RESEARCH ON CROPS (Q3) ISSN: 0972-3226

https://www.scopus.com/sources.uri

MANUSCRIPT SUBMISSION: 20 Januari 2022

Nutrient uptake and yield of rice (*oryza sativa***) applied with mycorrhizal fungi using different doses of nitrogen and phosporus fertilizers**

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ABSTRACT

Abstract: Rainfed rice fields in general often lack water and nutrients that are difficult for their roots to reach. The Vesicular Arbuscular Mycorrhiza (VAM) fungus can assist in absorbing water and nutrients to increase the efficiency of nitrogen and phosphorus fertilizers. The purpose of this study was to determine the uptake of N, P, and K nutrients and the yield of rainfed lowland rice applied with VAM at different doses of N and P. This research used a completely randomized block design with two factors and three replications. The first factor was nitrogen with a dose of 0, 45, 90, and 135 kg/ha,

while the second factor was phosphorus at 0, 25, 50, and 75 kg/ha. The authors conducted this study in rainfed rice fields in Demangan, Sambi, Boyolali, Central Java, Indonesia, from March 30, 2019, to July 14, 2019, at an altitude of 113 m above sea level. The results showed that VAM could increase the uptake of nitrogen, phosphorus, and potassium and increase yield using N and P fertilizers at 90 kg/ha and 75 kg/ha, respectively. This research shows that the use of N and P fertilizers at 90 kg/ha and 75 kg/ha, respectively, is sufficient to cultivate rainfed rice applied with mycorrhizal fungi.

Keywords: Growth, nitrogen, phosphorus, rainfed lowland rice, vesicular arbuscular mycorrhizae

INTRODUCTION

Rainfed land uses rainwater for irrigation and differs from irrigated rice fields. Rainfed rice fields have a low available P content due to groundwater leaching (Meng *et al*., 2018). In general, improper agricultural management, long-term application of chemical fertilizers, and inefficient fertilizer use decrease the soil productivity of rice fields.

Drought stress is one of the most destructive abiotic stresses affecting plant growth and development. Drought stress affects physiological processes, biochemical changes, formation of secondary metabolites, significantly accumulates endogenous reactive oxygen species (ROS), and increases toxin levels (Hasanuzzaman *et al.,* 2017).

Drought stress greatly reduces rice grain yields and vegetative growth (Ahadiyat, Hidayat, and Susanto 2014; Maisura *et al*. 2014). Water-scarce conditions generally reduce grain size, grain weight, and seed formation rates (Kumar *et al*., 2014; Raman *et al*., 2012). Drought stress during the booting, flowering, and terminal stages can interfere with floret initiation, cause grain sterility, lower grain weight, and ultimately lower grain yield (Acuña, Lafitte, and Wade 2008). The rate of grain yield loss depends on the duration of water scarcity, plant growth stage, and stress intensity (Gana, 2011; Kumar *et al*., 2014).

One of the efforts to overcome drought stress is microbial-based technology, such as vesicular-arbuscular mycorrhizae. Mycorrhizae can act as a link between plant roots and soil moisture, especially during the dry season. Plants withmycorrhizal roots showed increased nutrient absorption than those without (Narwal *et al.* 2018). Abd-Alla *et al*. (2014) stated that mycorrhizae increase nutrient uptake by expanding the absorption range through external hyphae that can reach 8 cm outside the root system, exploiting micropores as the outer diameter of small hyphae is less than 20% of the root hair diameter, and increasing the surface area of the absorption system.

Hernández and Munné-Bosch (2015) added that the application of mycorrhizae increased the absorption rate and content of phosphorus in seeds. Phosphorus is crucial in the photosynthetic cycle as it helps activate Ribulose 1,5 bisphosphate carboxylase oxygenase (Rubisco) and the Calvin cycle. Mycorrhizal fungal exudates can affect P leaching in the soil to ensure the availability of P to plants and may affect the absorption of other macronutrients (Tran *et al*., 2020).

The purpose of this study was to determine the uptake of N, P, and K nutrients and the yield of rainfed lowland rice applied with VAM fungi at different doses of N and P.

MATERIALS AND METHODS

The authors conducted this study in rainfed rice fields in Demangan, Sambi, Boyolali, Central Java, Indonesia, from March 30, 2019, to July 14, 2019. This study used a completely randomized block design with two factors and three replications. The first factor was urea with a dose of 0, 45, 90, and 135 kg/ha, while the second factor was phosphorus at 0, 25, 50, and 75 kg/ha. The planting process used three seeds. The authors selected one plant at 14 days old and used KCl as the fertilizer with a dose of 50 kg/ha.

Before planting, each plot was evenly sown with 5 kg of mycorrhizal fungus. The length and width of the experimental plots were 200 cm and 120 cm, respectively. The water level was 5 cm deep, with the plants spaced 20 cm x 20 cm apart. The authors weeded the plants at 2 and 4 weeks after planting and controlled pests and diseases using organic pesticides. The harvest criterion was the seed shells above the panicle being clean and firm. Observations included nitrogen, phosphorus, leaf potassium uptake, and grain weight per plot. Nutrient uptake in leaves was calculated using the formula: leaf nutrient uptake $=$ leaf tissue nutrient content x leaf dry weight.

Statistical analysis used the Anova SAS 9.1 program and continued with Duncan's Multiple Distance Test (DMRT) at a probability level of 5%.

RESULTS AND DISCUSSION

Leaf nitrogen uptake

Nutrient uptake is the ability of plants to absorb nutrients from the soil and convert them into plant parts. The higher the plant's ability to absorb nutrients, the higher the yield obtained (Wang et al. 2014). The nitrogen and phosphorus fertilizers had a significant effect on nutrient uptake. Table 1 shows that without nitrogen fertilizer,there was no difference in nitrogen uptake using 45,75,and 90 kg/ha of phosphorus fertilizer. However, 135 kg/ha of nitrogen fertilizer combined with 50 and 75 kg/ha of phosphorus fertilizershowed higher nitrogen uptake than with 0 kg/ha and 25 kg/ha of phosphorus fertilizer.

Applying N and P fertilizers with a dose of up to 45 kg/ha and 75 kg/ha, respectively, did not improve nitrogen uptake. Starting from 90 kg/ha of N fertilizer, a significant increase in N uptake was seen. Dakshina Murthy *et al*. (2015) and Permanasari *et al*. (2016)) also reported increased rice nutrient uptake with increased fertilizer doses. In addition to soil nutrient supply, mycorrhizal fungi also affect nutrient uptake (Narwal *et al*. 2018). This finding matches with the findings of Cavagnaro, Barrios-Masias, and Jackson (2012); Lee and Muneer (2014), and (Narwal *et al.* 2018), stating that the application of mycorrhizal fungi increases nitrogen uptake and content in seeds and plants.

Dose of N	Dose of P (kg/ha) Mean						
(kg/ha)		25	50	75			
	1.730 g	3.037 fg	2.267 g	2.437 g	2.367		
45	3.740 fg	4.433 $e-g$	$5.200 d-g$	$5.130 d-g$	4.625		
90	$7.150 c-f$	8.107 c-e	8.647 cd	9.997 bc	8.475		
135	10.773 bc	13.320 b	17.480 a	19.250 a	15.205		
Mean	5.848	7.224	8.398	9.203			

Table 1. Leaf nitrogen uptake of rice applied with mycorrhizal fungi using different doses of nitrogen and phosporus fertilizers (ug/mm2/sec)

Note: the numbers followed by the same letter show no significant difference at the 5% DMRT level.

Leaf phosphorus uptake

The nitrogen and phosphorus fertilizers affect phosphorus uptake. Table 2 shows no difference in phosphorus uptake between plants administered with 25, 50, and 75 kg/ha of phosphorus fertilizer and the control plants. The same applies to plants administered with 45 kg/ha and 90 kg/ha of nitrogen fertilizer. However, plants administered with 135 kg/ha of nitrogen fertilizer showed the highest phosphorus nutrient uptake when combined with 50 kg/haof phosphorus fertilizer. The results significantly differ from 0, 25, and 75kg/ha of phosphorus fertilizer. The highest phosphorus uptake was 5.821 g/mm2/second, achieved by applying nitrogen and phosphorus fertilizers at 135 kg/ha and 50 kg/ha, respectively. The lowest phosphorus uptake was 1,150 g/mm2/second, shown in plants without N and P fertilizers.

Phosphorus uptake was not affected by the dose of P fertilizer; higher doses did not increase P uptake. Phosphorus uptake of rice plants fertilized with phosphorus was relatively low, presumably due to a high increase in fertilization which decreased nutrient uptake and the ability of rice plants to absorb phosphorus. Continuous application of phosphate fertilizers causes P accumulation, reducing plant response to phosphate fertilization. This fact matches the findings of (Liu *et al*. 2020). Besides reducing P efficiency, P accumulation can also affect the availability of other nutrients. In plants, P is an integral part of its cellular activities. Phosphorus is crucial in plant metabolism processes, such as cell division, development, photosynthesis, sugar breakdown, nutrient transport, and metabolic pathway regulation (Bagyaraj, Sharma, and Maiti 2015). Arbuscular mycorrhizal fungi can increase the efficiency of P fertilizer absorption. This fungus can achieve symbiosis with roots and is crucial in ecological and agronomical plant growth. According to Bolduc and Hijri (2011), nutrient uptake in plants with mycorrhizal roots is more efficient than plants without mycorrhizae. This condition is due to the absorption and transport of nutrients by mycorrhizae.

Herlina and Syafruddin (2016) stated that mycorrhizae increase nutrient uptake by expanding the absorption range through external hyphae that can reach 8 cm outside the root system, exploiting micropores as the outer diameter of small hyphae is less than 20% of the root hair diameter, and increasing the surface area of the absorption system. Plants with mycorrhizae usually perform better than those without mycorrhizae. Mycorrhizae can effectively increase the absorption rate of macronutrients (N, P, K, Ca, Mg, and Fe) and micronutrients (Cu, Mn, and Zn) of plant root hairs. This condition improves plant metabolism, indicated by increased plant crown growth (Permanasari *et al*. 2016).

Pamuna, Darman, and Pata'dungan (2013) stated that the dry weight and P uptake of corn plants without mycorrhizae would increase along with the increase in the dose of SP-36. The higher the P concentration in the soil, the larger the role of mycorrhizae in absorbing P. Nazirah *et al*. (2018) added that mycorrhizae are crucial for plants as they increase phosphorus uptake and drought resistance. Upland plants, including Situbagendit, have a positive response to mycorrhizae.

Dose of N		Mean			
(kg/ha)		25	50	75	
	1.150 f	1.992 d-f	1.707 ef	1.305 f	1.538
45	2.095 d-f	$2.482c-f$	$3.384b-d$	1.828 d-f	2.447
90	2.172 d-f	$3.040 b - e$	3.942 bc	$2.686 b-f$	2.960
135	2.709 b-f	3.714 bc	5.821 a	4.040 b	4.071
Mean	2.031	2.807	3.713	2.464	

Table 2. Leaf phosphorus uptake of rice applied with mycorrhizal fungi using different doses of nitrogen and phosporus fertilizers (μ g /mm²/sec)

Note: the numbers followed by the same letter show no significant difference at the 5% DMRT level.

Leaf potassium uptake

The nitrogen and phosphorus fertilizers affect potassium nutrient uptake. Without the nitrogen fertilizer, phosphorus fertilizer with a dose of 0 to 75 kg/ha did not improve potassium uptake. The same is true for nitrogen fertilization at 45 and 90 kg/ha. However, 135 kg/ha of nitrogen fertilizer combined with 50 kg/ha of phosphorus fertilizer provided the highest potassium uptake and showed different results from 0 kg/ha and 25 kg/ha of phosphorus fertilizer (Table 3). Nitrogen plays a dominant role in potassium absorption, while mycorrhizae do not. Various references state that mycorrhiza plays a role in nitrogen and phosphorus absorption, but its role in potassium absorption is still unclear. Researchers have thoroughly studied the effects of mycorrhizal symbiosis for nitrogen and phosphorus uptake (Plassard and Dell 2010). However, few have studied the possible effects of mycorrhizae on potassium uptake (Ruíz-Sánchez *et al*. 2011). Baslam, Garmendia, and Goicoechea (2011) stated that the K content increased in rice plants administered with mycorrhizae.

Table 3. Leaf potassium uptake of rice applied with mycorrhizal fungi using different doses of nitrogen and phosporus fertilizers (μ g /mm²/sec)

Dose of N		Mean			
(kg/ha)					
	6.33e	8.83 c-e	7.10 de	10.19 $c-e$	
45	10.85 c-e	11.26 c-e	13.22 b-d	10.37 c-e	11.42

Note: the numbers followed by the same letter show no significant difference at the 5% DMRT level.

Grain yield

The nitrogen and phosphorus fertilizers do not affect grain yield. The application of nitrogen fertilizer at 0, 45, 90, and 135 kg/ha showed the same results (Table 4). Plants applied with mycorrhizae only require 45-90 kg/ha of nitrogen fertilizer. Indriani, Susilawati, and Islami (2011) stated that mycorrhizae can help the host plant absorb the nutrients needed for photosynthesis, while the host photosynthesizes for the mycorrhizal fungi. The inoculation of mycorrhizal fungi can increase the yield of soybeans, peanuts, green beans, corn, and sweet potatoes (Hadianur, Syafruddin, and Kesumawati 2016). Rini, Pertiwi, and Saputra (2017)) stated that utilizing mycorrhizae as biological fertilizers containing microorganisms greatly reduces nutrients absorbed by colloidal soils due to low pH or Al and Fe activity. In the long term, administering mycorrhizae will greatly benefit soil fertility.

Table 4. Grain yield per plot (200 cm x 120 cm) of rice applied with mycorrhizal fungi using different doses of nitrogen and phosporus fertilizers (g)

Dose of N		Dose of P (kg/ha)				
(kg/ha)		25	50	75		
	523.3 bc	510.0c	786.7 a-c	556.7 a-c	594.2 b	
45	767.3 a-c	$701.0 a-c$	768.3 a-c	768.7 a-c	751.3 ab	
90	847.0 a-c	961.3 ab	729.3 a-c	926.7 a-c	866.1a	
135	940.0 $a-c$	874.7 a-c	659.7 a-c	988.0 a	865.6 a	
Mean	769.4 a	761.7 a	736.0 a	810.0 a		

Note: the numbers followed by the same letter show no significant difference at the 5% DMRT level.

CONCLUSION

Based on the results and discussion of paddy fields treated with mycorrhizae at a dose of 5 kg per plot (200 cm x 120 cm) with different doses of N and P fertilizers, it can be concluded that:

1. The highest leaf nitrogen uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 75 kg/ha at 19,250 g/mm2/sec.

- 2. The highest leaf phosphorus uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 50 kg/ha of 5.821 g/mm2/sec.
- 3. The highest leaf potassium uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 50 kg/ha of 21.32 g/mm2/sec.
- 4. The highest grain yield per plot (200 cm x 120 cm) was achieved at a dose of N 135 kg/ha combined with a dose of P 75 kg/ha of 988.0 g.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of National Education of the Republic of Indonesia, which has provided funding for this research through the Research Grant of the Directorate General of Higher Education of the Ministry of National Education of the Republic of Indonesia Decree Number T/140/E3/RA.00/2019 with Contract Agreement number 09/ L6/AL/SP2H/Research/2019.

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Article submission : 20 Januari 2022

23 Februari 2022 : Return of paper for revision

MANUSCRIPT REVISED:

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leaf nutrient uptake = leaf tissue nutrient content \times leaf dry weight.

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RESULTS AND DISCUSSION

Leaf nitrogen uptake

Nutrient uptake is the ability of plants to absorb nutrients from the soil and convert them into plant parts. The higher the plant's ability to absorb nutrients, the higher the yield obtained (Wang et al. 2014). The nitrogen and phosphorus fertilizers had a significant effect on nutrient uptake. Table 1 shows that without nitrogen fertilizer,there was no difference in nitrogen uptake using 45,75and 90 kg/haof phosphorus fertilizer. However, 135 kg/ha of nitrogen fertilizer combined with 50 and 75 kg/ha of phosphorus fertilizershowed higher nitrogen uptake than with 0 kg/ha and 25 kg/ha of phosphorus fertilizer.

Applying N and P fertilizers with a dose of up to 45 kg/ha and 75 kg/ha, respectively, did not improve nitrogen uptake. Starting from 90 kg/ha of N fertilizer, a significant increase in N uptake was seen. Dakshina Murthy *et al*. (2015)and Permanasari *et al*. (2016)) also reported increased rice nutrient uptake with increased fertilizer doses. In addition to soil nutrient supply, mycorrhizal fungi also affect nutrient uptake (Narwal *et al*., 2018). This finding matches with the results of Cavagnaro*et al*. (2012), Lee and Muneer (2014) and Narwal *et al.* (2018), stating that the application of mycorrhizal fungi increases nitrogen uptake and content in seeds and plants.

Leaf phosphorus uptake

The nitrogen and phosphorus fertilizers affect phosphorus uptake. Table 2 shows no difference in phosphorus uptake between plants administered with 25, 50 and 75 kg/ha of phosphorus fertilizer and the control plants. The same applies to plants administered with 45 kg/ha and 90 kg/haof nitrogen fertilizer. However, plants administered with 135 kg/ha of nitrogen fertilizer showed the highest phosphorus nutrient uptake when combined with 50 kg/haofphosphorus fertilizer. The results significantly differ from0, 25 and75kg/ha ofphosphorus fertilizer. The highest phosphorus uptake was 5.821 g/mm2/second, achieved by applying nitrogen and phosphorus fertilizers at 135 kg/ha and 50 kg/ha, respectively. The lowest phosphorus uptake was 1,150 g/mm2/second, shown in plants without N and P fertilizers.

Phosphorus uptake was not affected by the dose of P fertilizer; higher doses did not increase P uptake. Phosphorus uptake of rice plants fertilized with phosphorus was relatively low, presumably due to a high increase in fertilization which decreased nutrient uptake and the ability of rice plants to absorb phosphorus. Continuous application of phosphate fertilizers causes P accumulation, reducing plant response to phosphate fertilization. This fact matches the findings of (Liu *et al*., 2020). Besides reducing P efficiency, P accumulation can also affect the availability of other nutrients. In plants, P is an integral part of its cellular activities. Phosphorus is crucial in plant metabolism processes, such as cell division, development, photosynthesis, sugar breakdown, nutrient transport, and metabolic pathway regulation (Bagyaraj*et al.*, 2015). Arbuscular mycorrhizal fungi can increase the efficiency of P fertilizer absorption. This fungus can achieve symbiosis with roots and is crucial in ecological and agronomical plant growth. According to Bolduc and Hijri (2011), nutrient uptake in plants with mycorrhizal roots is more efficient than plants without mycorrhizae. This condition is due to the absorption and transport of nutrients by mycorrhizae.

Herlina and Syafruddin (2016) stated that mycorrhizae increase nutrient uptake by expanding the absorption range through external hyphae that can reach 8 cm outside the root system, exploiting micropores as the outer diameter of small hyphae is less than 20% of the root hair diameter, and increasing the surface area of the absorption system. Plants with mycorrhizae usually perform better than those without mycorrhizae. Mycorrhizae can effectively increase the absorption rate of macronutrients (N, P, K, Ca, Mg and Fe) and micronutrients (Cu, Mn, and Zn) of plant root hairs. This condition improves plant metabolism, indicated by increased plant crown growth (Permanasari *et al*., 2016).

Pamuna*et al*. (2013)stated that the dry weight and P uptake of corn plants without mycorrhizae would increase along with the increase in the dose of SP-36. The higher the P concentration in the soil, the larger the role of mycorrhizae in absorbing P. Nazirah *et al*. (2018)added that mycorrhizae are crucial for plants as they increase phosphorus uptake and drought resistance. Upland plants, including rice variety SituBagendit, have a positive response to mycorrhizae.

Leaf potassium uptake

The nitrogen and phosphorus fertilizers affect potassium nutrient uptake. Without the nitrogen fertilizer, phosphorus fertilizer with a dose of 0 to 75 kg/ha did not improve potassium uptake. The same is true for nitrogen fertilization at 45 and 90 kg/ha. However, 135 kg/ha of nitrogen fertilizer combined with 50 kg/ha of phosphorus fertilizer provided the highest potassium uptake and showed different results from 0 kg/ha and 25 kg/ha of phosphorus fertilizer (Table 3). Nitrogen plays a dominant role in potassium absorption, while mycorrhizae do not. Various references state that mycorrhiza plays a role in nitrogen and phosphorus absorption, but its role in potassium absorption is still unclear. Researchers have thoroughly studied the effects of mycorrhizal symbiosis for nitrogen and phosphorus uptake (Plassard and Dell, 2010). However, few have studied the possible effects of mycorrhizae on potassium uptake (Ruíz-Sánchez *et al*., 2011). Baslam*et al*. (2011) stated that the K content increased in rice plants administered with mycorrhizae.

Grain yield

The nitrogen and phosphorus fertilizers do not affect grain yield. The application of nitrogen fertilizer at 0, 45, 90 and 135 kg/ha showed the same results (Table 4). Plants applied with mycorrhizae only require 45-90 kg/ha of nitrogen fertilizer. Indriani*et al*. (2011) stated that mycorrhizae can help the host plant absorb the nutrients needed for photosynthesis, while the host photosynthesizes for the mycorrhizal fungi. The inoculation of mycorrhizal fungi can increase the yield of soybeans, peanuts, green beans, corn, and sweet potatoes (Hadianur*et al*., 2016). Rini*etal*. (2017) stated that utilizing mycorrhizae as biological fertilizers containing microorganisms greatly reduces nutrients absorbed by colloidal soils due to low pH or Al and Fe activity. In the long term, administering mycorrhizae will greatly benefit soil fertility.

CONCLUSION

Based on the results of this study, it can be concluded that (1) The highest leaf nitrogen uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 75 kg/ha at 19,250 g/mm2/sec., (2) The highest leaf phosphorus uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 50 kg/ha of 5.821 g/mm2/sec., (3) The highest leaf potassium uptake was achieved at a dose of N 135 kg/ha combined with a P dose of 50 kg/ha of 21.32 $g/mm2/sec$. and (4) The highest grain yield per plot (200 cm x 120 cm) was achieved at a dose of N 135 kg/ha combined with a dose of P 75 kg/ha of 988.0 g.

ACKNOWLEDGEMENTS

The author would like to thank the Ministry of National Education of the Republic of Indonesia, which has provided funding for this research through the Research Grant of the Directorate General of Higher Education of the Ministry of National Education of the Republic of Indonesia Decree Number T/140/E3/RA.00/2019 with Contract Agreement number 09/ L6/AL/SP2H/Research/2019.

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Table 1. Leaf nitrogen uptake (μ g/mm2/sec)of rice applied with mycorrhizal fungi using different doses of nitrogen and phosphorus fertilizers.

Figures followed by the same letter show no significant difference at P=0.05 DMRT level.

Dose of N	Dose of P (kg/ha)			Mean	
(kg/ha)	θ	25	50	75	
	1.150f	$1.992d-f$	1.707ef	1.305f	1.538
45	$2.095d-f$	$2.482c-f$	3.384b-d	1.828d-f	2.447
90	$2.172d-f$	$3.040b-e$	3.942 bc	$2.686b$ -f	2.960
135	$2.709b-f$	3.714 bc	5.821a	4.040b	4.071
Mean	2.031	2.807	3.713	2.464	

Table 2. Leaf phosphorus uptake (µg /mm2/sec)of rice applied with mycorrhizal fungi using different doses of nitrogen and phosphorus fertilizers.

Figures followed by the same letter show no significant difference at P=0.05 DMRT level.

Table 3. Leaf potassium uptake (μ g/mm2/sec)of rice applied with mycorrhizal fungi using different doses of nitrogen and phosphorus fertilizers.

Dose of N		Mean			
(kg/ha)	0	25	50	75	
$\overline{0}$	6.33e	8.83 c-e	7.10 de	$10.19c - e$	8.11
45	$10.85c - e$	11.26c-e	$13.22b-d$	$10.37c - e$	11.42
90	11.38c-e	$11.83c - e$	13.54 bc	$12.54b-e$	12.32
135	$11.63c - e$	14.85 bc	21.32a	18.43ab	16.55
Mean	10.04	11.69	13.79	12.88	

Figures followed by the same letter show no significant difference P=0.05 DMRT level.

Table 4. Grain yield (g) per plot (200 x 120 cm) of rice applied with mycorrhizal fungi using different doses of nitrogen and phosphorus fertilizers.

Dose of N		Mean			
(kg/ha)	θ	25	50	75	
	523.3 bc	510.0c	786.7a-c	556.7a-c	594.2b
45	$767.3a-c$	$701.0a-c$	$768.3a-c$	768.7a-c	751.3ab
90	847.0a-c	961.3ab	$729.3a-c$	$926.7a-c$	866.1a
135	$940.0a-c$	874.7a-c	$659.7a-c$	988.0a	865.6a
Mean	769.4a	761.7a	736.0a	810.0a	

Figures followed by the same letter show no significant difference at P=0.05 DMRT level.

3 Maret 2022: Received the Revised

Acceptance of paper for publication : 17 Maret 2022

Print ISSN: 0972-3226 Online ISSN: 2348-7534

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