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by Asmiaty Sahur

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Growth response of pepper (*Piper nigrum* L.) on application Arbuscular Mycorrhizal Fungi (AMF) and the shallot filtrate

A Sahur, Nasaruddin and Muthmainnah

Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin, Jl. Perintis Kemerdekaan KM 10, Makassar 90245, Indonesia

E-mail: asmiatyasmiaty@gmail.com, asmiaty.sahur@unhas.co.id

Abstract. This study aimed to detemine the effect of Arbuscular Mycorrhiza Fungi (AMF) and shallot filtrate on the growth of pepper plant. The study was conducted in the district of Tompobulu, Bantaeng Regency, South Sulawesi Province from November 2018 to March 2019. The trial was conducted in the form of two factors factorial experiment (F2F) based on randomized block design (RBD) with AMF as the first factor consisted of 4 levels: 0, 2, 4, and 6 g/polybag and shallot filtrate as second factor consisted of 4 levels of 0, 10, 20, 30%. The experimental results show that the interaction of AMF 2 g/polybag + 10% of shallot filtrate resulted in the highest average plant height (60.19 cm) at 18 weeks after planting. Treatment of AMF 6 g/polybag + shallot filtrate of 10% produced the highest chlorophyll b (390.41 μmol.m^{-2}), total chlorophyll (818.64 μmol.m^{-2}), and stomatal density (24.63 mm^{2}). Application of AMF 4 g/polybag + 30% of shallot filtrate resulted in the largest stomatal aperture area (26.28 mm^{2}). AMF 6 g resulted in the highest average of root length (31.17 cm) and percentage of root infected with mycorrhizal (7.82%).

1. Introduction

Indonesia is one of the largest pepper producer in the world, especially black pepper (Lampung black paper) produced in Lampung and white pepper (Muntok white paper) that comes from Bangka Balitung [1]. Pepper production in Indonesia tends to increase in the last 4 years. In 2015, 81.50 thousand tons, up 1.57% in 2016 ie 82.80 thousand tons and in 2017 reached 84.50 thousand tons, up 2.01% from the previous year, then in 2018 again experienced an increase of 0, 82% amounting to 85.20 thousand tons [1].

Pepper grown in planting material from cuttings which can be done directly or indirectly. Planting cuttings pepper origin require longer periods of time. Provision of plant growth regulator (PGR) can be used to accelerate the development of plant organs [2]. One of the natural plant growth regulator that can be used is the shallot filtrate. Shallot contain allicin and vitamin B1 which can accelerate the growth of new shoots, riboflavin can help the process of plant growth and auxin, and rhizokalin to stimulate root growth [3].

Planting pepper has several problems, among which pepper has a shallow root and flourish only in the area of the soil surface, so that the absorption of nutrients and nutrients are not optimal. Pepper has the ability nutrient absorption and fixation of the car in the soil that is low enough, so that the necessary control efforts to overcome it.

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Mechanical control can be done by the use of biological agents. Application of arbuscular mycorrhiza fungi (AMF) can be an alternative to improve plant nutrient uptake [4]. Mycorrhiza is a kind of fungus symbiosis with the roots of plants to improve the uptake of nutrients N, P, and K and improve the efficiency of the use of groundwater, increasing the osmotic pressure of plant cells on the ground that the water level is low enough, so that the plant can continue life and able to increase the rate of vegetative growth and crop production [5].

2. Methodology

The study was conducted in the district of Tompobulu, Bantaeng Regency, South Sulawesi Province, located at an altitude of 400 m above sea level, temperatures ranging from 23 – 29 °C and monthly rainfall of about 282.34 mm. The study was carried out from November 2018 to March 2019.

The tools used were spades, meter, water hoses, sprayer, hoes, paranet 70%, cutter, knife, scissors, stationery, cameras, rope, plastic, blender, electron microscopy, CCM-200 plus, glass preparation and analytical balance.

The materials used are pepper 4 months old, polybags (20 x 35 cm), mycorrhizal fungi arbuscular (granular form), shallot, decoprima, goat manure, rice husks, bamboo, paper labels, water, soil layer of top soil, and distilled water.

2.1. Experimental design

The experiment was arranged in the form of two factors factorial experiment based on a randomized block design (RBD). This experiment consisted of two factors: the first factor was the application of arbuscular mycorrhiza fungi (AMF) with 4 levels ie 0 g AMF as a control (M0), 2 g of AMF per polybag (M1), 4 g of AMF per polybag (M2), and 6 g of AMF per polybag (M3) while the second factor was the application shallot filtrate with 4 levels ie 0 g shallot + 100 ml of distilled water as a control (B0), 10 g shallot + 100 ml of distilled water (B1), 20 g shallot + 100 ml of distilled water (B2), and 30 g shallot + 100 ml of distilled water (B3). So there were 16 combinations of treatments. Each treatment was repeated 3 times, and each experimental unit consisted of 3 plants. If there is a treatment that significantly, then followed by a further test of Honestly Significance Difference (HSD) test with $\alpha = 0.05$.

2.2. Implementation of the research

2.2.1. Preparation of the shallot filtrate. Making the filtrate onion, prepared 10 g, 20 g and 30 g shallot and added to each 100 ml of distilled water, then crushed using a blender, and filtered to obtain a filtrate.

2.2.2. Selection of planting material. The planting material used was 4 months old pepper seedlings, derived from local varieties of plants cultivated by previously acquired from local farmers.

2.2.3. Applications arbuscular mycorrhiza fungi (AMF). Application of the AMF according to the dose to be used (2 g, 4 g, 6 g) and given to the planting medium by mixing in the planting hole in polybags. Application was done at planting time.

2.2.4. Applications of the shallot filtrate. The shallot filtrate on the pepper seedlings was done by spraying on each plant. Spraying was done every two weeks.

3. Results

3.1. Number of leaves

The measurement results the average number of leaves for four-time observation (P1, P2, P3, P4) show that the AMF and the shallot filtrate as well as their interaction effect had no significant effect on

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the number of leaves. Number of leaves increased with the increased of AMF dose and concentration of shallot filtrate (Figure 1).

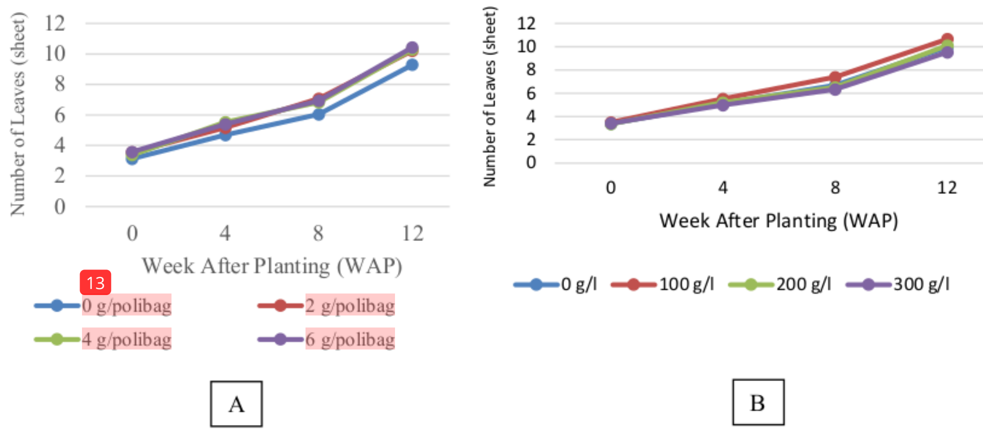

Figure 1. The number of leaves starting from the first measurement up to four measurements at (A) treatment Arbuscular Mycorrhiza Fungi (AMF) and (B) shallot filtrate.

3.2. Plant height

The observation on the average height of the plants show that the treatment of AMF was not significant, but the treatment of the shallot filtrate and interaction with AMF treatment gave significant effect.

Table 1. Average of plant height at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate.

Observation	Mycorrhizal (M)	The shallot filtrate (B) b0	b1	b2	b3	HSD 0.05
8 WAP	m0	29.39*ab*	29.17*ab*	27.80*b*	30.36*ab*	10.58
	m1	32.76*ab*	39.27*a*	35.89*ab*	26.94*b*	
	m2	32.39*ab*	32.96*ab*	36.33*ab*	26.83*b*	
	m3	31.67*ab*	36.06*ab*	32.67*ab*	35.86*ab*	
12 WAP	m0	42.44*bcd*	41.94*bcd*	38.20*bcd*	42.54*bcd*	17.39
	m1	45.83*abcd*	60.19*a*	53.89*ab*	33.26*d*	
	m1	50.92*abc*	45.42*abcd*	46.63*abcd*	36.19cd	
	m3	46.11*abcd*	47.39*abcd*	42.00*bcd*	46.56*abcd*	

Numbers followed by the same letter are not significantly different at HSD test α =0.05. WAP = Weeks After Planting.

Table 1 shows that treatment with AMF 2 g per polybag and shallot filtrate of 10% (M1B1) produces an average of the largest plant height (60.19 cm) and the average of the lowest plant height (33.26 cm) in the treatment of AMF 2 g and filtrate onion 30% (M1B3).

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3.3. Leaf chlorophyll index

The observation of the average leaf chlorophyll index showed that the AMF and the filtrate onion effect no significant effect on leaf chlorophyll index, but significant interaction. Table 2 shows that treatment with filtrate AMF 4 g of onion 10% (M2B1) produced an average of the largest leaf chlorophyll index (160.85) and the average of the lowest chlorophyll index (135.99) in the treatment of AMF 2 g and the shallot filtrate of 10% (M1B1).

Table 2. Average of leaf chlorophyll index at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate.

Mycorrhizal (M)	The shallot filtrate (B)				$HSD_{0.05}$
	b0	b1	b2	b3	
m0	142.75*ab*	139.94*b*	146.29*ab*	148.72*ab*	20.53
m1	147.02*ab*	135.99*b*	152.58*ab*	148.58*ab*	
m2	143.31*ab*	160.85*a*	145.00*ab*	150.61*ab*	
m3	145.03*ab*	154.38*ab*	145.21*ab*	150.14*ab*	

Numbers followed by the same letter are not significantly different at HSD test α =0.05.

3.4. Chlorophyll a, b, and total chlorophyll

The observation of the average chlorophyll a, chlorophyll b, and total chlorophyll showed that the AMF and the filtrate onion effect is not significant, but the interactions significantly affected chlorophyll a and total chlorophyll. There was no effect on observations of chlorophyll b.

Table 3 shows that the treatment M2B1 (AMF 4 g + shallot filtrate 10%) resulted in the largest average of chlorophyll a (544.34 μmol.m^{-2}) and the smallest average was shown by M1B1 (514.73 μmol.m^{-2}). The highest average value for the chlorophyll b parameter was shown by M2B1 (390.41 μmol.m^{-2}) and the smallest was M1B1 (342.07 μmol.m^{-2}), the largest average for total chlorophyll was M2B1 (818.64 μmol.m^{-2}) and the smallest was M0B0 (783.53 μmol.m^{-2}).

Table 3. Average chlorophyll a, chlorophyll b and total chlorophyll at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate

Chlorophyll	Mycorrhizal (M)	The shallot filtrate (B)				$HSD_{0.05}$
		b0	b1	b2	b3	
Chl a	m0	523.59*ab*	526.67*ab*	528.01*ab*	531.27*ab*	22.79
	m1	528.85*ab*	514.73*b*	535.44*ab*	530.77*ab*	
	m2	524.30*ab*	544.34*a*	526.43*ab*	533.13*ab*	
	m3	526.47*ab*	537.54*a*	526.67*ab*	532.56*ab*	
Chl b	m0	355.41	360.54	362.34	367.63	ns
	m1	363.75	342.07	374.56	366.81	
	m2	356.51	390.41	381.75	370.74	
	m3	359.88	370.76	360.23	369.83	
Chl Tot	m0	783.53*b*	788.74*ab*	790.93*ab*	796.43*ab*	38.53
	m1	792.35*ab*	768.76*b*	803.47*ab*	798.73*ab*	
	m2	784.72*b*	818.64*a*	788.29*ab*	799.57*ab*	
	m3	788.35*ab*	807.01*ab*	788.69*ab*	798.61*ab*	

Numbers followed by the same letter are not significantly different at HSD test α =0.05.

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3.5. Leaf area
The observation of the average leaf area show that the treatment of AMF, the shallot filtrate, and its interaction had no significant effect on leaf area.

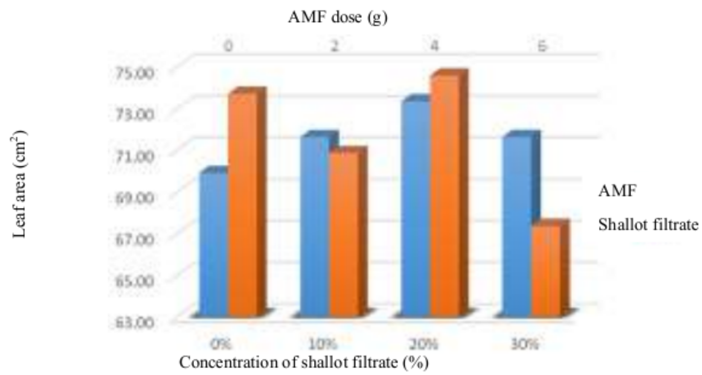

Figure 2. Leaf area at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate.

3.6. Stomata density
The observation of the average of stomatal density show that the shallot filtrate treatment had a very significant effect as well as AMF treatment and interaction between the treatments which significantly affected the density of stomata.

Table 4. Average density of stomata at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate

Mycorrhizal (M)	The shallot filtrate (B)				HSD 0.05
	b0	b1	b2	b3	
m0	21.23*abc*	21.66*ab*	22.08*ab*	16.14*bc*	7.24
m1	18.26*abc*	19.53*abc*	17.41*abc*	15.29*bc*	
m2	14.01*c*	16.99*bc*	16.56*bc*	18.26*abc*	
m3	15.71*bc*	24.63*a*	17.83*abc*	15.71*bc*	

Numbers followed by the same letter are not significantly different at HSD test α =0.05.

3.7. Stomatal aperture
The observation of an average area of stomatal aperture show that the shallot filtrate treatment had a highly significant effect, while treatment of AMF and the interaction between the two factors significantly affected the stomatal aperture.

Table 5. Average of stomata aperture at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate

Mycorrhizal (M)	The shallot filtrate (B)				HSD 0.05
	b0	b1	b2	b3	
m0	0.00035*c*	0.00027*c*	0.00024*c*	0.00044*bc*	0.000276
m1	0.00039*c*	0.00031*c*	0.00024*c*	0.00062*a*	
m2	0.00023*c*	0.00042*bc*	0.00037*c*	0.00070*a*	

m3	0.00034*c*	0.00027*c*	0.00023*c*	0.00034*c*

Numbers followed by the same letter are not significantly different at HSD test α =0.05.

Table 5 shows that treatment with AMF 4 g/polybag and shallot filtrate of 10% (M2B3) produced the largest average area of stomata openings (26.28 mm^2), while the smallest average stomatal aperture size (14.82 mm^2) in the treatment of AMF 6 g/polybag and the shallot filtrate of 20% (M3B2).

3.8. The root length

The observation of the average length of the roots show that the AMF treatment had a highly significant effect and the shallot filtrate treatment had significantly effect. On the contrary, interaction between the two factors had no significant effect on root length.

Table 6 shows that treatment of AMF 6 g (M3) produces an average of the highest root length (31.17 cm) and the average of the lowest root length (22.72 cm) in the treatment of AMF 0 g (M0). While the treatment of the filtrate onion produce an average of the highest root length B1 (10%) with a 27.33 cm root length and average root length was lowest for the treatment B3 (30%) with a length of 23.25 cm root.

Table 6. Average length of roots at different doses of Arbuscular Mycorrhizae Fungi (AMF) and different concentration of shallot filtrate

Mycorrhizal (M)	The shallot filtrate (B)				Average	$HSD_{0.05}$
	b0	b1	b2	b3		
m0	23.39	24.20	19.86	23.44	22.72*b*	4.14
m1	24.17	26.41	22.98	19:08	23.16*b*	
m2	28.78	27.06	21.50	21.33	24.67*b*	
m3	32.66	31.67	31.22	29.13	31.17*a*	
Average	27.25*a*	27.33*a*	23.89*a*	23:25*a*		
$HSD_{0.05}$	4.41					

Numbers followed by the same letter are not significantly different at HSD test α =0.05.

3.9. Percentage of infected root

The observation of the average of the infected root percentage by the AMF show that the treatment had a highly significant effect on the percentage of mycorrhizal infected roots. On the other hand, the shallot filtrate and its interaction with AMF did not affect the parameter.

Table 7. Average Percentage of infected roots by Mycorrhizal fungi at different doses of Arbuscular Mycorrhizae Fungi (AMF)

Mycorrhizal (M)	Average	$HSD_{0.05}$
m0	0.71*c*	1.69
m1	5.14*b*	
m2	6.68*a*	
m3	7.82*a*	

Numbers followed by the same letter are not significantly different at HSD test α =0.05. Data transformation result ($\sqrt{x} + 0.5$).

Table 7 shows that treatment of AMF 6 g/polybag (M3) produced an average of the highest percentage of infected root (7.82%), while the lowest average percentage of infected root (0.71%) was shown in the treatment of AMF 0 g/polybag (M0).

4. Discussion

Mycorrhizal symbiosis with the roots of plants can increase the absorption of nutrients N, P and K and improve the efficiency of the use of groundwater, increasing the voltage value osmotic plant cells on the ground that the water level is low enough, so that the plant could go on and be able to increase the rate of vegetative growth and crop production. Nasaruddin [6] stated that good soil structure makes the roots are well developed and the more comprehensive as well as increasing the field of absorption of the nutrients, because the AMF can extend and expand the reach of the roots, then the uptake of plant nutrients increases and crop yields will also rise because of mycorrhizae not only to take phosphorus in soil with low nutrient content but also on perbuhana unavailable becomes available. AMF association with plants to make efficient use of fertilizers. This is in accordance with the opinion Sudiarti [7], which states that the granting of mycorrhizal fungi were able to save 50% use of nitrogen fertilizers, phosphate fertilizers by 27% and 20% potassium fertilizer.

The filtrate red onions contain growth regulators have a role like Indol Acetic Acid (IAA). This is in accordance with the statement Masitoh [8], that the IAA is the most active auxin for various plants and play an important role in the growth of the optimal pacing. The filtrate onion as a natural growth regulator was instrumental in the growth and development of the pepper plant. According Rahayu and Berlian [9], onion bulbs contain vitamin B1, thiamine, riboflavin, nicotinic acid, and contains plant growth regulator auxin, gebirelin and rhizokalin to stimulate root growth.

Mycorrhiza and shallot filtrate containing hormones that play a role in the physiological processes of the pepper plant. Auxin and gibberellin are hormones that alleged role in extending the road between books pepper seedlings. Auxin plays an active role in encouraging the enlargement of cells stem segments, while the more active gibberellins as controlling plant growth, especially in the division and stimulate and promote the magnification stem segments cells and the formation of chlorophyll in the leaves [10].

5. Conclusions

Based on the results obtained, it can be summed up as follows:
1) Treatment of shallot filtrate show good effect on root length parameter.
2) Arbuscular mycorrhiza fungus (AMF) treatment effect on both the parameters of the number of roots, root length, and the percentage of mycorrhizal infected roots.
3) Interaction between arbuscular mycorrhiza fungi and filtrate shallots show good influence on parameters of plant height chlorophyll a, chlorophyll b, and total chlorophyll, stomatal density and stomatal aperture area.

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