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Growth Improvement of Mung Bean (*Vigna Radiata* (L.) Wilczek R.) by Application of Mycofer and Phosphate Fertilizer

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Abstract The objective of the research was to observe the effect of mycofer inoculation and the phosphate fertilizer on growth improvement of mung bean (*Vigna radiata* (L.) R. Wilczek). The research method used experimental method, which used randomize block design 2 x 7 factorial with 3 replications. The first factor was mycofer inoculation (M), which consisted of two levels, i.e. without mycofer inoculation (m_0) and with mycofer inoculation (m_1). The second factor was adding of phosphate fertilizer (P), which consisted of seven levels of doses, i.e. without adding of phosphate fertilizer (p_0), 25 kg/ha (p_1), 50 kg/ha (p_2), 75 kg/ha (p_3), 100 kg/ha (p_4), 125 kg/ha (p_5) and 150 kg/ha (p_6). The observation parameter included the plant height, the leaf area, the dry weight, the number of pods, the seeds weight and the percentages of the root infection. The result showed that there was interaction between mycofer inoculation and the adding phosphate fertilizer to increase the plant height, the number of pods and the seeds weight. Phosphate fertilizer dose 75 kg/ha (p_3) was the best dose for increasing the growth of mung bean plants inoculated mycofer on all parameters observed, except best phosphate fertilizers dose for the parameters of dry weight was 50 kg[1] / ha (p_2).

Keywords: mycofer, phosphate fertilizer, growth, mung bean.

Introduction

Like other legumes, mung beans are high in protein, having around 25% of the seed dry weight and its amino acid profile is complementary to cereal grains [1]. Mung bean plays an important role in protein supplement in the cereal based low-protein diet of the people of Asia, but the acreage and production of mung bean are steadily declining [2].

For optimal growth, plants need phosphorus (P) as a macro-nutrient elements. Phosphorus absorbed by plants in the form of phosphate, is an integral component of important compounds of the plant cell, including sugar phosphate intermediate respiration and photosynthesis, and phospholipids that is part of plant cell membrane; is a nucleotides component used in energy metabolism such as ATP and in DNA and RNA. Phosphorus deficiency in plants can inhibit the growth of young plants with symptoms that are shown as dark green leaves color, malformed, death of tissue called necrotic [3].

Plants need phosphorus for growth, utilization of sugar and starch, photosynthesis, nucleus formation and cell division. Phosphorus compounds are involved in the transfer and storage of energy within plants. Energy from photosynthesis and the metabolism of carbohydrates is stored in phosphate compounds for later use in growth and reproduction [4]. P in the soil often present in the form is not available or in the form of the only available outside the rhizosphere that P application in the soil needed to support plant productivity [5].

Mycofer is a mixture of various kinds of Arbuscular Mycorrhizal Fungi (AMF) *as Glomus etunicatum, Glomus manihotis, Gigaspora margarita* and *Acaulospora tuberculata*. Arbuscular mycorrhizas is symbiotic microbes most essential for many plants especially in conditions of low P in the soil, affecting the development, nutrient uptake, water relations and crop productivity [6]. The result of a research showed that a plant in symbiosis with mycorrhiza absorp

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nutrients from soil solution more effectively, more quickly in assimilation of the phosphate element and increase the absorption of N, S, Zn, and other elements [7]. Thus FMA in mycofer can contribute to the growth of plants, especially in improving the efficiency of the use of phosphate fertilizers.

Research Methods

Research Materials

The materials used were seed of mung bean (*V. radiata* (L.) R. Wilczek), mycofer (*Gigaspora* sp., *Glomus manihotis*, *Glomus etunicatum* and *Acaulospora* sp.), latosol soil, TSP fertilizer (48% P_2O_5), urea (45% N) and KCl (60% K_2O); KOH solution 10%, HCl 1%, alkaline H_2O_2, carbolic acid fuchsin, sodium hypochlorite solution 5%; distilled water .

Design of Experiments

The method used was experimental method with randomized block design, factorial pattern 2 x 7. The first factor was the inoculation mycofer (M) which consisted of two levels (m_0 = without mycofer and m_1 = the mycofer 7.5 g). The second factor was the dose of phosphate fertilizer (P), which consisted of 7 levels (p_0 = without phosphate fertilizer (TSP) ; p_1 = 25 kg/ha; p_2 = 50 kg/ha; p_3 = 75kg/ha ; p_4 = 100 kg/ha ; p_5 = 125 kg/ha and p_6 = 150 kg/ha). Each treatment consisted of three repetitions.

Procedures

Preparation of Growing Media, Seeding Seed and Inoculation Mycofer

Soil that has been sterilized weighed 2 kg/polybag and given basic fertilizer (50 kg/ha urea and 50 kg/ha KCl) [8]. TSP fertilizer spread on growing media adjusted to the treatment. Seeds germinated using media that was a mixture of soil: compost: sand (1: 1: 1). Mycofer 7.5 g inserted in the planting hole as deep as 3 cm. Sprouts grown into a planting hole that had been inoculated mycofer.

Test of Mycorrhizal Infection Degree

Staining method the roots of Kormanik and McGraw`s was used to determine the percentage of mycorrhizal infection [5]. Roots washed and cut into pieces 1 cm long and then soaked in solution of KOH 10% and heated in a water bath. Roots were rinsed with distilled water and then soaked in alkaline H_2O_2. Then roots soaked in HCl solution 1% for 10-20 minutes and then a solution of HCl is discarded. Carbolic fuchsin was added at the root and heated at a temperature of ± 90 ° C.

Observed Parameters and Data Analysis

The parameters observed in this study were the plant height (cm), leaf area (cm^2), number of pods, seed weight , plant dry weight (g), percentage of root infection were performed at 10 weeks after planting. Data were analyzed using ANOVA and Duncan's Multiple Range Test (DMRT), α = 5%.

Results

The result of ANOVA indicated that the interaction between mycofer inoculation and phosphate fertilizer affect height of plant, number of pods and weight of seeds significantly. The result of DMRT is presented in Table 1, 2 and 3 .

Table 1. Average of Plant Height (cm) in Treatment of Mycofer Inoculation and Phosphate Fertilizer

Treatment	Dose of P fertilizer (kg/ha)						
	p_0 (0)	p_1 (25)	p_2 (50)	p_3(75)	p_4(100)	p_5(125)	p_6(150)
m_0 (without mycofer)	27.00a A	30.00a AB	32.00a BC	32.67a BC	36.00a C	34.67a C	33.67a BC
m_1 (mycofer)	36.00b A	42.67b B	49.00b CD	52.33b D	49.33b CD	44.33b BC	39.67a AB

Tabel 2. Average of Pods Number in Treatment of Mycofer Inoculation and Phosphate Fertilizer

Treatment	Dose of P fertilizer (kg/ha)						
	p_0 (0)	p_1 (25)	p_2 (50)	p_3(75)	p_4(100)	p_5(125)	p_6(150)
m_0 (without mycofer)	6.67a A	7.00a AB	7.33a AB	8.00a AB	8.33a AB	8.67a B	8.33a AB
m_1 (mycofer)	7.33b A	8.67b B	9.67b BC	10.67b C	10.00b BC	9.00a B	8.67a B

Tabel 3. Average of Seeds Weight in Treatment of Mycofer Inoculation and Phosphate Fertilizer

Treatment	Dose of P fertilizer (kg/ha)						
	p_0 (0)	p_1 (25)	p_2 (50)	p_3(75)	p_4(100)	p_5(125)	p_6(150)
m_0 (without mycofer)	3,37a A	3,62a A	3,68a A	3,99a A	4,23a A	4,32a A	4,29a A
m_1 (mycofer)	3,88b A	4,49b B	4,95b BC	5,74b D	5,42b CD	4,84a B	4,80a B

Note: Means are followed by the same letters (uppercase in horizontal direction and lower case in vertical direction) showed no significantly different according to DMRT at the $P < 0.05$ level of significance

Table 1 shows that in the treatment of without mycofer (m_0), the average of plant height at all doses of P fertilizer significantly different with control (p_0) except on 25 kg/ha P (p_1). In treatment using mycofer (m_1), the average of plant height at all doses of P fertilizer significantly different from the control (p_0) except on p_6. Treatment combination of mycofer with 75kg/ha P (m_1p_3) produces an average of the highest plant height is 49.33 cm .

Table 2 and 3 shows that the average of pods number and seed weight on treatment using mycofer is higher and significantly different with treatment without mycofer at doses of phosphate 0 kg/ha to 100 kg/ha (p_0, p_1, p_2, p_3 and p_4). Treatment combination of mycofer with 75kg/ ha P (m_1p_3) produces average of the highest pods number and seeds weight, respectively of 10.67 pods and 5.74 g.

The result of ANOVA indicate that mycofer inoculation and phosphate fertilizers significantly affected leaf area and dry weight of plant but both these factors indicate no interaction. The result of DMRT is presented in Table 4. Table 4 shows that the average of leaf area and dry weight in plant inoculated mycofer (m_1) respectively of 374.86 cm^2 dan 2.94 g is higher than the average of leaf area on without mycofer treatment (m_0) respectively of 315.14 cm^2 and 2.42 g significantly different. Phosphate fertilizer application of 75 kg/ha (p_3) gives average of the highest leaf area is 383.17 cm^2, significantly different of 0 kg/ha P (p_0) and 25 kg/ha P (p_1). While the average of the highest dry weight is 2.93 g obtained at phosphate fertilizer application of 50 kg / ha (p_2), significantly different of all treatment except on dose of 75 kg/ha P (p_3).

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Table 4 Average of Leaf Area (cm^2) and Dry Weight of Plant in Treatment of Mycofer Inoculation and Phosphate Fertilizer

Treatment	Average of leaf area (cm^2)	Average of Dry Weight (g)
m_0 = without mycofer	315.14 a	2.42 a
m_1 = mycofer	374.86 b	2.94 b
p_0 = without P fertilizer	262.50 a	2.39 a
p_1 = 25 kg/ha P fertilizer	313.33 ab	2.69 ab
p_2 = 50 kg/ha P fertilizer	367.83 bc	2.93 c
p_3 = 75 kg/ha P fertilizer	383.17 c	2.92 c
p_4 = 100 kg/ha P fertilizer	377.00 c	2.85 ab
p_5 = 125 kg/ha P fertilizer	365.67 bc	2.59 ab
p_6 = 150 kg/ha P fertilizer	345.50 bc	2.42 a

Note: Means are followed by the same letters in a group of same treatment and the same coloums showed no significantly different according to DMRT at the $P < 0.05$ level of significance

Percentage of mycorrhizal infection

The observation of the percentage of root infection of mung bean plants inoculated mycofer can be seen in Figure 1.

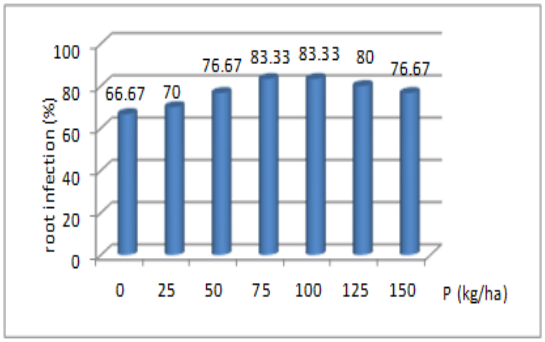

Figure 1. Average of Root Infection (%) in Variation of Phosphate Fertilizer Dose

Figure 1 shows that average of the highest mycorhizal infection found in doses of 75 kg/ha P and 100 kg/ha P treatment that is equal to 83.33%. The percentage of root infection increases with increasing doses of P fertilizer to dose of 100 kg/ha P and begin to decrease at a higher P doses (125 kg/ha and 150 kg/ha)

Discussion

The effect of the interaction between mycorrhizal with phosphate fertilizers in increasing the growth of mung bean plants i.e. on plant height, number of pods and weight of seeds (Tables 1, 2 and 3) are due to the compatibility of host plants with mycofer inoculant and phosphate fertilizers are used. External mycorrhizal hyphae of mycofer can expand the area of absorption. Mycorrhizal hyphae diameter is relatively small (average size of 2-5 μm) easily break through the pores of the soil that can't be penetrated by the root hairs [9]. Phosphorus uptake is also regulated by enzymes called phosphatases [10]. It is known that mycorrhizae produce phosphatase enzyme capable of catalyzing the hydrolysis of insoluble phosphate compound contained in the soil. Enhanced phosphatase activities resulted in an increment in P availability in P-deficient soil [11]. As stated also by [12] that mycorrhizal fungi, especially AMF have a role in obtaining phosphorus from organic material and mineral by increasing the diffusion zone around root and production of phosphatase enzyme.

Phosphorus (P) is an essential nutrient needed for plant growth, promotion of early root formation and root development, formation and seed development [3, 12]. This is indicated by the value of the highest average in the number of pods (10.67 pods) and seed weight (5.74 g) with mycorrhizal treatment and dose of phosphate 75 kg/ha (p_3) as seen in Table 1 and 2. Phosphorus also plays an important role in the process of cell division in the meristem tissue so that if the element P is increased, the cell division will also rise and the effect on plant height [7]. This is indicated by mung bean plants inoculated mycofer with a dose of phosphate 75 kg/ha (p_3), had the highest average of plant height is 52.33 cm (Table 1).While on treatment without mycofer inoculation, the

highest average of height plants (36.00 cm) was found at phosphate dose 100 kg/ha (p_4).

The average of leaf area (Table 2) of plants inoculated mycofer (374.86 cm^2) is higher of 19% than the plants without mycofer inoculation (315.14 cm^2). This is due to the role of mycorrhizae in increasing the absorption of nutrients, especially P element for supporting the generative and vegetative growth such as the development of the leaves of the host plant. Phosphate fertilizer application on dose 75 kg/ha (p_3) gives the best results in improving leaf area is 383.17 cm^2.

The plants were grown under conditions of low P will result in lower leaf area which negatively affects light interception and growth [8] so that the control plants (without P) has an average of the lowest leaf area is 262.50 cm^2. Total leaf area affected by the number of leaves and leaf size of the individual. As reported by Lynch et al. (1991) that reduction in the number of leaves and smaller individual leaf size result in the reduction of the total leaf area [13]. P deficiency can inhibit the activity of apical meristems and leaf initiation [14] and the rate of cell division [15] as well as the expansion of epidermal cells [16] resulting in a reduced number and leaf area.

Table 4 shows that the average dry weight in plants inoculated mycofer of 2.94 g, is higher of 21% than the dry weight of plants without mycofer inoculation of 2.42 g. The increase in dry weight is proportional with increasing dose of phosphate were applied and started to decline at doses higher P (120 kg / ha and 150 kg/ha). The highest average of dry weight found in phosphate application 50 kg/ha (p2) and 75 kg/ha (p3) of 2.93 g. This is due to the role of mycorrhizae in increasing the absorption of nutrients from the soil. As reported by [17] that increase the absorption of nutrients by the presence of mycorrhiza often associated with an increase in dry weight of a few folding on plant has a high dependence on mycorrhizal. Macro nutrients are absorbed mycorrhizal especially P greatly contribute in increasing the dry weight of the host plant and [18] reported that P improves the root growth which has a great effect on the overall plant growth performance. A good and optimum supply of P is associated with increased root growth due to which the plants explore more soil nutrients and moisture.

Figure 1 indicate that the highest percentage of root infection found in the treatment of phosphate fertilizers p_3 (75 kg/ha) and p_4 (100 kg/ha) is 83.83%. Mycorrhizal infection has decreased at doses of 125 kg/ha P (p_5) and 150 kg/ha P (p_6). On treatment without P (0 kg/ha P) showed that the lowest percentage of root infection is 66.67%. According to [19] this relates with role of mycorrhizal that usually have a maximum effect on the growth of host plant when the level of P in the soil solution can hardly be absorbed or not even able to be absorbed by plants. As well as a statement of [3] that a key factor in the extent of mycorrhizal association with the plant root is the nutritional status of the host plant. Moderate deficiency of a nutrient such as phosphorus tends to promote infection, whereas plants with abundant nutrients tend to suppress mycorrhizal infection.

The highest mycorrhizal infection rate is 83.33 % resulted in the highest growth of mung bean plants (Table 1-4). In general applications of phosphate at higher doses, namely at 125 kg/ha and 150 kg/ha led to the growth of host plant decreases (Table 1-4). Thus the higher the level of mycorrhizal infection resulted in the growth of host plants are better due to the increased absorption of P. The ability of mycorrhiza in increasing P uptake was associated with increased activity of phosphatase enzymes in a low P availability [19]. Phosphatase activity increases with increasing levels of root mycorrhizal infection on the roots of host plants. Thus under conditions of low P, the mycorrhizal infection will increase, followed by an increase in phosphatase activity so that the plant can absorb P more effective.

Conclusion

Based on the results of this study, it could be concluded that there was interaction between mycofer inoculation with phosphate fertilizer application on plant height (94%), number of pods (60%) and weight of seeds (70 %). Phosphate fertilizer dose 75 kg/ha (p_3) was the best dose for increasing the growth of mung bean plants inoculated mycofer on all parameters observed, except bEst phosphate fertilizers dose for the parameters of dry weight is 50 kg / ha (p_2).

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