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The amelioration of tin tailings with arbuscular mychorrizal fungi and liquid biofertilizer for pepper cultivation

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Abstract: The sand tailings from tin mining activities, which is low productivity, could be improved for plant cultivation by ameliorating with materials which contains mutual microorganisms. The aims of this research were to investigate the influences of arbuscular mychorriza dosages and liquid biofertilizer concentration on the growth of pepper in tin tailings media. This research was conducted to determine the effect of mycorrhizal dosages and biofertilizer concentration on the growth of pepper at sand tailings media in polybags. It was conducted at the Experimental and Research Station of Bangka Belitung University, Bangka. This study used Completely Randomized Design with two factors and three replications. The first factor was mycorrhizal dosages (0; 10; 20 g/plant), and the second one was liquid biofertilizer concentration (0;1;2;3 mL/L). The pepper growth variables were observed at 16th weeks after planting. The results showed the treatment of arbuscular mycorrhizae dosage was significantly influences on plant's height, stem diameter, number of internode, number of leaf, total leaf area, and percentage of infected root. Amelioration of 20 g/plant arbuscular mycorrhizae was the most effective for pepper growth. Biofertilizer concentration was no significantly influences on all of the variables. Combination of 10 g/plant arbuscular mycorrhizae and 2 mL/L of biofertilizer gave the highest pepper stem height, and combination of 20 g/polybag of mycorrhizae and 2 mL/L of biofertilizer showed the widest of pepper leaf area on sand tailings media.

1. Introduction

Tin mining activities produced ex-tin mining land which has dominated by tailings. Sand tailings is type of tailings that are the largest percentage of ex-mining land [1]. Sand tailings contains a high sand fraction of 92%, 2% loam, and 6% clay [1] causing low water absorption. The chemical characteristics of sand tailings are low soil pH and low availability of nutrients [2]; with a total N content of 0.01%, phosphorus content of 0.15 ppm, potassium 0.03 me [3]. The tailing materials have lost the soil colloids. Tin ores are separated from other materials using high pressure spraying so that the soil colloids are leached out [4].

Although classified as low productivity land, sand tailings have the potential to be used for agricultural use including pepper cultivation because it is quite extensive. Sand tailings for pepper cultivation needs amendment, one of them is by adding microorganisms that can improve the chemical quality of the soil. Soil microorganisms that can be developed include live fungi in mutually beneficial plant roots such as mycorrhiza.

Mycorrhizal fungi play a crucial role in enhancing plant growth and survival through enhancing plant nutrient uptake, water relations, ecosystem establishment, plant diversity, and productivity of plants [5], increasing tolerance to drought, as well as root's pathogen, and providing access for the plants to utilize the unavailable nutrients to be available, and reduce metal concentration on soil [6,7]. The application of mycorrhizal fungi together with endophytic bacteria to pepper seedlings in podzolic soil showed better vegetative growth at certain treatment combination [8]. Inoculation of pepper rooted cuttings with inoculum containing the G. *mosseae* spores and fungal structures with sorghum (*Sorghum bicolor* L.) roots and soil, led to a successful inoculation of black pepper rooted cuttings [9].

Biofertilizer is the substances that containing living microorganisms which is increase microbial activity in the soil [10-12]. Biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil

Based on the description above, planting pepper in sand tailings requires soil amendment to support its growth such as giving mycorrhiza and liquid biological fertilizers. Therefore, this study was conducted to look at the effect of mycorrhizal dosage and liquid biofertilizer concentration on the growth of pepper plants in sand tailings media.

2. Materials and Methods

The research has conducte d at the Experimental and Research Station of Bangka Belitung University, Bangka Island, Indonesia from May until September 2016. This experiment used factorial completely randomized design, with two factors and three replications. The first factor was arbuscular mychorrizae dosage, consists of 0; 10, and 20 g/plant. The second factor was biofertilizer concentration, consists of 0; 1; 2, and 3 mL/L.

Planting media is a mixture of 6.3 kg/polybag sand tailings from ex-tin mining site, 3.7 kg/polybag top soil, and 5 kg/polybag chicken manure compost. Firstly, the media was sterilized by steaming. Furthemore, media was innoculated with mycorrhizal culture according to the treatment level. The media was incubated in the field for two weeks before planting. The seedlings of pepper cv. Lampung Daun Kecil was three months old after cutting propogation. The seedlings were planted in mixed media. Application of biofertilizer was two weeks before planting, and 2, 6, and 10 weeks after planting. The maintanence of cultivated plant were watering, pest and disease controlling, weed controlling, and fertilizing with inorganic fertilizer using 15 g urea/plant, 15 g SP36/ plant, and 15 g KCl/plant. The data was analyzed using analysis of variance (ANOVA) 95% and 99% and Duncan's Multiple Range Test (DMRT) α=0.05.

3. Results and Discussion

The result of ANOVA was shown that the dosage of arbuscular mycorrhizal application in media has significant effect in increasing plant height, stem diameter, number of internoda, number of leaf, leaf area, and the persentage of infected roots, but it has no significant effects to other variables. The treatment of concentration of liquid biofertilizer has no significant effects to all variables. The interaction of arbuscular mycorrhizal dosage and biofertilizer concentration had only significant effect to increase plant height and increase leaf area.

The average of pepper growth in various AM fungi dosage is shown in Table 1. The level of 20 g/plant AM fungi resulted in a higher pepper growth variable than 0 and 10 g/plant, except increasing of plant height and total chlorophyll contents. Based on DMRT analysis, the average of increasing of stem diameter, internoda number, and leaf area of pepper plant which were applied with 20 g/plant

arbuscular mycorrhizal had significant different from 0 and 10 g/plant. The increasing of plant height, leaf number, percentage of roots infected of pepper in level of 20 g/plant of arbuscular mycorrhizal has no significant different from 10 g/plant and 0 g/plant.

The result obtained by the treatment of mycorrhizal dosage affected the growth of pepper plants. This is similar to the work of Ferry et al. [7], that the application of 60 g/plant mycorrhizal with organic compost of 80% water hyacinth and 20% zeolite showed the best growth of shrub pepper on sandy tin tailings. Mycorrhizae is a microorganisms that are symbiotic with plant roots; mycorrhiza gets assimilate from plants. For its host, mycorrhiza plays a role in increasing the surface area of contact with the soil, thereby accelerating the absorption of water and plant nutrients.

Table 1. Average of variable values of growth pepper in sand tailings media with arbuscular mychorrizal dosage application and the DMRT results.

AM Dosage (g/plant)	Plant height (cm)	Stem dia-meter (mm)	Inter-noda numbers	Leaf number	Leaf area (cm^2)	Percentage of infected roots (%)	Total of chloro-phyll content (mg/g)	Root bio-mass (g)	Shoot bio-mass (g)	Shoot-root ratio
0	32.18b	1.03b	32.58b	28.24b	399.79b	1.34b	22.682	1.0025	7.636	22.682
10	38.34a	1.09b	37.15b	33.93ab	478.1b	4.17a	23.103	1.1558	10.386	23.103
20	37.74a	1.32a	45.88a	40.18a	626.02a	5.47a	21.763	1.4133	11.628	21.765

Notes: Number followed by the same letters in the same column show no significant difference based on the Duncan's Multiple Range Test (DMRT) α=0.05

Wang [13] confirm from previous studies that AMF exhibit significant positive effects, such as increased plant survival, enhanced growth and nutrition, improved soil structure and quality, and greater plant re-establishmen. According to Fuad et al. mycorrhizae cause nutrient supply and absorption by plants to be optimal. Hazra et al. [14], only treatment combination of B2 of four endophytic bacteria isolates and 10 g/plant mycorrhizae showed better vegetative growth of pepper in podzolic soil

According to Table 3, pepper growth tend to increase in line with the increasing of biofertilizer concentrations. This increase occurs from 0 to 2 mL/L. Increasing the concentration of 2 mL/L to 3 mL/L decrease of acceleration of pepper growth in all variables, except for root biomass. The results also showed that the concentration of 2 mL/L biofertilizer produced the best pepper growth in the sand tailings ex tin-mining media. This result was different with other study. Kartikawati et al. [15] reported that the application of 50 ml nitrogen fixing bacterium and phosphate solubilizing bacteria i.e. *Azotobacter* and *Penicillium* together with 10 g/plant of arbuscular mycorrhizal fungi in undisturbed soil has vegetative growth of pepper was better than the control. The difference result might be caused by the liquid biofertilizer dosages used in this study were low enough to give a positive effect.

Table 2. Average of variable values of growth pepper in sand tailings media with biofertilizer concentration application.

Biofertilizer concentration (mL/L)	Plant height (cm)	Stem diameter (mm)	Inter-noda numbers	Leaf number	Leaf area (cm^2)	Percentage of infected roots (%)	Total of chloro-phyll content (mg/g)	Root biomass (g)	Shoot bio-mass (g)	Shoot -root ratio
0	32.82	1.06	33.56	29.99	429.11	2.76	15.60	1.21	8.023	7.2
1	37.83	1.25	39.79	33.19	440.14	2.81	12.84	0.97	9.226	10.2
2	39.29	1.28	42.89	38.61	593.95	4.57	17.05	1.25	11.372	9.1
3	34.41	1.01	37.91	34.67	542.01	4.51	14.82	1.34	10.371	8.4

The effect of interaction of arbuscular mychorrizal dosage and biofertilizer concentration has significant to increase plant height and leaf area. In general, there was a trend that the increasing of

biofertilizer concentration to 2 mL/L and the increasing of arbuscular mychorrizal dosage tended to increase the pepper growth, but the increasing of biofertilizer to 3 mL/L tended to decrease the pepper growth (Tabel 4). The role of mycorrhizae in maximizing the addition of mineral fertilizers was reported by Hegazy *et al.* [8] in their study of the use of mycorrhizae on tomato in Sinai.

Biofertilizer contains living microorganisms to increase the uptake of nutrients for plant. Biofertilizer may have nitrogen fixers such as Rhizobium, Azospirillum, Azotobacter, and phosphate solubilizing bacteria such as Bacillus, Psedomonas, and Aspergillus [12]. Biofertilizers differ from chemical fertilizers for not directly supply nutrients to plants [12].

Table 3. Average of variable values of growth pepper in sand tailings media with arbuscular mychorrizal dosage and biofetilizer concentration treatments, and the DMRT results.

AM Dosage (g/plant)	Biofertilizer concentration (mL/L)			
	0	1	2	3
		Plant height (cm)		
0	22.83 c	38.77 ab	31.67 abc	33.44 ab
10	38.78 ab	41.22 ab	43.36 a	30.00 bc
20	36.86 ab	33.50 abc	42.83 a	37.78 ab
		Stem diameter (mm)		
0	0.72	1.27	1.16	0.96
10	1.12	1.16	1.14	0.97
20	1.33	1.33	1.53	1.10
		Internoda number		
0	19.00	37.06	40.83	33.44
10	35.89	39.11	36.00	37.61
20	45.78	43.22	51.83	42.67
		Leaf number		
0	16.22	29.83	38.33	28.56
10	33.22	31.45	33.06	38.00
20	40.55	38.28	44.44	37.44
		Leaf area (cm^2)		
0	218.01c	512.87bc	471.37bc	396.90bc
10	503.17bc	405.02bc	390.05bc	614.15b
20	566.14b	402.53bc	920.44a	614.97b
		Total of chlorophyll content (mg/g)		
0	15.63	12.89	16.22	14.74
10	14.14	13.25	19.28	16.70
20	17.01	12.38	16.68	13.02
		Root biomass (g)		
0	0.80	0.85	1.22	1.13
10	1.53	1.02	0.86	1.21
20	1.30	1.03	1.66	1.67
		Shoot biomass (g)		
0	4.39	7.57	10.75	7.84
10	12.08	10.44	7.37	11.65
20	8.14	9.67	16.00	12.70
		Percentage of infected roots (%)		
0	0.7	0.7	3.25	0.7
10	1.97	3.86	5.13	5.75
20	5.60	3.86	5.34	7.09

Note: Number followed by the same letters in the same column or same rows in the same variable show no significant difference based on the Duncan's Multiple Range Test (DMRT) α=0.05

4. Conclusion

Arbuscular mycorrhizal fungi effects to early growth of pepper in sandy tailings media. The best pepper growth was obtained in the planting medium with 20g/plant AM fungi. The treatments of biofertilizer

concentration in media has no effect to pepper growth. It is likely that the liquid biofertilizer dosages used in this study were low to give the positive result. The treatment combination of 10 g mychorrizal/plant and 2 mL/L biofertilizer results in better growth of pepper than other treatments.

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