P0), which had values of 42.48±0.28 g for fresh weight, 22.96±0.63 g for dry weight, 0.12±0.00% for P concentration, and 1.63±0.00 mg/plant for P uptake. Treatments M3P1 and M3P2 were the second- and third-most effective, respectively, in increasing fresh weight (M3P1: 64.63±0.61 g; M3P2: 63.06±0.17 g) and dry weight (M3P1: 29.99±0.23 g; M3P2: 29.10±0.76 g). This trend indicates that the mixed mycorrhizal treatment (M3) consistently promoted greater plant growth and nutrient uptake compared to the single-source treatments (M1 and M2). The M0P2 treatment (carbofuran alone) showed the poorest plant growth and P uptake, with a mean fresh weight of 41.78±0.38 g, dry weight of 20.35±0.14 g, P concentration of 0.10±0.00%, and P uptake of 1.56±0.01 mg/plant, which was even lower than the control.

The level of plant fresh and dry weight was correlated with the level of AMF infectivity and AMF spores and also correlated with the level of soil nutrients. Pearson's correlation analysis indicated positive and significant correlations between the fresh and dry weight of plants with AM infectivity (r=0.883; $p<0.01$ and r=0.834; $p<0.01$), AMF spore density (r=0.631; $p<0.01$ and r=0.668; $p<0.01$). In addition, Pearson's analysis showed positive and significant correlations between the fresh and dry weight of plants with organic C (r=0.751; $p<0.01$ and r=0.676; $p<0.01$), total N (r=0.865; $p<0.01$ and r=0.860; $p<0.01$), available P (r=0.715; $p<0.01$ and r=0.735; $p<0.01$), and total P (r=0.867; $p<0.01$ and r=0.719; $p<0.01$) in the soil. This indicated that higher levels of organic C, total N, available P, and total P in the soil significantly contributed to the increase in the fresh and dry weight of plants. Plant P concentration and uptake were also positively correlated with soil available P (r=0.756; $p<0.01$ and r=0.843; $p<0.01$, respectively), highlighting the importance of the relationship between soil P availability and its uptake by plants.

Discussion

Effects of the application of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on soil chemical characteristics

The results showed that the combined application of AMF, rock phosphate, compost, and carbofuran significantly improved soil chemical properties. The M3P3 treatment, resulted in the highest increases in soil pH, organic C, total N, total P, and available P compared to the control (M0P0). This indicated that the M3P3 treatment was more effective in enhancing soil nutrient levels than all other treatments. According to Sammama et al. (2022), the intercropping of soft wheat and faba beans was more effective in raising soil nutrient levels than monocropping of soft wheat or faba beans. The study showed that nitrogen levels in intercropped soft wheat increased by 50.65% compared to soft wheat monocropping and by 34.88% compared to faba bean monocropping. Similarly, soil phosphorus concentration in soft wheat planted in intercrops was 55.81% higher than in soft wheat monocropping and 48.89% higher than in faba bean monocropping. Sammama et al. (2022) also found that the addition of AMF inoculation further enhanced soil nutrients compared to treatments without mycorrhizal inoculation (control).

AM inoculation increased soil nutrients such as C, N, and P (Fall et al. 2022). The mycorrhizal hyphae are significantly smaller than root hairs and can penetrate soil pores inaccessible to roots (Chandra et al. 2025). This network effectively functions as an extension of the root system, increasing the soil volume explored for nutrients by up to several hundred times (Han et al. 2025). Click or tap here to enter text. Similarly, Kuyper et al. (2021) reported that hyphae in AM enhanced the absorption of water and nutrients from soil pores that were generally difficult for plants to reach. This demonstrated that mycorrhizal associations played an important role in the decomposition and mineralization of plant organic matter and in the mobilization of nutrients, especially nitrogen, for the benefit of their host plants. Birhane et al. (2023) reported that mycorrhizal plants had more and longer fine roots than non-mycorrhizal plants, indicating that the absolute increase in carbon acquisition due to mycorrhizal symbiosis could exceed the relative allocation of carbon between roots and shoots.

The application of rock phosphate in the M3P3 treatment helped plants by providing Phosphorus (P) nutrients in the soil. Rock phosphate, activated by soil microbes, including arbuscular mycorrhizal fungi, functioned as a source of slow-release phosphorus and natural fertilizer, which increased plant growth and productivity by stimulating enzyme activity and aiding phosphate solubilization (Boutasknit et al. 2024). Arbuscular mycorrhiza (AM) assisted in dissolving inorganic phosphorus into orthophosphate forms that could be absorbed by plant roots (Ahmed et al. 2021).

The application of rice straw compost in the M3P3 treatment contributed to increasing organic matter content in soil, improving soil structure, and enhancing microbial activity. This is supported by research from Soetedjo et al. (2020) reported that the application of a 500 g/polybag dose of organic waste increased the phosphorus and nitrogen content in alfisol soil compared to without the addition of organic waste. This increase in nutrients could have occurred because organic matter decomposed when it was submerged in the soil. Some of the submerged organic matter was utilized by soil microorganisms as an energy source, which in turn helped maintain nutrient availability in the soil; some oxidized, releasing CO_2 into the atmosphere; and some, along with the microorganisms, died and became residue left in the soil.

The synergistic interaction among these three elements-AMF, rice straw compost, and rock phosphate, was the key to their effectiveness. The compost nourished the AMF, which in turn mobilized the insoluble phosphorus from the rock phosphate (Duan et al. 2022)**.** This made P available to the host plant, even in soils where it would typically be fixed and inaccessible. This integrated approach not only boosted P uptake but also enhanced the plant's overall ability to acquire other macro and micronutrients, leading to improved plant growth, dry matter production, and overall yield (Kumari et al. 2024).

Table 2. Effect AM inoculum, rock phosphate, compost and carbofuran on AM spore density and AM infectivity

Treatment	**AM infectivity (%)**	**AM spore density (spores/ 100 g soil)**
F Value		
M	0.01 **	0.01 **
P	0.01 **	0.01 **
M×P	0.01 **	0.01 **
Interaction (P×M)		
M0P0	36.33±0.58 j	224.67±0.58 l
M1P0	40.67±1.15 h	263.67±0.58 k
M2P0	37.67±0.58 i	293.00±1.00 j
M3P0	47.00±1.00 e	337.00±1.00 h
M0P1	41.33±1.15 h	341.67±1.53 f
M1P1	45.00±1.00 f	352.67±1.15 d
M2P1	47.67±0.58 e	336.00±1.00 h
M3P1	60.33±0.58 b	363.67±1.53 b
M0P2	34.67±0.58 k	218.67±0.58 m
M1P2	41.67±0.58 h	331.00±1.00 i
M2P2	43.67±0.58 g	345.33±1.15 e
M3P2	57.33±0.58 c	356.67±0.58 c
M0P3	47.67±0.58 e	339.00±1.00 g
M1P3	48.00±1.00 e	353.67±0.58 d
M2P3	55.33±0.58 d	353.67±0.58 d
M3P3	68.67±0.58 a	369.00±1.00 a

Note: **: $p<0.01$, *: $p<0.05$, and ns: Not significant. Means followed by the same letter are not significantly different at a significance level of 5% by Duncan's Multiple Range Test (DMRT)

Table 3. Effect of AM inoculum, rock phosphate, compost and carbofuran on plant growth in the pot experiment

Treatment	**Plant height (cm)**	**Plant fresh weight (g)**	**Plant dry weight (g)**	**P concentration (%)**	**P uptake (mg/plant)**
F Values					
M	0.01*	0.01**	0.01**	0.01 **	0.01 **
P	0.17 ns	0.01**	0.01**	0.01 **	0.01 **
M×P	0.35 ns	0.01**	0.01**	0.01 **	0.01 **
Interaction (P×M)					
M0P0	62.92±1.23	42.48±0.28 kl	22.96±0.63 h	0.12±0.00 g	1.63±0.00 k
M1P0	66.00±2.15	44.91±0.41 j	23.61±1.20 gh	0.13±0.01 f	1.73±0.00 j
M2P0	66.66±1.78	46.17±0.51 i	24.11±0.57 fg	0.14±0.01 e	1.76±0.00 i
M3P0	71.16±4.26	47.45±0.47 h	26.52±0.83 e	0.14±0.01 e	1.73±0.00 j
M0P1	64.09±6.51	44.55±0.65 j	25.93±0.68 e	0.12±0.00 fg	1.78±0.00 h
M1P1	68.19±5.14	46.79±0.78 hi	23.15±0.35 gh	0.15±0.00 d	1.76±0.01 i
M2P1	76.33±2.65	48.51±0.53 g	23.49±0.15 gh	0.15±0.00 de	1.83±0.01 f
M3P1	76.71±6.70	64.63±0.61 b	29.99±0.23 b	0.18±0.01 b	2.48±0.00 b
M0P2	62.42±4.18	41.78±0.38 l	20.35±0.14 i	0.10±0.00 h	1.56±0.01 l
M1P2	65.76±7.12	43.17±0.67 k	24.88±0.28 f	0.12±0.01 fg	1.73±0.01 j
M2P2	70.80±4.76	46.04±0.17 i	28.51±0.04 cd	0.15±0.00 de	2.07±0.00 d
M3P2	72.00±1.24	63.06±0.17 c	29.10±0.76 bc	0.17±0.01 c	2.34±0.00 c
M0P3	66.23±5.42	55.11±0.19 f	25.93±0.28 e	0.16±0.00 d	1.80±0.00 g
M1P3	69.76±3.48	56.15±0.60 e	28.33±0.54 cd	0.16±0.00 d	1.89±0.01 e
M2P3	71.50±3.67	58.40±0.48 d	27.75±0.41 d	0.16±0.00 d	2.34±0.01c
M3P3	77.66±10.93	67.45±0.82 a	35.09±0.88 a	0.19±0.00 a	2.77±0.01 a

Note: **: $p<0.01$, *: $p<0.05$, and ns: Not significant. Means followed by the same letter are not significantly different at a significance level of 5% by Duncan's Multiple Range Test (DMRT)

An interesting finding was that the M3P2 treatment, which combined AM from monocropping and intercropping systems (M3) with carbofuran application (P2), showed the third-highest increase in soil chemical properties. This suggests that AMF have the potential to tolerate carbofuran, allowing them to maintain their beneficial effects on soil nutrient levels even with carbofuran application. Assaf et al. (2009) found that the application of metalaxyl fungicide at 50 g/kg soil could not eliminate AM propagules in the soil. This supports the findings of this experiment, indicating that AM can survive exposure to certain doses of pesticides and that different AM species may respond differently.

Effects of the application of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on mycorrhiza infectivity and spore density

The M3P3 treatment indicated the highest mycorrhizal colonization rate and spore density. This indicates that this treatment produced higher mycorrhizal infection and spore density than other treatments. Research by Sammama et al. (2022) reported that in the intercropping system, mycorrhizal colonization increased significantly in both soft wheat and faba bean plants. Soft wheat experienced a rise in colonization from 48.68% to 53.94%, while faba bean increased from 52.02% to 71.74%, compared to their monocropping values. Faba bean plants had a higher colonization rate than soft wheat plants in the intercropping arrangement. Additionally, the number of spores per 100 g^{-1} of soil increased by 56.02% in faba bean and 101.38% in soft wheat in co-inoculation and intercropping systems compared to monocropping. This aligns with a study by Lu et al. (2023), which reported that intercropped maize had 125% more AMF communities than monocropping maize. However, intercropped soybean had only 25% of the AMF community observed in monocropping soybean. This demonstrates that diverse mycorrhizae are more effective in increasing spore density and mycorrhizal infection. Ghorui et al. (2023) also supported this, reporting that the diversity and abundance of mycorrhizal fungi were significantly influenced by environmental and agricultural practices. Sustainable practices like intercropping, reduced tillage, and the use of organic amendments were critical for maintaining and enhancing mycorrhizal diversity (Chaudhary et al. 2025).

The M3 treatment also demonstrated the ability to tolerate carbofuran, as shown by the increase in spores and mycorrhizal infections in the M3P3 treatment, which were higher than in the other treatments. This is supported by a study by Buysens et al. (2015), which found that the addition of fungicides did not affect the presence of mycorrhizal spores. Similar findings were reported by Baysal and Miller (2009), who explained that, at the first harvest, 10 days after pesticide application, the shoot diameter and dry weight of shoots and roots were smaller in the control group. However, after AMF inoculation, plant growth showed improvement.

Following M3P3, the treatment M3P1 showed a higher level of mycorrhiza infectivity and spore density than the treatment M3P2, indicating that the addition of rock phosphate and rice straw compost (P1) contributed to higher mycorrhiza infectivity and spore density than the application of carbofuran (P2). This was supported by research Boutasknit et al. (2024), who reported that during the recovery phase (rehydrated 5 days after drought stress), the intensity of mycorrhizal colonization on carob seeds increased by 8% in the AMF+RP (Rock Phosphate) treatment and increased by 2% in the AMF+VC (Vermicompost)+RP (Rock Phosphate) treatment compared to the application of mycorrhizae alone. Yang et al. (2018) also reported that the application of cow manure and maize straw compost significantly increased AM root colonization and Extra Radical Hyphae (ERH) density compared to the control. The addition of rice straw compost could increase the activity of mycorrhizal fungi because it was a source of essential nutrients and organic carbon that supported metabolism, colonization, and spore production (Ma et al. 2024). In contrast, research by de Novais et al. (2019) reported that the application of the herbicides dicamba and glufosinate and the fungicides benomyl and fenhexamide decreased the length and density of Extra Radical Mycelium (ERM), while some mycorrhizosphere bacteria increased the frequency of anastomosis and hyphal branching. Thus, application of pesticide alone (without combination with organic matter) impacted to adverse effect on to host plant and AMF. Research by Vasilikiotis et al. (2020) had reported that the level of AMF colonization in the organic management system in the research station site with the treatments of resident vegetation, compost, and no fumigation, was higher than in the conventional management system with the treatments of bare soil, no compost, and fumigation. The AMF colonization was 73.2% in the organic management system and 62.1% in the conventional management system. Thus, the study of (Vasilikiotis et al. 2020) supports the present findings that the combined application of rock phosphate and rice straw compost resulted in significantly higher AM colonization than carbofuran application.

Effects of the application of arbuscular mycorrhizal inoculum, rock phosphate, compost, and carbofuran on plant growth of maize crops

The M3P3 (mycorrhizal spores from monoccropping and intercropping systems with rock phosphate, rice straw compost, and carbofuran) treatment showed a significant effect to increase plant growth, tissue phosphorus concentration, and phosphorus uptake. This indicated that a mixture of AM spores obtained from different planting systems (monocropping and intercropping systems) gave higher plant growth than from a single source. This was supported by Ishaq et al. (2021), who reported that the intercropping system was able to provide a better environment for a diverse and abundant population of mycorrhizal fungi. In an intercropping system, the combined root secretions from different plants created a complex chemical environment in the soil, which in turn supported a greater variety of AMF species and increased the overall diversity of the mycorrhizal community (Zhang et al. 2024). In intercropping systems, the arrangement of mycorrhizal hyphal networks can become stronger, increasing the abundance of mycorrhizal spores on plant roots, which in turn enhances plant growth (Hu et al. 2019).

In the M3P3 treatment, a combination of AM spores from the rhizosphere of maize in monocropping and intercropping systems (M3) was mixed with rock phosphate, rice straw compost, and carbofuran (P3). The addition of rock phosphate in this combination helped provide nutrients for the plants, particularly phosphorus (P). Wahid et al. (2019) also confirmed that the association of AMF with manure given RP compost fertilizer had a positive effect on the biomass of shoots (3.04 g) and roots (2.62 g). The use of microorganisms, including AMF, could be one approach in the rock phosphate dissolution process (Hazzoumi et al. 2022). Mycorrhizal Fungi excreted organic acids and enzymes, such as phosphatases (Rawat et al. 2021), that could dissolve the insoluble compounds in rock phosphate (Ahmed et al. 2021).

The highest increase of soil fertility and plant growth in the treatment M3P3 was also contributed from the rice straw compost effect. The addition of rice straw compost in the M3P3 treatment increased soil fertility and plant growth. This aligns with the research by Soetedjo (2017), who reported that application of that the application of buried rice straw was able to increase the growth and yield of sweet corn. The use of compost enhanced nutrient availability and promoted functions such as respiration, photosynthesis, and the antioxidant system (Mbarki et al. 2020).

The highest plant growth in the M3P3 treatment indicated that the inoculated mycorrhiza and maize plant demonstrated the tolerance to carbofuran, which was applied at a two-fold dose. This result, supported by Yan et al. (2021), also reported that seeds coated with carbosulfan had a mycorrhizal colonization rate of 70% after 49 days.

The limitations and external validity

The study's limitations and external validity were influenced by several factors, including the specific type of soil, the location of the inoculum source, and the specific crop and varieties utilized. This experiment exclusively used the specificity of soil type, namely Alfisol soil, which was characterized by low productivity, low organic carbon (0.9%), and low available phosphorus (6.2 ppm). The finding that the combined M3P3 treatment significantly improved soil fertility was directly valid for other Alfisol-type soils but had limited applicability to other soil orders (e.g., more fertile Mollisols or extremely weathered Oxisols).

The second factor limiting the study's external validity was the specific geographical source of the inoculum. The AMF spores were isolated from the rhizosphere of maize fields in a specific region (Sukosari Village, Central Java). The effectiveness of the mixed inoculum (M3) depended on the specific AM species present in that local source, and it was not guaranteed that this specific combination of AMF would perform identically in other geographical locations with different native microbial communities and environmental pressures.

The final factor limiting the external validity of this study was the specificity of the plant types and varieties used. In this experiment, AMF boosted plant fresh weight by 58.7%; this effect was expected to change significantly across other maize varieties or different plant species, even within the same soil type. Therefore, these findings held the greatest external validity for maize cultivated in Alfisol soil under similar climatic conditions.

In conclusion, the treatment of M3P3, which combined a mixture of AMF spores from the maize rhizosphere in monocropping and intercropping systems (M3) with a mixture of rock phosphate, rice straw compost, and carbofuran (P3), showed the highest increase in soil nutrient status (organic C (89.4%), Total N (75%) and avalaible P (26.5%)), maize plant growth (plant fresh weight (58.7%), plant dry weight (52.8%), and P uptake (69.9%)), AMF infection (89.01%) and AMF spore density (62.24%), compared to the control. The treatment combination of M3P1 resulted in the second-highest level to all variables for soil nutrient status and maize growth. These results confirmed that the M3 inoculation was more effective compared to M1 (AMF spores from maize rhizosphere in monocropping system) and M2 (AM spores from maize rhizosphere in intercropping system) in supporting plant growth and enhancing soil nutrients, besides the contribution in increasing nutrient uptake. Further research is needed to reveal the functional capacity of AMF in increasing plant growth with high tolerance or resistance to carbofuran in the natural field conditions. For smallholders growing crops like maize, it was necessary to use a synergistic combination of readily available farm resources to enhance soil fertility and plant nutrient uptake.

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