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Enhanced of Root Nodules, Uptake NPK, and Yield of Peanut Plant (*Arachis hypogaea* L.) using Rhizobium and Mycorrhizae Applications

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Abstract | Peanut plants use nitrogen sources from the atmosphere with the help of Rhizobium bacteria. Rhizobium was requiring P elements for root nodules formation. This study aims to determine the effects of the application of mycorrhizae at different levels that play an essential role in increasing P elemental uptake and rhizobia inoculants to improve N element fixation and root nodule formation. Rhizobia inoculant applications were allotted to main plots and levels of mycorrhizae to the subplots under each main plot. Three types of rhizobia applications viz., soaking (A1), spraying (A2), soaking and spraying (A3) and four levels of mycorrhizae doses viz., 0 g plant^{-1} (M0), 10 g plant^{-1} (M1), 15 g plant^{-1} (M2), 20 g plant^{-1} (M3). The results indicated that the application of Rhizobium at various doses of mycorrhizae together had a significant effect on plant height 70 d (A2M1 = 53.96 cm), leaf number 70 d (A1M3 = 42.75), leaf N content (A3M3 = 5.36 g 100 g^{-1}), leaf P content (A3M3 = 0.418 g 100 g^{-1}), leaf K content (A3M3 = 0.273 g 100 g^{-1}), number of root nodules (A3M0 = 120), and weight of fresh pods plant^{-1} (A1M3 = 33.67 g). That application can enhance root nodules and had increased NPK uptake.

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Introduction

Peanut (*Arachis hypogaea* L.) is grown in more than 100 countries over 22 × 10^6 ha in the tropical and sub-tropical countries (Ahmed *et al.*, 2016). Peanut productivity in tropical countries, including Indonesia, India, and countries in Africa, is generally almost the same, between (0.7 to 1.3) t ha^{-1} of dry pods (Sumarno, 2015). Kementerian Pertanian (2017) reports that productivity of peanuts has fluctuated throughout 5 year in the period 2013 to 2017 with the average productivity of 1.318 t ha^{-1}. Peanut productivity in Indonesia is relatively low, compared to USA, China, Australia and Argentina, which have reached more than 2 t ha^{-1} (Kurniawan *et al.*, 2017; Sumarno, 2015). The efforts to increase peanut production are carried

out by improving the formation of root nodules. Phosphorus plays an essential role in the development of root nodules. Peanut plants need essential nutrients such as N, P and K. Legume plants are provided with natural potential for atmospheric nitrogen fixation when associated with a suitable microbial partner, rhizobia (Rehman *et al*., 2019).

Nitrogen (N) is an essential nutrient for the growth of peanut plants, but N availability in tropical regions such as Indonesia is relatively low (Budiono *et al*, 2019). Nitrogen, which forms a protein compound, cannot be used directly by plants, although in the atmosphere, there is N_2 around 80 %. Nitrogen nutrients can enter the plant in the form of nitrat (NO3-) and ammonium (NH4+) and amino acids (Purbajanti *et al*., 2019). Among the soil bacteria a unique group called Rhizobia has a beneficial effect on the growth of plants. It can live either in the soil or within the root nodules of host legumes (Kukkamalla and Vardhan, 2016).

The symbiosis between peanut plants and Rhizobium is a major ecological process in the nitrogen cycle. This symbiosis allows peanut plants to colonize N-limited environments (Bouznif *et al*., 2019). The nitrogen source in the soil, the longer becomes insufficient for plant needs. Nitrogen needs can source from N fertilizer and N_2 fixation from the atmosphere. The nitrogen is derived from their association with symbiotic rhizobia that fix atmospheric nitrogen gas into ammonia. The symbiotic nitrogen fixation has been purported to facilitate legume colonization (Simonsen, 2017). Peanuts have a high nitrogen fixation capacity. The N element obtained by nitrogen fixation is a cheap source and can help reduce production costs. Rhizobium inoculation to peanut can increase nitrogen fixation. Peanut can form root nodule by fast-growing rhizobia such as *Rhizobium giardini* and *Rhizobium tropic* (Taurian *et al*., 2006). Rhizobium, as an inoculant, can produce effective root nodules in peanut plants. Rhizobia may promote plant growth through nitrogen fixation. Anteneh (2017) found that inoculation of *Bradyrhizobium* is necessary to improve root nodule and seed yields.

Mycorrhizae is a type of soil microbe that can have a symbiosis with plants. It has an essential contribution to soil fertility by increasing plants' ability to absorb nutrients; especially P. Mycelium mycorrhizae can extend to areas outside the rhizosphere and enlarge the root area to absorb nutrients. Mycorrhizal colonization in plant roots can expand the absorption area of the roots with the presence of external hyphae which grow and develop through plant root feathers (Muhammad *et al*., 2020; Sukmawati *et al*., 2020). Therefore, water and nutrients can be transported by hyphae tissue to be absorbed by plants (Abd-Alla *et al*., 2014; Yaseen *et al*., 2016).

The research is planned to evaluate the beneficial effect dual inoculation of Rhizobium and mycorrhizal on increased root nodule formation and NPK uptake and peanut production.

Materials and Methods

The field work was conducted in 2018 during the dry season (mid-May through August) on the rainfed fields located at Bolosingo Village, Pacitan District, Pacitan Regency, Indonesia. The location geographically situated at an altitude of 50 m above sea level. Total rainfall at this location is approximately 250 mm $year^{-1}$ and an average maximum daily temperature was varied between 24 °C to 28 °C. The soil's chemical properties in the experiment area were analyzed (Table 1) as an illustration of the initial soil conditions.

Table 1: *Initial soil chemical properties.*

Soil chemical properties	Initial soil
pH (H_2O)	5.640
Organic-C (%)	2.377
Organic Matter (%)	3.087
Total N (%)	0.179
C/N ratio	13.265
Total P_2O_5 (mg 100 g^{-1})	16.333
P_2O_5 Bray (mg 100 g^{-1})	6.634
Total K_2O HCl 25 %(mg 100 g^{-1})	13.099

Field experiment started with the ploughing process to homogenize the soil quality. Hoeing and ploughing the land two times to obtain loose soil. There were 36 plots with a size of (2.5 x 3) m each to accommodate the number of treatments and three replications.

The cultivar of peanut plant used is Kelinci. The application of mycorrhizae doses using "mikoriza plus" (solid commercial biofertilizer, CV Wahana Sukses, Malang, Indonesia) contains mycorrhizal plus *Trichoderma* sp., *Bacillus subtilis,* and *Pseudomonas fluorescent*. Mycorrhizae fertilizer dose was applied

at planting. The application of Rhizobium using "Legume" (commercial liquid biofertilizer, PT Central Biotech Indonesia) that contain Rhizobium and Phosphorus Solubilizing Bacteria (PSB). It was soaking the seeds in a "Legume" biofertilizer solution with a concentration of 30 mL L^{-1} within (10 to 15) sec and it is executed before planting. Spraying biofertilizers is carried out on plants aged 25 d and 45 d after planting with a concentration of 2 mL L^{-1}.

The study used split plot design which was repeated three times with the application factor of Rhizobium (A) occupying the main plot consisting of soaking (A1), spraying (A2), soaking and spraying (A3). The factor of mycorrhizae fertilizer dose per plant (M) allocated to the subplots consisted of 0 g (M0), 10 g (M1), 15 g (M2), and 20 g (M3)). Observations were made on growth and yield variables including plant height, number of branches, leaf area index, levels of N, P, K leaves, number of root nodules, the weight of root nodules, number of pods per plant, and weight of pods per plant.

Data collected about nodule formation, NPK uptake, growth and production parameters were statistically analysed using SPSS 25.0. The treatment means were compared using analysis of variance technique, followed by Duncan's Multiple Range Test (DMRT) at 5 % of probability level ($p < 0.05$).

Results and Discussion

Nodule formation

The number of root nodules per plant was significantly different on the application of Rhizobium in various doses of mycorrhizae (Fig. 1) whereas the weight of nodules was not affected by both of treat applied, but the percentage of effective root nodules was difference significantly on doses of mycorrhizae (Table 2).

The number of root nodules formed varied not along with the increase in mycorrhizae dose given (Fig. 1). The forming of root nodules increased at first then decreased in 20 g mycorrhizae dose combined with the application of soaked Rhizobium (A1M3) and sprayed (A2M3). On the other hand, declined in the beginning then increased at a dose of mycorrhizae 20 g combine with Rhizobium soak and spray (A3M3). Peanut seed that soaked with Rhizobium and planted directly will occur mortality of Rhizobium bacteria since have to adjust to the environmental conditions of the soil until the seeds germinate and form roots.

The formation of roots helps the Rhizobium to colonize and infect the roots so that the Rhizobium mortality rate decreases. Likewise, the occurrence of mycorrhizal infections in the roots of peanut plants can increase the tolerance of plants to drought, so that the amount of Rhizobium can develop and have an impact on the formation of root nodules and nitrogen fixation (Meng *et al.*, 2015).

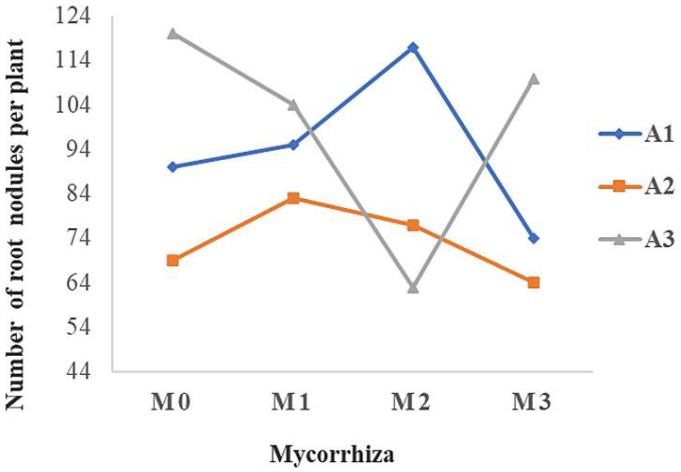

Figure 1: *Interaction between the application of Rhizobium and mycorrhizae doses to number of root nodules per plant.*

Table 2: *Percentage of effective nodules and root nodules weight per plant at Rhizobium application and various doses of mycorrhizae.*

Treatment	Effective root nodules (%)	Weight root nodules (g)
A1	86.25	3.54
A2	81.25	0.83
A3	80	1.72
	ns	ns
M0	76.67 a	2.08
M1	80.00 a	2.01
M2	83.33 ab	1.95
M3	90.00 b	2.08
		ns

Means followed by the same letter within the same column are not significantly different at DMRT 5 %.

The existence of the Rhizobium indigenous population can help the treatment of Rhizobium spray application. However, if the community is large, it will inhibit the rhizobia inoculant applied (Chemining'wa *et al.*, 2011). This is indicated by the application of Rhizobium spray at various doses of

mycorrhizae which produce the lowest number of root nodules.

Rhizobium inoculation in peanut plants is not only affected by the method of application but many factors influence. The physical, chemical and biological conditions of the soil determine the effectiveness of the symbiotic relationship between Rhizobium bacteria and their host plants. Similarly, the quality of inoculants applied. In the infection phase, Rhizobium actively grows and divides itself so that it is highly dependent on the availability of nutrients outside of plant cells. Therefore, the lack of essential nutrients when the infection phase can limit the formation of root nodules.

It can be seen in Table 2, the Rhizobium application has no significant influence on effective root nodules and weight of nodules. However, the soak application (A1) is the highest 86.25 % and 3.54 g, respectively. In otherwise, the mycorrhizae dose has significantly different. The effective nodules varied from (76.67 to 90.00) %. Maximum effective nodules were recorded under treatment 20 g (M3) and minimum with without mycorrhizae 0 g (M0). Without mycorrhizae, nutrient uptake is only at the tip of the hair root and the nutrients absorbed are only in the available form. While mycorrhizal fertilizer was added with mycorrhizal hyphae which grew widely with plant root tissue. Nutrients absorbed by tissue hyphae are brought to the tips of root hairs to be exchanged with polysaccharides. The level of hyphae in absorbing nutrients is much broader than root tissue without mycorrhizae (Adinurani *et al.*, 2019; Marzban *et al.*, 2017).

The effective nodule percentage indicates that Rhizobium actively fixation nitrogen. The more percentage of the nodule effective shows the more nitrogen is tethered. According to Labidi *et al.* (2015), Suryantini (2015) that an effective symbiotic relationship can be identified by cut the nodule in the initial period of flowering, and observing the color. Effective root spots are large and have a bright red color on the inside. Red pigment is leghemoglobin which shows active nitrogen fixation.

NPK uptake

N, P, and K elements of the leaves of peanut plants were significantly affected by the application of Rhizobium to the administration of various doses of mycorrhizae (Table 3).

The data in Table 3 shows the trend of increasing N, P and K uptake in leaves as the doses of mycorrhizae increase. The symbiosis of mycorrhizae with plant roots has an essential role in plant growth, both ecologically and agronomically. These roles include increasing P uptake and other nutrients, such as N, K, Zn, Cu, Mn, and Mg in the soil (Ortas, 2010; Mukhongo *et al.*, 2017). In this research, the highest absorption of N, P and K in leaves was achieved by application of the spray soak Rhizobium together with 20 g of mycorrhizae (A3M3). The highest plant nutrient uptake occurs when the plant reaches its maximum vegetative point (Utami *et al.*, 2020). Nitrogen-fixing activity in peanut plants starts at 25-30 days of age and reaches a maximum near the end of the plant growth phase.

Table 3: *The levels of N, P and K elements of peanut plant leaves as an influence from the application of Rhizobium at various doses of mycorrhizae.*

Treatment	N (g 100 g^{-1})	P (g 100 g^{-1})	K (g 100 g^{-1})
A1M0	3.19 a	0.366 a	0.231 a
A1M1	3.75 cd	0.376 d	0.238 c
A1M2	3.81 d	0.384 e	0.241 d
A1M3	4.16 e	0.394 g	0.244 e
A2M0	3.35 ab	0.371 b	0.232 b
A2M1	4.31 e	0.386 f	0.254 f
A2M2	4.37 e	0.394 g	0.256 g
A2M3	4.73 f	0.405 i	0.259 h
A3M0	3.54 bc	0.373 c	0.233 b
A3M1	4.93 fg	0.399 h	0.267 i
A3M2	4.99 g	0.407 i	0.270 j
A3M3	5.36 h	0.418 j	0.273 k

Means followed by the same letter within the same column are not significantly different at DMRT 5 %.

Peanut plants really need N and P elements. Phosphorus is required for nodule formation and nodule activity in maximum. Therefore, plants that obtain nitrogen symbiotically require more Phosphorus in number than plants fed N fertilizer. Inoculation of Rhizobium together with mycorrhizae has a synergistic effect on root nodule formation and nitrogen fixation. Based on research by Yakubu *et al.* (2010) that administering P fertilizer as much as 40 kg P_2O_5 ha^{-1} increased the number of root nodules and the number of fixated N was 169 % compared to controls.

Growth parameters

The statistically significant interaction between Rhizobium application and a dose of mycorrhizae

were found in the height of plant (Fig. 1) and number of leaf (Fig. 2) on 70 d after planting (DAP).

It can be seen in Figure 2 that the treatment of Rhizobium by soaking and spray applied with 10 g of mycorrhizae (A3M1) encourages the best plant growth because it produces the highest peanut plants (53.96 cm), although statistically not significantly different from the treatment of Rhizobium soak spray without mycorrhizae (A3M0), the application of rhizobium spray with 20 g of mycorrhiza (A2M3) and with 10 g of mycorrhizae (A2M1). The same study states that Rhizobium inoculation is capable of spurring plant heights, improving nodulation, plant growth and yield (Kurniaty *et al.*, 2013; Malinda *et al.*, 2019). Spraying Rhizobium on peanut plants without fertilization gave the best growth response to all observed variables (Prayoga *et al.*, 2018).

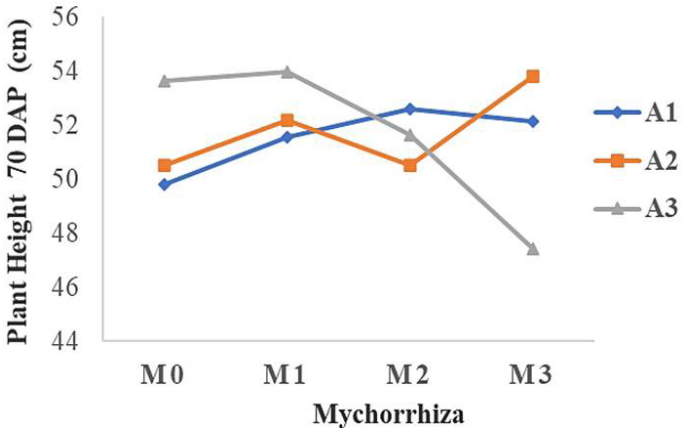

Figure 2: *The interaction between the application of Rhizobium and mycorrhizae doses to plant height 70 d after planting.*

Soaking the seeds before planting makes Rhizobium adaptable to the rhizosphere environment which makes it easier to develop and infect roots. If an application was continued by spraying encourages Rhizobium in the process of forming root nodules. Rhizobium plays a necessary role in N_2-fixation from the atmosphere to form ammonium compounds (Sari and Prayudyaningsih, 2015). Most of the ammonium compounds that contain proteins will be converted into amino acids which then become the nitrogen compounds needed by plants for plant growth and development.

Figure 3 shows that the combination treatment of Rhizobium application by soaking and 20 g mycorrhizae (A1M3) produced the highest number of leaves 42.75. Double inoculation of Rhizobium with mycorrhizae has a synergistic effect on root nodule formation and nitrogen fixation, especially in P deficient soil. In this caused, element P is required for nodule formation and maximum nodule activity. Plants that get symbiotic nitrogen require more significant amounts of P than fertilized N plants (Haro *et al.*, 2018; Jin *et al.*, 2016). Therefore the administration of mycorrhizae greatly helps the absorption of P elements in the soil needed for plant development, including leaf formation and a number of branches.

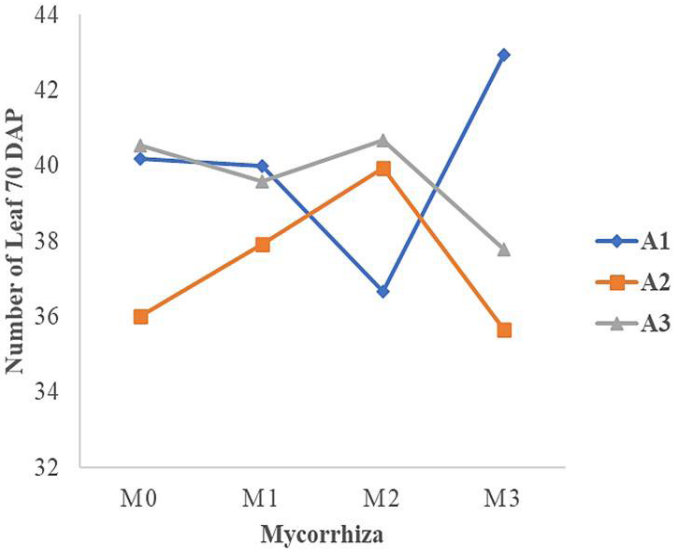

Figure 3: *The interaction between the application of Rhizobium and mycorrhizae doses to number of leaf 70 d after planting.*

Table 4: *Number of branches and Leaves Area Index of peanut plants as affected by Rhizobium application and doses of mycorrhizae.*

Treatment	Number of branches					Leaf area index
	14 dap	28 dap	42 dap	56 dap	70 dap	70 dap
A1	2.07	6.43	6.77	8.78	8.90	7.0
A2	2.07	6.42	6.70	8.23	8.38	6.9
A3	2.20	6.73	7.05	8.42	8.53	7.6
M0	2.11	6.51	6.82	8.67	8.77	6.1
M1	2.22	6.51	6.89	8.80	8.95	7.4
M2	2.02	6.64	6.84	8.11	8.27	7.6
M3	2.09	6.44	6.80	8.33	8.42	7.5

The number of branches of peanut plants at all time of observation was not significantly affected by the application of Rhizobium or mycorrhizae doses, either in combination or individually, likewise for the leaf area index (Table 4).

Although the effect of each factor was not significantly different on the number of branches, however, increase the most on the Rhizobium soak application (329.9 %), and without mycorrhizae (315.6 %). The increase in the number of branches will affect photosynthate transport from leaves to other parts of the plant for the better and have a positive impact on pod formation. But, the leaves will give its photosynthesis results, especially to pods in the same branch. The differences of the peanut's plant structure (main stem, types and the number of branches) determinate of pod number and pod weight on each structure (Giayetto *et al.*, 2013).

The largest Leaf Area Index (LAI) was achieved by application of spray soak (A3) and a mycorrhizae dose of 10 g (M2) which is 7.6. The application of Rhizobium and mycorrhizae increased LAI by 75.12 %. Large LAI values will absorb more solar radiation and do the photolysis process of water. It is because the leaves are the main part of the plant that photosynthesis and produces assimilates. Photosynthetic activity is related to source capacity which is characterized by the growth rate of leaf area index, chlorophyll content, and stomata density (Purnamawati and Manshuri, 2015).

Table 5: *Number of pods per plant on the Rhizobium application and various doses of mycorrhizae.*

Treatment	Number of pods
A1	21.92
A2	22.62
A3	24.45
	ns
M0	21.69
M1	23.60
M2	22.49
M3	24.24
	ns

Production parameters

The application of Rhizobium at various doses of mycorrhizae both in combination and independently did not significantly affect the number of pods per plant. The obtained result is tabulated in Table 5.

The number of pods per plant was not statistically significantly different in the application of Rhizobium, and mycorrhizae doses due to the peanut plants need more Ca (Phatak, 2010) than N elements or P elements on pod formation phase. Ahmed *et al.* (2019) stated that there was a significant difference in the number of pods per plant in peanuts treated with microdoses of NPK. This shows that the number of pods per plant does not depend on the NPK element (Tian *et al.*, 2019).

The data obtain that Rhizobium soaked spray (A3) and mycorrhizae doses of 20 g (M3) had the highest number of pods, 24.45 and 24.24, respectively. Calcium plays a role in promoting fertilization. Hence it is possible that calcium may exert an early effect on peanut reproduction by increasing the successful fertilization of the distal ovule, thereby increasing the number of pods. Whereas P serves to accelerate flowering and ripening of fruit and seeds, an element of N and Ca needed also when stage flowering.

There was a significant interaction between the application of Rhizobium at various doses of mycorrhizae to fresh pod weight (Fig. 4).

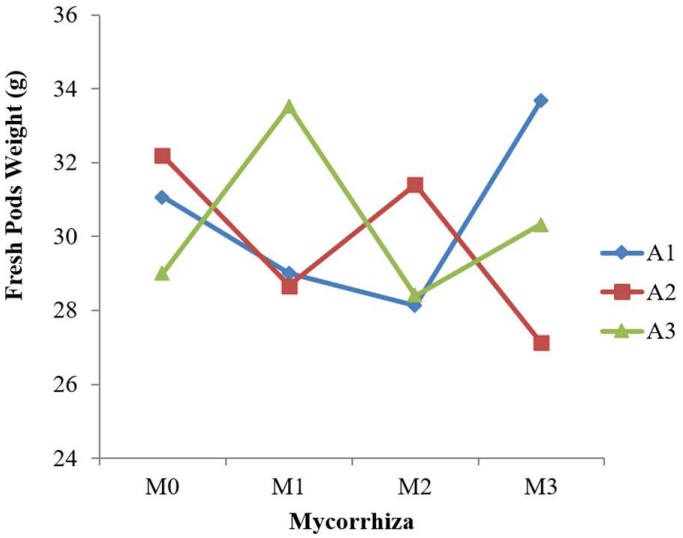

Figure 4: *Effect of interaction between Rhizobium application and mycorrhizae doses to fresh pods weight per plant.*

The analysis result reveals that soak Rhizobium applicated with 20 g mycorrhizae (A1M3) had the highest fresh pods weight per plant (33.67 g) although no different significant with combination treat between spray soak Rhizobium and 10 g mycorrhizae (A3M1) namely 33.53 g. Based on the data in Table 4, two factors applied either in combination or individually did not affect the number of pods, so the number of pods did not determine the weight of the pod. An increase in the number of pods per plant is not always followed by filling pods (Al-Jobori and Al-Hadithy,

2014). This is because at harvest not all pods are in the filling phase of the seeds, especially in pods that develop from flowers with the last anthesis process (Maji *et al*., 2017; Yaseen *et al*., 2016). The increasing number of pods per plant will disrupt the filling of previously formed pods, resulting in many unfill pods.

Conclusions and Recommendations

It is concluded that peanut should instead be grown with dual inoculation of mycorrhizae along with Rhizobium. The result showed that inoculation also exhibited a significantly positive response to the growth and yield of peanut. Mixed of mycorrhizal doses + Rhizobium application had increased NPK uptake. The dual inoculation of mycorrhizal doses and Rhizobium also enhanced root nodules of peanut plant indicating that their combination may have a potential role to improve the crop productivity.

Combine of the mycorrhizal doses and Rhizobium have a potential role in improving crop productivity by root nodule formation. It is also recommended to know the relation between NPK uptake and nodule formation, especially the effective root nodule.

Novelty Statement

Inoculation of peanuts with Rhizobium is mostly done using solid (granule) form inoculums. Meanwhile, the liquid rhizobium inoculum is rarely used and its application is sprayed on the soil during planting. Rhizobium needs time to adapt to environmental conditions for N_2 fixation. Based on this, this study attempted to increase the effectiveness of N_2 fixation, accelerate Rhizobium infection, and formation of root nodules by soaking the seeds combined with spraying to obtain the maximum number of pods. Vesicular Arbuscular Mychorizae is added along with the application of Rhizobium, to increase nitrogen uptake.

Author's Contribution

PGA and EDP conceived and designed the study, elaborated intellectual content, literature search, manuscript review and manuscript preparation. PGA and SR performed experimental studies. PGA acquired data, and performed data and statistical analysis. DDS, KS and RHS elaborated the intellectual content, literature search, manuscript preparation, and manuscript review. RHS performed manuscript editing and guarantor. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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