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Co-application of Arbuscular Mycorrhizae via Seed Coating and Phosphorus Fertilizer for Enhancing Growth, Yield, and Nutrient Uptake in Ultisols for Maize

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ABSTRACT

A field trial was conducted to determine the efficiency of AMF inoculation for enhancing growth and yield performance of maize, root colonization, soil available P, and P uptake in Ultisols amended with different P dosages. This experiment was conducted in a 3 x 3 factorial scheme under complete randomized design with three AMF inoculation methods and three different P levels (0, 60, and 120 kg P_2O_5 ha^{-1}) in five replications. Data were collected on plant growth, yield parameters, soil available P, P uptake, and root colonization. Results showed that inoculation via seed coating at 60 kg P_2O_5 ha^{-1} gave a significant ($p<0.05$) increase in growth traits (average of 25%), biomass production, grain yield (average of 30%), soil P availability, P uptake, and root colonization. Inoculation via seed coating combined with 60 produced the same values of all the tested parameters with and seed coating inoculation and soil inoculation at120 kg P_2O_5 ha^{-1}. Thus, seed coating was as effective tool as soil inoculation for AMF delivery and capable of reducing 50% of P fertilizer use. For these reasons, co-application of P fertilizer and AMF inoculation via seed coating could be practiced to improve corn yields on poor Ultisols.

Keywords: AMF inoculation, maize growth, soil phosphorus, seed coating, Ultisols

INTRODUCTION

Corn contributes roughly two thirds of the energy in human diets worldwide, along with rice and wheat, demonstrating the crop's economic importance and nutritional worth (Cassman *et al*., 2003). The opportunity to increase corn production is tremendous, especially in Ultisols. The reason is that the Ultisols acreage is about 45.8 million ha or 24% of the land area of Indonesia. However, Ultisols have disadvantages such as low organic matter content, pH less than 5, and aluminium (Al) toxicity, the low concentrations of extractable phosphorus (P) due to fixation by aluminium (Al) and/or iron (Fe) oxides, resulting in the inhibition of root growth and function and finally reducing crop yields (Pineros *et al*., 2011).

Corn relies on phosphorus (P) as a crucial nutrient for its growth and development. In areas featuring Ultisols soil type and employing intensive farming techniques, farmers use highly soluble P fertilizers like super phosphate to achieve increased corn yields. Despite their effectiveness, these fertilizers come at a high cost and may lead to environmental issues (Schröder *et al*., 2011). Following their application, a significant amount of the phosphorus from the fertilizer undergoes a transformation into a non-extractable form, primarily due to the soil's low pH.

Deficiency of P due to retention of this nutrient by other elements in the soil such as Fe, Al or Ca so that it it becomes unavailable to plants is a major problem in Ultisols. This problem can be solved by introducing arbuscular mycorrhizal fungi (AMF). This fungus develops the extensively external mycelium that grows beyond the nutrient depletion zones and colonizes plant roots. Establishing a colonization enhances the surface area, leading to a heightened capacity for the absorption and uptake of P. For instance, mycorrhiza can contribute significantly, providing up to 80% of the acquired phosphorus (Sharma *et al*., 2023).

Introducing AMF propagules into contact with seeds or roots or also called inoculation can be accomplished via soil inoculation or seed coating (O'Callaghan, 2016). Soil inoculation is the most common and simplest way of inoculation, but also proved the least efficient method. The reasons are that first, the AMF inoculum can only be applied to plants that have grown actively. Second, its application in the field requires more time, energy and higher cost, and third, it is not known exactly the number of spores applied (Rocha *et al*., 2019a). The current method of inoculation is impractical due to the indiscriminate distribution of the inoculum across extensive surface areas, leading to elevated costs per plant.

The beneficial effects of introducing AMF into soil on enhancing plant growth and nutrition under greenhouse experiment and yield of various crops under field conditions have been investigated (Rocha *et al*., 2019a). However, the capability of AMF inoculation via seed coating to promote different crop growth and yield is still poorly understood (Rocha *et al*., 2019b).

The application of seed coating, where a specific active compound envelops the seed, has the potential to reduce the required amount of inoculum with precision (Rocha *et al*., 2019a, 2019c; Accinelli *et al*., 2018a, 201b). Marwanto *et al*. (2020) carried out a greenhouse experiment focused on seed coating related to AMF. The objective was to identify the most effective blends of PVA (polyvinyl alcohol) +TS (tapioca starch) as adhesive agents for inoculating AMF spores via seed coating, aiming to enhance spore viability and their beneficial effects on maize in the greenhouse. The results indicate that seed coating with a 50% PVA+50% TS blend increased mycorrhizal root infestation, AMF spore density, and phosphorus levels in shoots. The optimal seed coating identified in the greenhouse study was then applied in a field experiment, and the findings are detailed in this article.

Mycorrhizal inoculum coating seeds has been shown by Oliveira *et al*. (2016) to be equally effective as soil inoculation in increasing the dry weight of wheat shoots and seed spikes. Higher nutrient concentrations were observed in plants that were seed coated for inoculation, especially in P. As a result, seed coating presents itself as a potential technology for AMF application in open-air agricultural fields.

Limited information exists regarding the impact of P fertilizer and mycorrhizal inoculation on the growth and yield of maize in non-sterilized soil, particularly in field conditions where applied AMF must contend with the native AMF population. Therefore, Therefore, the study on collaborative participations between AMF and maize as the host plant grown under field conditions is imperative to optimize the positive impact of AMF. This study sought to determine the efficiency of AMF application for enhancing growth and yield performance of maize, root colonization, soil available P, and P uptake in Ultisols amended with different P dosages.

MATERIALS AND METHODS

Planting preparation

A field trial was performed in 2019 at the Research Plot of Agriculture Faculty located on the outskirts of the Bengkulu University campus, Indonesia. Before beginning the experiment, the soil and cow dung was analysed primarily for its chemical properties. The soil used was Ultisols with the following characteristics: pH (H_2O) 4.9, 3.43% organic matter, 0.19% total nitrogen (N), 4.71 ppm extractable phosphorus (P), 0.60 me 100 g^{-1} exchangeable potassium (K), and 0.49 me/100 g exchangeable aluminium (Al). The cow dung properties were C/N ratio 10, 1.28% total N, 0.60% available P, 1.65% total K, and 0.43% calcium (Ca).

The land at the experimental site was prepared so that it could be planted. Following that, the land was split into three blocks, each with nine plots. Every plot had dimensions of 3.75 m × 1.50 m, and there was a 1.0 m gap between blocks and 0.50 m between plots. All plots received a basal fertiliser containing 225 kg N ha^{-1} of urea, 120 K_2O/ha of KCl, and 10 tonnes ha^{-1} of cow dung.

Experimental design

The study employed a randomized complete block design with a 3x3 factorial arrangement, including three AMF spore inoculations (via seed coating, conventional soil inoculation, and without AMF spore inoculation) and three P application rates (0, 60, and 120 kg P_2O_5 ha^{-1}). Non-inoculated maize plant without P application was grown as a control. There were a total of 27 plots in the experiment because each treatment combination was duplicated three times.

Arbuscular mycorrhiza fungus inoculation and seed sowing procedure

The carrier for AMF spores was a sterile mixture of zeolite and peat (1:1 v/v). The'mother inoculum' consisted of spores from the AMF species *Glomus mossae*, suspended in water. This mixture was in sterile conditions combined with a little quantity of the agent for introducing inoculum, resulting in an inoculum with a spore density of 200 spores/g. In order to ensure that the AMF spores were uniformly dispersed across the carrier particles' surface, the volume of AMF spore suspension and carrier medium was fine-tuned to ensure that the solution nearly saturated the medium.

For determine the initial seed quality determination, the hybrid maize cultivar BISI seed was obtained from a local market and subjected to a germination test at the Agronomy Laboratory of Bengkulu University. Batches of high-quality maize seeds, with germination rates exceeding 90%, underwent a surface sterilization process. This involved immersing the seeds in a 3% H_2O_2 solution for five minutes, succeeded by five rinses with sterilized water. In the transfer chamber, the seeds were then left to air dry for the entire night on sterile filter paper. After misting the maize seed with deionized water, it was coated by progressively spraying a 50% polyvinyl alcohol (PVA) + 50% tapioca starch (TS) blend as a binding agent (Marwanto *et al*., 2022). An inoculum carrier containing AMF spores was then added, and the process continued until the seed was uniformly coated. By weight, the ratio of inoculum carrier, adhesive, and maize seed was 10:1:1. An average of at least 300 AMF spores were loaded into each coated maize seed. The approximate number of spores per gram of the inoculum carrier was determined using the Most Probable Number (MPN) method. Field tests were then conducted using the coated seeds.

Inoculated plants via soil inoculation and seed coating received the same amount of 1.50 g of inoculum carrier containing 300 spores of *Glomus mossae*. Soil inoculation was done at sowing by directly applying the inoculum into the planting hill. The inoculum was placed 2 cm below one uncoated seed. One corn seed were sown in each plant hill at a spacing of 75 cm x 30 cm between plants. Phosphorus was applied as single superphosphate at 0, 60, and 120 kg P_2O_5 ha^{-1}. Crop care included the use of glyphosate as a pre-planting herbicide followed by manual weed control during the crop cycle. Pesticides such as Pyrethroids, Triazoles were applied on corn plots to control insect pests, diseases such as gray leaf spot and northern corn leaf blight, respectively.

Plant harvest, sampling and measurements

In the field, seeds with and without inoculation were planted with the three P application rates and grown until maturity stage. At the R4-R5 phenological stage (60 days after planting), plant growth, biomass production, P uptake, and root colonization were determined. While at maturity stage (125 days after planting), grain weight per plot and its components (100 seed weight, grain weight per cob and soil available P were determined from sampled plants.

Using a metre ruler, plant height was measured from base to highest leaf tip. Entire maize plants were severed at the soil surface. Maize plants were completely removed at the soil surface, weighed, oven-dried at 70 ^{0}C for 72 hours in tagged paper pouches, and then dry mass was recorded. After being cleaned, the roots were weighed, placed in tagged paper pouches, oven-dried at 70 ^{0}C for 72 hours, and dry mass was noted. To measure the grain yield and its components, plants were randomly selected from each plot in 1 m^2 area and all the yield components were recorded on them.

To assess P uptake, plant leaves underwent cleaning with purified water and were then oven-dried at 70 °C until an unchanging weight was achieved. The dried foliage's were powdered and subjected to digestion using a 2:1 blend of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Calorimetry was employed to determine the P content within the processed plant samples.

To determine root colonization, chosen roots were sliced into segments of 1-2 cm in length and mounted on slides for microscopic examination (x250). A hundred root segments were chosen without bias from each specimen, and mycorrhizal mycorrhizal presence in the roots was determined using the standard protocol.

After harvest, soil samples (0- to 15-cm layer) were collected and sieved (2 mm), air-dried, and analysed for bicarbonate-extractable (Olsen) P concentrations with standard procedure.

Statistical data analysis

The collected data underwent a statistical analysis using variance assessment. Post-hoc examination of means among treatments was performed using the DMRT approach (*p<0.05*). Statistical analyses were conducted using SPSS software version 20 (IBM SPSS Inc.).

RESULTS AND DISCUSSION

Results show that AMF inoculation combined with P application strongly affected growth and biomass related traits (Table 1). Inoculation via seed coating combined with 60 kg P_2O_5 ha^{-1} produced comparable figures of all the tested attributes to seed coating and soil inoculation at 120 kg P_2O_5 ha^{-1}, but had greater magnitudes of the traits compared to the rest of the treatments. Data obtained in this study were parallel with the results reported by Rocha *et al*. (2019a). They explained that the beneficial impact of AMF inoculation was probably greatest at low presence of nutrient, especially P. These results suggest that AMF inoculation via seed coating was only effective in promoting maize growth in soil treated with a half dose of recommended P. Thus, seed coating for AMF inoculation was capable of reducing a half amount of applied P without disturbing maize growth and biomass production. dosages.

Identical to the case of corn growth, AMF inoculation combined with P application greatly influenced plant yield and its components (Table 2 and 3). Inoculation via seed coating combined with 60 kg P_2O_5 ha^{-1} produced similar magnitudes of all the tested parameters to seed coating and soil inoculation at 120 kg P_2O_5/ha, but had higher values of the traits compared to the rest of the treatments. This response means that AMF inoculum introduced via seed coating was successful to induce P uptake under medium P application. The probable reasons for this success is thar unlike soil inoculation, seed coating provides readily available AMF spores at the seed-soil interface and immediate accessibility of AMF spore at seed germination and initial plant development stages. Therefore, the extended soil network of fungal hyphae produced from AMF spore will colonize the roots (Table 3) and subsequently exploration of more soil volume was successfully established. With more developed roots, P acquisition and uptake was enhanced. On the contrary, less effective of soil inoculation under reduced P fertilization might be due to conversion of soluble P to non-extractable form under low soil pH in this study after its application (Table 4) as also explained by Schröder *et al*. (2011). The pH (H_2O) of soil used in this study was 4.90 so that the conversion of soluble P to non-extractable form was inevitable. Table 4 showed that the magnitudes of water extractable P soil in soil inoculation treatments were lower that those in seed coating inoculation irrespective of applied P doses except at 120 kg P_2O_5 ha^{-1}. This result also suggests that AMF inoculation via seed coating was only effective in promoting maize yield and its components in soil applied with a half dose of recommended P fertilization. Moreover, AMF inoculation via seed coating was able to reduce applied P by 50%. These results agree with previous study by Marwanto *et al*. (2020) who reported that introduction of AMF in soils with elevated P applicatin rates had little effects on the harvest of wheat in open-field cultivation. The improvements in plant growth and yield resulting from the use of coated microbial fertilizer at reduced rates of chemical P fertilizer inputs are pertinent to sustainable and eco-friendly agricultural practices.

Similar to findings in prior studies, the introduction of AMF through inoculation led to yield increases of up to 32% in corn (Sene *et al*., 2023). In this current research, the inoculant triggered even greater improvements, reaching an average increment of 40%. These outcomes highlight that incorporating the inoculant, particularly through seed coating in Ultisols soils with limited available P, leads to substantial enhancements in corn grain yield.

Table 1. Maize growth under different AMF inoculation and P application at maturity stage

Treatment	P_2O_5 (kg ha^{-1})	Plant height (cm)	Shoot fresh weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)
	0	116.9 d	10.33 e	11.70 e	4.5 e
Control	60	127.5 c	15.34 d	15.34 d	6.0 d
	120	126.1 c	14.80 d	14.80 d	6.7 d
	0	125.8 c	25.30 c	25.30 b	8.9 c
Soil inoculation	60	125.5 c	33.90 b	33.90 b	10.9 b
	120	156.4 a	44.91 a	44.91 a	13.1 a
	0	146.8 b	24.54 c	24.54 c	11.9 b
Seed coating	60	163.6 a	44.34 a	44.34 a	15.2 a
	120	164.1 a	45.21 a	45.21 a	14.9 a

Note: No significant differences between numbers in the same column when followed by the same letters based on the Duncan test at the 5% level.

Table 4 lists the average values of phosphorus uptake, water extractable P-soil, and root colonization in maize under different AMF inoculation and P applications. The treatments were positively affected these traits. The application of seed coatings led to increased levels of these characteristics and the highest were noticed at 60 kg P_2O_5 ha^{-1}, which was on par with the seed coating and soil inoculation at 120 kg P_2O_5 ha^{-1}, yet demonstrated a markedly higher level compared to other treatments ($p<0.05$). These result of this study with specific case of root colonization was parallel with that reported by Sene *et al*. (2023). The higher values for these parameters were associated with the immediate accessibility of AM spores delivered via seed coating as explained by Colla *et al*. (2015). The explanation was supported by data from a preliminary greenhouse study conducted by Marwanto *et al*. (2020), which also serves as an integral part of this experiment. The obtained data showed that root colonization by fungi, sporulation of AMF, and P levels in shoots was higher in AMF inoculated plants via seed coating than via soil inoculation.

Table 2. Maize grain yield components under different AMF inoculation and P application at maturity stage

Treatment	P_2O_5 (kg/ha)	100 grain weight (g)	Shoot fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})
Control	0	126.9 c	0.427 e	0.267 e
	60	127.5 c	0.853 d	0.622 d
	120	126.1 c	1.191 cd	0.742 c
Soil inoculation	0	125.8 c	1.547 c	0.996 bcd
	60	125.5 c	2.016 b	1.278 b
	120	156.4 a	2.898 a	2.189 a
Seed coating	0	146.8 b	1.511 c	1.049 b
	60	163.6 a	3.022 a	2.315 a
	120	164.1 a	2.827 a	2.285 a

Note: No significant differences between numbers in the same column when followed by the same letters based on the Duncan test at the 5% level

Table 3. Maize grain yields under different AMF inoculation and P application at maturity stage

Treatment	P_2O_5 (kg ha^{-1})	Grain weight per cob (g)	Grain weight (ton ha^{-1})
Control	0	37.46 c	2.37 c
	60	36.60 c	2.47 c
	120	38.20 c	2.53 c
Soil inoculation	0	39.53 c	2.30 c
	60	36.27c	2.90 c
	120	53.53 a	4.53 a
Seed coating	0	45.45 b	3.45 b
	60	54.06 a	4.35 a
	120	52.75 a	4.26 a

Note: No significant differences between numbers in the same column when followed by the same letters based on the Duncan test at the 5% level

Table 4. Phosphorus uptake, water extractable P-soil, and root colonization in maize under different AMF inoculation and P application at maturity stage.

Treatment	P_2O_5 (kg ha^{-1})	P Uptake (mg plant^{-1})	Water Extractable P-soil (mg kg^{-1})	Root Colonization (%)
Control	0	10.01 c	5.75 e	5.25 e
	60	10.16 c	7.00 e	7.50 d
	120	9.67 c	15.75 d	8.50 d
Soil inoculation	0	9.50 c	27.50 c	13.30 c
	60	10.05 c	47.75 b	39.50 b
	120	15.01 a	46.00 a	58.25 a
Seed coating	0	13.85 b	28.40 c	13.50 c
	60	16.02 a	46.75 a	63.00 a
	120	15.84 a	44.25 a	59.50 a

Note: No significant differences between numbers in the same column when followed by the same letters based on the Duncan test at the 5% level

CONCLUSION

Inoculation with AMF via seed coating improved plant growth, yield and its components, root colonization, P uptake, and soil P availability under reduced P fertilization, whereas the efficacy of seed coating under full dose of P fertilization was lower. AMF inoculation via seed coating reduced P fertilizer requirement by 50% in poor P Ultisols soils. Hence, further researches regarding AMF inoculation via seed coating in maize and other crops are necessary to obtain better understanding of its benefits.

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