Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2021.196.202

Research Article
Effect of Indigenous Arbuscular Mycorrhizal Formulations with Cow Dung on Phosphorus Uptake, Growth and Yield of Local Corn (*Zea mays* L.) in Marginal Land

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Abstract
Background and Objective: Bladygrass (*Imperata cylindrica*) that grows predominantly on marginal dry land can be utilized as an indigenous inoculum source of arbuscular mycorrhiza. It has many benefits, including helping plants absorb nutrients and water. Likewise, cow dung, which contains macro and micronutrients. Has the potential to fix of physical dan biological soil as well as soil fertility. The current research was carried out to study the effect various of arbuscular mycorrhizal formulations with cow dung to phosphorus uptake, growth and yield of local corn (*Zea mays* L.) plant in marginal land. **Materials and Methods:** The study to analyze the effect of indigenous arbuscular mycorrhizal inoculum has been carried out in 2018 in The Field Laboratory of Faculty of Agriculture, Haluoleo University. The design used in this study was Randomized Block Design (RBD) in three groups with a single factor pattern, by using arbuscular mycorrhizal and cow dung treatments. **Results:** The result showed that in the rhizosphere there are various arbuscular mycorrhizal genus which is effective in increasing local corn yields in marginal dry land when combined with cow dung, too increasing of P plant uptake and available P in one month-old plant rhizosphere. **Conclusion:** The use of indigenous arbuscular mycorrhizal inoculum combined with cow dung of 80 g per planting hole to corn plant resulted in dry seed yield increasing to 3.85 t ha^{-1}, whereas in plants without arbuscular mycorrhizal inoculation and without cow dung did not produce seeds.

Key words: Phosphorus absorbtion, root colonization, arbuscular mycorrhizal fungi Cow dung, dryland, indigenous, dungs, marginal, organic fertilizer

Citation: Hasid, R., M.J. Arma, A. Nurmas, Halim and M.W. Arsana, 2021. Effect of indigenous arbuscular mycorrhizal formulations with cow dung to phosphorus uptake, growth and yield of local corn (*Zea mays* L.) in Marginal land. Asian J. Plant Sci., 20: 196-202.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Formulation of arbuscular mycorrhizal with cow dung is very important in crop cultivation system. Cow dung contains a high amount of nutrients essential for plants. It was reported by Huang *et al.*[1] that chemical characteristics of the cattle dung were pH 7.86, N 28.7 g kg^{-1}, C/N 10.7, $NH4^{+}$ 0.02 g kg^{-1}, NO_3^{-} 0.7 g kg^{-1}. According to Kuntyastuti *et al.*[2], cow dung has higher pH (6.21), C/N ratio is less than 10, which means that the nutrients contained in both fertilizers can be quickly utilized by crops. According to Zaman *et al.*[3], cow dung application have been found to be increased the total N, available P, exchangeable K, Ca, Mg, available S, Zn and B contents in soils and biomass yield of plant. The use of organic fertilizers is one means of increasing sustainable crop production and reduces the use of inorganic fertilizers to improve plant growth[4]. In addition to dung, arbuscular mycorrhiza can be used to increase plant yields. According to Aguegue *et al.*[5], the arbuscular mycorrhizal fungi (*G. cubens, R. intraradices* and *F. mosseae*) does not present a significant effect on the growth of corn seedlings when there is no potassium, phosphorus and nitrogen complementary application. The study proves the potential use of the arbuscular mycorrhizal fungi with organic fertilizers like cow dung organic fertilizer.

Arbuscular Mycorrhizal (AM) has a very big role in symbiosis with plant roots. Arbuscular mycorrhizal associations with plants can directly increase plant growth and productivity[6-11]. The presence of AM can increase the availability of nutrients especially phosphate[12], environmental stress tolerance[13] status and uptake of nutrient soil[14-16].

Arbuscular Mycorrhizal (AM) is a type of fungi symbiotic with plant roots and both benefit each other. Bladygrass (*Imperata cylindrica*) is categorized as weeds whose range of proliferation is wide. Bladygrass are capable of adapting to any type of soil and inclement climate. Researches identify mycorrhiza arbuscula symbiosis with the bladygrass roots had reported, the result showed that on vegetation of bladygrass was found several genuses of arbuscular mycorrhizal, with the infecting capability ranging from 56.67-86.67%, the number of spores ranging from 359-401 per 100 g soil. Arbuscular mycorrhizal were found to be dominated by the genus of *Glomus* and *Acaulospora*[17]. These results indicate support for utilizing bladygrass rhizosphere soil as a source of arbuscular mycorrhizae. The current research was carried out to study the effect various of arbuscular mycorrhizal formulations with cow dung to phosphorus uptake, growth and yield of local corn (*Zea mays* L.) plant in marginal land.

MATERIALS AND METHODS

Research design: This research was conducted at the Field Laboratory and Agrotechnology Laboratory of the Agricultural Faculty, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia, in 2018. This research used a Randomized Block Design (RBD) in three groups with single factor pattern, consisting of eight treatments (Table 1). The total number of experimental units consists of 24 units. Each experimental unit was a plot measuring 3m×4 m, with spacing between group 50 cm and the distance between treatment plots in each group of 30 cm. Parameters observed were corn plant growth and productivity, P uptake and available P.

Materials and tools: The materials used throughout the study were corn seeds, cow dung and arbuscular mycorrhizal inoculum. The tools used were hoes, machetes, scopes, measuring tapes, scales, ovens, scissors, petri dishes, measuring cups, object glasses and microscopes, Erlenmeyer, multilevel filters with pore sizes of 355, 125 and 15 μm, beaker, micro pipette, drop pipette, camera and stationeries. Formulate arbuscular mycorrhizae with cow dung. Arbuscular Mycorrhizal (AM) indigenous inoculum formulation plus dung based on the requirement of dung per planting hole with spacing of 75 cm × 20 cm which equaled the dosage of cow dung per hectare. The cow dung used has nutrient content C organic 16.49%, N total 1.65%, P 0.84%, K 0.79%, Na 0.32%, Ca 1.79%, C/N 10, KTK 95.83 me 100 g^{-1} and organic material 28.54% . The indigenous AM inoculum is isolated from bladygrass (*Imperata cylindrica*) with spore population densities ranging ±500 spores per 100 g soil. Further indigenous inoculum AM and cow dung were formulated as in Table 1.

Planting and arbuscular mycorrhiza inoculation: Inoculation of indigenous MA inoculum treatment plus cow dung was done simultaneously planting, by inserting the inoculum into the planting hole before the seeds were planted. Furthermore, the planting of corn seed using plant spacing of 75 cm × 20 cm as much as 1 grain of seed per planting hole.

Evaluation of growth dan yield of corn plant: The plant growth was evaluated at 15 and 30 day after planting (dap). Variables observed were plant height (cm, measured from crop stem base to the tip of the stem), diameter of stem (cm, measured at stem middle point using the sliding term), number of leaves (by counting all fully expanded leaves), leaf area (cm^2, = L × W × C, where, L is leaf

Table 1: Level of treatments indigenous arbuscular mycorrhizal formulations with cow dung

Arbuscular mycorrhizal formulation (g)	Cow dung		Arbuscular mycorrhizal plus cow dung (g)	Symbol
	Per planting hole (g)	Equality (t ha^{-1})		
0	0	0	-	K1
50	0	0	-	K2
0	80	5	-	K3
50	16	1	66	F1
50	32	2	82	F2
50	48	3	98	F3
50	64	4	114	F4
50	80	5	130	F5

length, W is leaf width and C is the constant), dry weight of crop biomass (dried in oven at 80°C for 2×24 hrs), yield of the crops and P uptake (at 30 dap) and available P.

Statistical analysis: Statistical analysis was performed using two-way analysis of variance (ANOVA) and further tests with Honestly Significant Difference (HSD α0.05).

Observation of arbuscular mycorrhiza: Spores of arbuscular mycorrhiza were extracted from soil by wet sieving and decanting method[18]. Infected roots were observed based on the slide method[19]. Root infections were calculated according to the following formula:

$$\text{Root infection (\%)} = \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \times 100$$

Application inoculum of arbuscular mycorrhiza to plant: The indigenous AM inoculum is isolated from bladygrass (*Imperata cylindrica*) with spore population densities ranging ±500 spores per 100 g of soil. Inoculation of indigenous MA inoculum treatment was done simultaneously planting, by inserting the inoculum into the planting hole before the seeds were planted. The planting of corn seed using plant spacing of 75 cm ×20 cm as much as 1 grain of seed per planting hole.

RESULTS

Corn plant growth: The observed corn plant growth included plant height, stem diameter, number of leaves, leaf area, stover dry weight and plant productivity. There was no significant effect on arbuscular mycorrhizal formulation with cow dung on plant growth at 15 days after planting (Table 2). Significant effect formulation of arbuscular mycorrhizal with cow dung to plant on all observed parameters was find on plant growth 30 days after planting (Table 3).

The results of the HSD α0.05 test in Table 3 show that in the plant height parameters 30 days after planting, there is a significant difference in plant height between plants treated with cow dung fertilizer compared to plants not treated with cow dung. The best treatment that obtained on formulation of Arbuscular Mycorrhizal (AM) with Cow Dung (CD) was 0 g AM+80 g CD (K_3) with plant height reaching 41.57 cm, not significantly different with the treatment formulations 50 g AM+16 g CD (F_1), 50 g AM+32 g CD (F_2), 50 g AM+48 g CD (F_3), 50 g AM+64 g CD (F_4) and 50 g AM+80 g CD (F_5) with plant heights, respectively 26.67, 32.00, 31.33, 40.27 and 39.93 cm. The highest stem diameter was obtained in the treatment of arbuscular mycorrhizal formulations of 50 g AM+64 g CD (F_4) and 50 g AM+80 g CD (F_4) with stem diameters achieved, respectively 1.50 and 1.37 cm. The highest number of leaves was achieved in the treatment of 50 g AM+80 g CD (F_5) and 0 g AM+80 g CD (K_3) with 10 leaves each per plant. The highest leaf area was obtained in the treatment of 50 g AM+64 g CD (F_4) and 50 g AM+80 g CD (F_4) with the leaf area achieved, respectively 3007.22 and 2731.02 cm^2 per plant. The highest plant dry weight was obtained in the treatment of 50 g AM+80 g CD (F_5) with a dry weight of 18.55 g per plant, which was not significantly different from the treatment of 50 g AM+64 g CD (F_4), 50 g AM+48 g CD (F_3) and 0 g AM+80 g CD (K_3) with dry weight achieved respectively 15.28, 11.89 and 15.40 g per plant.

There is no significant difference between level formulated of arbuscular mycorrhizal with cow dung on yield of plants, except compared with the formulation of arbuscular mycorrhizal non cow dung treatment (Table 3). The highest plant yield was 3.85 t ha^{-1} by. plant treated with 50 g arbuscular mycorrhizal +80 g cow dung, however no significant different with another level of formulation, except plant treated with formulation of 50 g arbuscular mycorrhizal +16 g cow dung. While at plant by treated formulation of non arbuscular mycorrhizal and non cow dung or using arbuscular mycorrhizal but non cow dung, was no seed production found (Fig. 1).

Phosphorus uptake and availability: Figure 2 shows that the highest plant Phosphorus uptake was obtained in the

Table 2: Vegetative growth of corn plant aged 15 days after planting with treatment in various formulations of arbuscular mycorrhizal plus cow dung

Formulation of arbuscular mycorrhizal (AM) and cow dung (CD)	Plant height (cm)	Diameter of the stem (cm)	Number of leaves (strands per plant)	Leaf area (cm^2 per plant)	Dry weight of stover (g per plant)
0 g AM+0 g CD (K_1)	7.40	0.42	5.67	200.83	4.49
50 g AM+0 g CD (K_2)	8.17	0.42	6.00	241.17	4.86
0 g AM+80 g CD (K_3)	9.30	0.52	5.67	323.87	5.16
50 g AM+16 g CD (F_1)	9.93	0.42	6.00	333.62	5.45
50 g AM+32 g CD (F_2)	9.20	0.38	6.33	278.36	5.31
50 g AM+48 g CD (F_3)	8.50	0.37	5.67	227.35	4.84
50 g AM+64 g CD (F_4)	9.50	0.52	6.33	380.72	5.45
50 g AM+80 g CD (F_5)	9.60	0.45	5.67	248.75	5.24
HSD $\alpha_{0.05}$	ns	ns	ns	ns	ns

Description: ns (not significantly different)

Table 3: Vegetative growth of corn plant aged 30 days after planting with treatment in various formulations of arbuscular mycorrhizal plus cow dung

Formulation of arbuscular mycorrhizal (AM) and cow dung (CD)	Plant height (cm)	Diameter of the stem (cm)	Number of leaves (strands per plant)	Leaf area (cm^2 per plant)	Dry weight of stover (g per plant)
0 g AM+0 g CD (K_1)	12.00^b	0.57^d	7.00^b	711.09^c	2.33^c
50 g AM+0 g CD (K_2)	10.87^b	0.67^c	7.33^b	747.00^{bc}	2.73^{bc}
0 g AM+80 g CD (K_3)	41.57^a	1.33^{ab}	10.00^a	2587.49^{ab}	15.40^a
50 g AM+16 g CD (F_1)	26.67^a	1.05^{bc}	8.33^{ab}	1765.21^{abc}	7.74^{bc}
50 g AM+32 g CD (F_2)	32.00^a	1.23^{ab}	9.00^{ab}	2435.23^{abc}	11.44^{ab}
50 g AM+48 g CD (F_3)	31.33^a	1.27^{ab}	9.33^{ab}	2031.00^{abc}	11.89^a
50 g AM+64 g CD (F_4)	40.27^a	1.50^{ab}	9.33^{ab}	3007.22^a	15.28^a
50 g AM+80 g CD (F_5)	39.93^a	1.37^a	10.00^a	2731.02^a	18.55^a

Description: Values in the same row followed by different letters indicate a significant difference according to HSD at $\alpha = 0.05$. AM (arbuscular mycorrhizal)

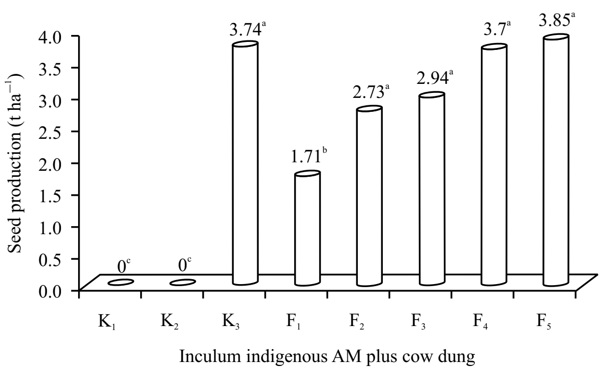

Fig. 1: Seed production of corn plant with treatment in various formulations of arbuscular mycorrhizal plus cow dung

Description: Values followed by different letters indicate a significant difference according to HSD at $\alpha = 0.05$, AM: Arbuscular mycorrhizal, CD: Cow dung, K_1: 0 g AM+0 g CD, K_2: 50 g AM+0 g CD, K_3: 0 g AM+80 g CD, F_1: 50 g AM+16 g CD, F_2: 50 g AM+32 g CD, F_3: 50 g AM+48 g CD, F_4: 50 g AM+64 g CD, F_5: 50 g AM+80 g CD

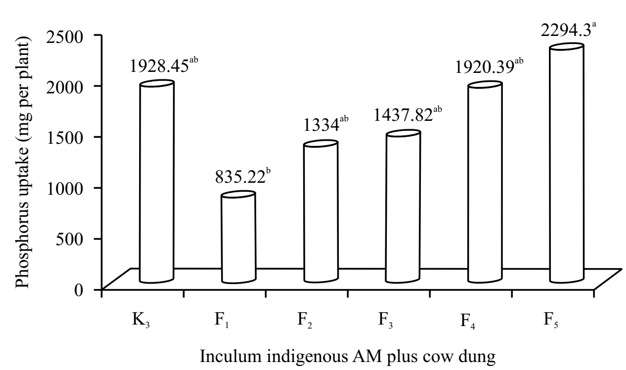

Fig. 2: Phosphorus uptake of corn plant with treatment in various formulations of arbuscular mycorrhizal plus cow dung

Description: Values followed by different letters indicate a significant difference according to HSD at $\alpha = 0.05$, AM: Arbuscular mycorrhizal, CD: cow dung, K_1: 0 g AM+0 g CD, K_2: 50 g AM+0 g CD, K_3: 0 g AM+80 g CD, F_1: 50 g AM+16 g CD, F_2: 50 g AM+32 g CD, F_3: 50 g AM+48 g CD, F_4: 50 g AM+64 g CD, F_5: 50 g AM+80 g CD

treatment of 50 g AM+80 g CD (F_5), that is 2294.3 mg per plant, which was significantly different from the treatment of 50 g AM+16 g CD (F_1) with Phosphorus uptake only reaching 835.22 mg per plant but not significantly different from other treatments. Likewise the available Phosphorus (Fig. 3), highest was obtained in the treatment of 50 g AM+80 g CD (F_5), which was not significantly different from the 0 g AM+80 g CD (K_3) treatment, with available Phosphorus respectively of 14.29 and 12.88 mg kg^{-1} soil.

As one form of increased nutrient uptake due to arbuscular mycorrhizal inoculation seen in Phosphorus uptake of plants. Phosphorus uptake in plants inoculated with arbuscular mycorrhizal is higher than plants without

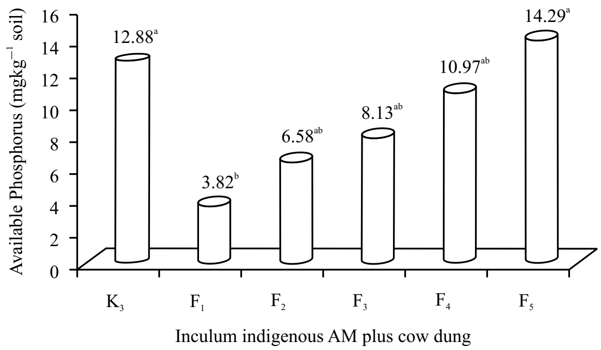

Fig. 3: Available phosphorus in planting medium with treatment in various formulations of arbuscular mycorrhizal plus cow dung

Description: values followed by different letters indicate a significant difference according to HSD at $\alpha = 0.05$, AM: Arbuscular mycorrhizal, CD: Cow dung, K_1: 0 g AM+0 g CD, K_2: 50 g AM+0 g CD, K_3: 0 g AM+80 g CD, F_1: 50 g AM+16 g CD, F_2: 50 g AM+32 g CD, F_3: 50 g AM+48 g CD, F_4: 50 g AM+64 g CD, F_5: 50 g AM+80 g CD

inoculation at the same dung dose (Fig. 2) although P is available in planting medium not a significant difference (Fig. 3). Phosphorus uptake of plant can be increased through its symbiotic with arbuscular mycorrhizae but Phosphorus uptake may proceed well if supported with adequate levels of Phosphorus in the soil.

DISCUSSION

The effect of inoculation arbuscular mycorrhizal to corn plant in the field showed that was able to well infect the plant in the field. In addition to the development of arbuscular mycorrhizal on plant roots, in corn plants have also increased growth and yield with the occurrence of root infections by arbuscular mycorrhiza. This is an indication that the symbiosis between arbuscular mycorrhizal and corn plants has been well.

The observed corn plant growth included plant height, stem diameter, number of leaves, leaf area, stover dry weight and plant productivity. Overall indicate there was no significant effect on arbuscular mycorrhizal formulation with cow dung on plant growth at 15 days after planting (Table 2). A significant effect was find on plant growth 30 days after planting (Table 3).

As in plant growth 30 days after planting, plant production in a significant increase in plant inoculated of arbuscular mycorrhizal combined with cow dung (Fig. 1). There were no significant differences in the yield of corn plant inoculated arbuscular mycorrhizal combined with dung in dose various. The difference in plant yield was only seen when compared with plant inoculated +16 g cow dung per planting hole (F_1), its seed yield only 1.71 t ha^{-1}, while the plant non inoculated and non fertilizers did not produce seeds. Whenever the use of AM inoculum combined with dung of 80 g per planting hole resulted in dry seed increasing, yield reach 3.85 t ha^{-1}. This is in line with the results of increased nutrient uptake due to arbuscular mycorrhizal inoculation seen in P uptake of plants. Phosphor uptake in plants inoculated with arbuscular mycorrhiza is higher than the plants without inoculation at the same cow dung dose (Fig. 2) although P is available in planting medium not significant difference (Fig. 3). Phosphor uptake of plant can be increased through its symbiotic with arbuscular mycorrhizae but P absorption may proceed well if supported with adequate levels of P in the soil. As the results of this study that the highest P uptake was obtained in the P available in the highest growing media, in the treatment of 50 g mycorrhizal arbuscular+80 g cow dung (F5, Fig. 2 and 3). The symbiosis of plants with arbuscular mycorrhizal, in addition, can help increase the P uptake of plant, can also increase the available P in the rhizosphere of plant, measurable before being absorbed by plants. The concentrations of P present in the soil solution can be very low and reach the minimum concentration that can be absorbed by the root, this occurs as a result of the phosphate absorption process present in the root of the plant. However, with symbiosis of plants with arbuscular mycorrhizal, the availability P on the soil of plant rhizosphere can be increased. The role of arbuscular mycorrhizal in increasing availability of P through the process of chemical modification by arbuscular mycorrhizae in plant roots, so that the plant exudes organic acids and acid phosphatase enzymes that spur the process of mineralization P. Gianfreda[20], characteristic compounds in the root exudates are organic acids such as citrate, malate, fumarate, oxalate and acetate, carbohydrates such as glucose, xylose, fructose, maltose, sucrose, galactose and ribose, inorganic compounds such as CO_2, inorganic ions, protons and anions as consequence of the root metabolic activity, phyto-ormones, amino acids and small peptides. All these compounds are generally soluble and as such usually promptly accessible to the rhizosphere and rhizoplane microorganisms. Arbuscular mycorrhizal fungi grow extensively in soil to form a well-developed hyphal network that absorbs Pi (via fungal high-affinity PiTs) from up to several centimeters from the root surface and can markedly extend the depletion zone. The P is translocated rapidly to the roots (probably as polyphosphate), overcoming the slow diffusion that occurs in the soil solution[21]. Under nonexcessive P availability, the mycorrhiza was more effective in P uptake than roots alone. Arbuscular mycorrhizal did not contribute to P uptake under excessive P. In this case, plants did not benefit from arbuscular mycorrhizal with regard to P uptake, since P availability was sufficient for independent root system absorption[22]. Arbuscular mycorrhiza is able to increase the growth and yield of plants. In symbiosis with corn plants, arbuscular mycorrhiza are connected to plant roots, helping plants to absorb more water along with phosphorus and other nutrients. The formation of hypha, arbuscules and vesicles in plant tissues accompanied by increased growth and yield of plants is an indication of the symbiotic occurrence of arbuscular mycorrhizae with plants as has been found in this research. The inward arbuscular mycorrhiza develops in plant roots cells extending and multiplying the hyphae, forming arbuscules and vesicles, outward forming spores and forming long and small hyphae that can help the plant in terms of water uptake, nutrient by penetrating layer deeper and small pore of soil that can not be reached and penetrated by plant roots.

The results obtained in this study are in line with the findings Shuaibu *et al.*[23], that sorghum growth and yield increase with increase of rate of both organic and inorganic fertilizer. Samanhudi *et al.*[24] reported treatments with mycorrhiza provide higher yields of plant than without mycorrhiza Akande *et al.*[25] reported, the combined application of organic fertilizer and mycorrhizal inoculation increase uptake of nutrients most especially in uptake of Phosphorus. Concomitant use of mycorrhizal fungi with organic amendments is required to enhance flow of nutrients to crops. The application of organic fertilizers favored AMF activity and diversity in the rhizosphere of maize and cowpea[26].

The plant yield will increase by application of cow dung on marginal land planting media. However, it will give a higher yield of the plant by using cow dung combined with an inoculum of arbuscular mycorrhizal. So, suggested to using arbuscular mycorrhizal and cow dung formulated.

CONCLUSION

The results of this study are important for marginal land, to increasing soil fertility, especially on dry land. The treatment that is applied to the plant has the ability to improve the physical, chemical and biological properties of soil which is important for sustainable agricultural development. The results of this study indicated that formulation of arbuscular mycorrhizal with cow dung as organic fertilizer increasing available Phosphorus, Phosphorus uptake, growth and yield of plant. The best treatment was obtained by using 50 g arbuscular mycorrhizal plus 80 g cow dung per planting hole equally with 5 t ha^{-1}.

SIGNIFICANCE STATEMENT

This study assessed the use of arbuscular mycorrhizal and cow dung for organic fertilization on marginal land which is known for its very low nutrient content. Formulation of Arbuscular mycorrhizal and cow dung potentially to increase soil fertility, which is effective in increasing available Phosphorus, Phosphorus uptake and plant yields. This manuscript presents new findings regarding the high potential of formulation of Arbuscular mycorrhizal and cow dung as a source of organic fertilizers on marginal land, which has not been reported so far. This study may help researchers to uncover the use of marginal land for higher productivity of corn cultivation that has not been explored by many researchers. Subsequently, a new theory on how the formulation of Arbuscular mycorrhizal and cow dung affect this crop development grown in nutrient-poor environments may be postulated.

ACKNOWLEDGMENT

The authors extend their gratitude to the Directorate General of Higher Education, the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for providing the Research Grant.

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