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RESPONS OF CACAO SEEDLINGS FERTILIZED WITH PAPUAN AYAMARU PHOSPHATE ROCK (PAPR) COMBINED WITH HUMIC ACID, INOCULATION OF AMF AND PHOSPHATE SOLUBILIZING BACTERIA

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ABSTRACT

The effect of Papuan Ayamaru Phosphate Rock (PAPR) dosage levels combined with inoculation of humic acid (HA), *arbuscular mycorrhizae fungi* (AMF), and Phosphate Solubilizing Bacteria (PSB) was studied on the growth of cocoa seedling. The treatment of PAPR consisted of 0, 1, 2, 4 g P_2O_5 / seedling and 2 g P_2O_5 SP-36 /seedling as control. Ultisol was collected from Jasinga, West Java and used as planting media, while the Upper Amazone Hybrid F-1 cocoa seeds were obtained from the Coffee and Cacao Research Center, Jember-East Java. The study was carried out for 16 weeks after planting under 60 % of shading net. All fertilization treatments which were inoculated with AMF produced better seedling growth compared to the seedling growth without AMF. The response of seedling growth and P available content of the media due to 4.0 g P_2O_5 plant^{-1} of PAPR was still linearly increased. The responses were improved by application of HA, AMF and PSB inoculation. Synthesis of organic acids by PSB inoculation and the release of acid phosphatase by AMF to the media was an indication that there was an external mechanism of P solubilization of the phosphate rock. The effectiveness of AMF, HA and PSB on shoot dry weight were 104.29%, 4.38% and 4.24% respectively on P uptake were 191.00%, 33.20% and 18.31% respectively, on root colonization were 681.82%, 10.26% and 6.17% respectively, on acid phosphatase activity were 30.07%, 7.58%, 7.34% respectively, and on PAPR efficiency were 104.29%, 438% and 4.24% respectively.

Key words: biofertilizer, inoculation, mycorrhizae, phosphate rock, SP-36

TANGGAP BIBIT KAKAO (*Theobroma cacao* L.) TERHADAP PUPUK FOSFAT ALAM PAPUA DAN KOMBINASI ASAM HUMAT, INOKULASI FMA SERTA BAKTERI PELARUT FOSFAT

ABSTRAK

Pengaruh tingkat dosis fosfat alam Ayamaru yang dikombinasikan dengan asam humat, inokulan fungi mikoriza arbuskular (FMA), dan bakteri pelarut fosfat terhadap pertumbuhan bibit kakao telah dilaksanakan di kebun Percobaan Cikabayan IPB Bogor dengan naungan paranet 60%. Perlakuan tingkat dosis fosfat alam Ayamaru yang digunakan adalah 0, 1, 2, 4 g bibit tanaman^{-1} P_2O_5 dan 2 g bibit tanaman^{-1} P_2O_5 SP-36 sebagai kontrol. Media tanam digunakan tanah Ultisol dari Jasinga Bogor sedangkan benih kakao yang digunakan adalah Upper Amazone Hybrid F-1 dari Pusat Penelitian Kopi dan Kakao Jember Jawa Timur. Pada umur 16 minggu setelah tanam, semua perlakuan pemupukan yang diinokulasi FMA memberikan pertumbuhan bibit kakao lebih baik daripada tanpa inokulasi FMA. Respon pertumbuhan bibit kakao dan kadar P tersedia hingga tingkat dosis 4g pot^{-1} P_2O_5 fosfat alam Ayamaru meningkat secara linier. Respon ini lebih meningkat dengan aplikasi asam humat, inokulasi FMA dan bakteri pelarut fosfat. Sintesis asam organik oleh inokulasi bakteri pelarut fosfat dan eksudasi asam fosfatase oleh FMA menunjukkan adanya mekanisme eksternal terhadap pelarutan pupuk fosfat alam. Efektivitas FMA, asam humat, dan bakteri pelarut fosfat terhadap bobot kering tajuk berturut-turut adalah 104.29%, 4.3%, dan 4.24%; terhadap serapan P berturut-turut 191.0%, 33.20%, 18.31%, dan terhadap kolonisasi akar berturut-turut 681.82%, 10.26%, serta 6.17%, terhadap aktivitas asam fosfatase berturut-turut 30.07%, 7.58%, 7.34%, sedangkan terhadap efisiensi fosfat alam Ayamaru berturut-turut 104.29%, 438% dan 4.24%.

Kata kunci: fosfat alam, inokulasi, mikoriza, pupuk biologi, SP-36

INTRODUCTION

The effort to increase cocoa production has been done continually in Indonesia, mostly through the intensification of plantation area. The intensification areal of cocoa plantation was started with high quality seeds in order to have a well grow and high production. As fertile areas became more limited, cocoa development has extended into the marginal land. This land is commonly dry areas, which has low level of fertility, therefore fertilizers should be supplied in the right amount.

Different from the phosphate rock in other locations, which is in the form of rock, the phosphate rock in Ayamaru Papua is in the form of soil. Subsequent research showed that the phosphate rock is composed of Crandallite mineral ($CaAl_3(PO4)_2(OH)_5.H_2O$). In Indonesia, this kind of phosphate rock deposit is only found in Ayamaru District, Papua (Schroo, 1963). According to Rumawas (1990) phosphate rock can be used directly as a source of P fertilizer after grinding in 100 mesh, dissolving with strong acid or humic acid, AMF inoculation and inoculating with phosphates solubilizing bacteria (PSB).

Inoculation of Arbuscula Mycorrhiza Fungi (AMF) can help plants in absorbing P especially from a number of forms which is not available in the rhizosphere (Smith, 2002). Research by Widiastuti & Baon (1994) showed that when Setia Bakti phosphate rock was given (13.36% P HCl 25%) it increased the dry-weight and N, P, K, Ca, Mg and Mn shoot uptake in both of non-inoculated and inoculated cocoa seedling with *Acaulospora delicata* and *Glomus fasciculatum*. Yahya *et al*. (2000) stated that cocoa seedling inoculated with AMF in general is more adaptive to environmental stress, and it is an effective way in increasing inorganic P effectiveness (FAO, 2005).

The release of P from phosphate rock could be increased by the inoculation of phosphates solubilizing bacteria (Jones & Farrar, 1999). However, the activity of phosphates solubilizing bacteria was influenced by the type of its mineral composition (Mikanová & Nováková, 2002). Fluorescence P inoculation could also increase P solubility in Ultisol applied with Tunis phosphate rock (Noor, 2003). Meanwhile, humic acid is known to be able to increase the effectiveness of natural phosphate by its ability to release anion PO_4^{3-} and cation Ca^{2+} from hardly dissolved mineral, to complex metal from solubility and to increase microbial metabolism (Mayhew, 2004), to influence new roots development and to enhance permeability of root membrane (Lulakis & Petsas, 1995).

Arbuscula Mycorrhiza Fungi (AMF) inoculation, humic acid (HA), phosphates solubilizing bacteria (PSB) and its combination are treatment alternatives to increase the solubility of Papuan Ayamaru phosphate rock. However, there is no research yet on the use of AMF, HA, and PSB in increasing an agronomical value of Ayamaru Papuan phosphate rock in cocoa seedling. Therefore, a research was conducted to study the response of cocoa seedling to Papuan Ayamaru phosphates rock (PAPR) fertilization and the combination of AMF inoculation, phosphates solubilizing bacteria, and humic acid.

MATERIALS AND METHODS

The experiment was carried out in the Cikabayan Field Laboratory IPB Bogor. It used factorial experiment with randomized complete design consisting of the levels of P_2O_5 dosage and combination of AMF inoculation, HA, and PSB. There were two factors i.e., the first factor was P_2O_5 dosages which consisted of 0, 1.0, 2.0, 4.0 g P_2O_5 phosphate rock/seedling and 2 g P_2O_5 SP-36/ seedling was used as control. The second factor was the combination of Mycover AMF inoculation (m), PSB isolate FT.3.2 (b), and 3.10^{-3} mL humic acid (h). Each combination of treatment was replicated three times and three experimental seedling plants were used in each experimental unit.

The Hybrid F-1 Upper Amazone Hybrid (UAH) cocoa seeds were obtained from The Indonesia Coffee and Cocoa Research Center, Jember East Java, which the

AMF Mycofer inoculants and isolate of PSB FT.3.2 were collected from the Laboratory of Forest Biotechnology and Environment, Bio Resources and Bio Technology Research Center, IPB Bogor, and Humega (6.26% HA) was supplied by the Green Planet Indonesia. Shading net of 60% level was used. Seedling media was Ultisol from Jasinga Bogor which contained exchangeable Al 17.03 cmol kg^{-1}. PAPR was collected from the Ayamaru District West Papua.

The growing media of Ultisol was dried, cleaned, and sieved with the size of 0.5cm x 0.5 cm. The cleaned soil was put into polybags of 20 cm x 30 cm-size containing 3 kg of soil per polybag. The phosphate rock was grounded until 100 mesh of particle and was applied into the growing media a week before planting according to the treatment dosage, along with the basic fertilizer of 2 g of Urea and 2 g of KCl per seedling. All fertilizers were mixed well with the growing media and watered for seven days of incubation period. Mycofer AMF inoculants were cultured in sterile zeolit media for 3 months with the *Pueraria japonicum* as host plant, using plastic cup with the capacity of 200 gram of zeolit each cup. PSB was cultivated in YMB media. The number of applied bacteria was determined by the method of flowing dish based on standard curve of the population, which came from the optical density value and Colony Forming Unit. Humega, in addition, was diluted up to the concentration of 3.10^{-3} mL. Dilution was done by using the aquadest and it was applied for 10 mL per seedling.

Cocoa seedling was grown in a plate with sterile zeolit media. After the seedling was one week old, it was transplanted into a polybag which was filled with the growing media. Twenty grams AMF inoculants (mixture of zeolit, spore, hyphal, and AMF colonization root) were filled into the planting hole of the polibag, subsequently the cocoa seedling as well and the hole was covered again. One milliliter of PSB inoculation and 10 mL of humic acid with 3.10^{-3} mL concentration were given at the root seedling zone, when the seedling was planted. Seedling was allocated under 60% of the seeding net for 16 weeks after planting, watered every day up to 100% field capacity throughout the experiment.

The observed variable were height of the seedling (cm) measured from stempbase up to the growing point, stem diameter (mm) measured at 5 cm aboved the root base, leaves number, root dry-weight (g), shoot dry-weight, shoot to root ratio, shoot P content (%), shoot P uptake (g), AMF colonization (%), acid phosphatase activity, P availability, P total, exchangeable Al, and pH of the media. The analysis of shoot P content, shoot P uptake, total P content, exchangeable Al, and pH of the media was conducted in the Chemist and Soil Fertility Laboratory of IPB, while acid phosphatase activity and AMF colonization were analysed separately at the Plant Physiology Laboratory and Laboratory of Forest Biotechnology and Environment, Bio Resources and Bio Technology Research Center, IPB Bogor.

Data were analyzed using analysis of variance. The Least Significant Difference (LSD) test and contrast orthogonal test were used to evaluate the differences among treatments. The effect of PAPR dosage levels on the growth of cocoa seedling was analysed by polynomial orthogonal test at 95% significant level. All data were analysed using SAS v 9.0.

RESULTS AND DISCUSSION

The dosage level of PAPR and mbh combination highly significant influenced ($P<0.001$) the growth of cocoa seedling by increasing the seedling height, and leaves number, however the interaction was not siginificant (Table 1). Phosphate rock with the dosage level of 2.0 g P_2O_5 gave the best result to seedling height, stem diameter and leaf number, while the highest root dry weight was produced by 4 g P_2O_5 phosphate rock/seedling plant. Shoot-root ratio and the highest shoot P content were resulted from seedling fertilized with 2 g P_2O_5 SP-36/ plant.

All parameters in the cocoa seedling with treatment combination of AMF inoculation ($^{+}$m) were significantly different compared to the treatment combination without AMF inoculation (-m). This indicate the important role of AMF in cocoa seedling. Miyakasa & Habte (2001) stated that cocoa was categorized as plant of arsbuscular mycorrizal fungi dependent. Linderman & Davis (2000) also asserted that arsbuscular mycorrizal fungi inoculation increased P uptake through extraradical hypha, and thus the efficiency of nutrient application in triggering the growth of cocoa seedlings is increased.

The effect of AMF inoculation in the treatment of mbh combination was higher than the role of PSB and humic acid (Table 1). AMF inoculation in the treatment of mbh combination increased the seedling's height, stem diameter, leaves number, shoot P content and root dry-weight by 49.60%, 28.43%, 70.21%, 25.00%, 117.78% respectively, but shoot-root ratio decreased by 11.85%.

The growth response of cocoa seedling caused by PARP dosage were: **y**= 43.16 + 2.66 x R^2 = 0.66 (seedling height), **y**= 8.27 +0.71x R^2= 0.64 (stem diameter), **y**= 11.15 + 2.76x - 0.54x^2 R^2 = 0.99 (leaves number), **y** = 0.21 + 0.22x R^2 = 0.98 (root dry-weight), **y** = 0.15 + 0.01x R^2 = 0.95 (shoot P content), and **y** = 3.98 + 0.25x - 0.05x^2 R^2 = 0.59 (shoot-root ratio).

The effect of treatment combination of AMF inoculation (+m) and without (-m), also PSB inoculation (+b) and without (-**b**) showed significant differences on shoot dry weight, shoot P uptake, root colonization, and acid phosphatase activity, whereas there was a significant difference only in root colonization between the application of humic acid (+h) and without humic acid (-h) (Table 2). However, the effectivity of AMF inoculation (m) in the treatment of mbh combination was higher than the role of

Table 1. Effect of PAPR dosage and combination of AMF, PSB, Humic Acid on seedling height, leaf number, stem diameter, shoot P content, root dry weight, and shoot-root ratio evaluated with LSD test and contrast orthogonal

Treatment	Seedling height (cm)	Stem diameter (mm)	Leaves number	Shoot P content (%)	Root dry-weight (g)	Shoot-root ratio
PAPR dosage (g P_2O_5/ seedling):						
Control 0	34.92 d	6.29 c	11.17 b	0.15 e	1.16 c	3.93 b
1	37.79 c	6.85 b	13.30 ab	0.16 d	1.49 b	4.31 ab
2	42.00 a	8.36 a	14.54 a	0.18 c	1.67 b	4.17 ab
4	41.54 ab	8.56 a	12.50 ab	0.19 b	2.07 a	4.06 b
SP36: 2 (g P_2O_5/ Seedling)	39.46 bc	8.18 ab	12.17 ab	0.21 a	1.48 b	4.66 a
Combination :						
mbh	44.43	7.68	15.37	0.20	1.96	4.09
mh	40.57	7.39	13.53	0.18	1.95	3.96
mb	41.87	7.25	13.00	0.17	1.59	4.21
bh	29.70	5.98	9.03	0.16	0.90	4.64
Contrast Orthogonal test:				.		
+m vs –m	**	**	**	**	**	*
+b vs –b	*	**	ns	**	**	ns
+h vs –h	**	*	ns	**	ns	ns
mbh vs others	**	**	**	**	**	ns
Effectiveness (%)						
mbh vs mh (b)	10.50	3.92	13.60	11.11	0.51	3.28
mbh vs mb (h)	7.07	5.93	18.23	17.65	23.27	-2.85
mbh vs bh (m)	49.60	28.43	70.21	25.00	117.78	-11.85

Note: The number in each column followed by the same letter is not significantly different in LSD Test for 95%.
m: AMF Mycofer; h:humic acid (3.10^{-3} mL) ; **b**: PSB Isolate FT.3.2 ;
*: significant, **: highly significant, ns: non-significant.

Table 2. Effect of several treatment combinations on shoot dry weight, shoot P uptake, root colonization, and phosphatase acid activity of cocoa seedling evaluated with Contrast Orthogonal test

Treatment	Shoot dry -weight (g/seedling)	Shoot P uptake (mg/seedling)	Root colonization (%)	Acid phosphatase activity (µg/g/hour)
Combination :				
mh	7.31	13.60	81	13.62
mb	7.30	12.08	78	13.59
bh	3.73	5.53	11	11.24
mbh	7.62	16.09	86	14.62
Contrast Orthogonal test:				
+ m vs – m	**	**	**	**
+ b vs – b	ns	**	**	**
+ h vs – h	**	ns	**	ns
mbh vs others	**	**	**	**
Effectiveness (%):				
mbh vs mh (b)	4.24	18.31	6.17	7.34
mbh vs mb (h)	4.38	33.20	10.26	7.58
mbh vs bh (m)	104.29	191.00	681.82	30.07

Note: **m**: Mycofer AMF; **h**: asam humat (3.10^{-3} mL); **b**: PSB isolate FT.3.2;
** : highly significant, ns : non-significant.

BPF (b) and humic acid (h). The efectivity of AMF inoculation in the treatment of mbh combination increased shoot dry weight, shoot P uptake, root colonization, and acid phosphatase activity by 104.29%, 191.00%, 681.82%, 30.07% respectively.

The interaction of ARP dosage and m, b, h, combination significantly influenced ($P<0.001$) shoot dry weight, shoot P uptake, root colonization and phosphatase acid activity of cocoa seedling (Figure 1). Mycorrhizal plants produced higher shoot dry weight and absorbed more P from growing media than plants without mycorrhiza (Raj *et al.* 1980). According to Bago *et al.* (2000), AMF inoculation could increase photosynthesis and plant biomass. Smith & Read (1997) stated that P translocation rate in mycorrizal roots could achieve 3-5 times higher than non-mycorrizal plants.

Many new roots with high permeability which resulted from humic acid application would get benefits for root colonization process by mycorriza as mycorrizal colonization generally formed in new roots (Sieverding, 1991). Phosphatase acid content in root depended on the high level of root colonization (r = 0.98). According to Ingrid *et al.* (2002) and Widiastuti *et al.* (2003), phosphatase acid content of root was increased by root colonization of AMF. Phosphatase acid was an important enzyme for plant physiological respons in increasing P solubility (Rao *et al.,* 1999).

Shoot dry-weight, shoot P uptake, root colonization, and acid phosphatase activity were the highest at all dosage levels of PARP as shown by seedling with mbh combination, whereas the lowest value was shown by seedling with the bh combination. The highest root colonization for each treatments of mh, mb, and mbh combination was achieved by dosage of phosphate rock at 1.78, 1.97, and 1.97 g P_2O_5, while the respons of root colonization to the treatment of bh combination was linear. Treatment which involved AMF, root colonization and activity of root acid phosphatase decreased by the increase of PARP dosage in growth media.

Interaction of phosphate rock dosage and m, b, and h combination significantly influenced the available P, total P content, pH media, and exchangeable Al The availability of P and exchangeable Al between the treatment of AMF (+m) combination and without FMA (- m) showed a significantly different, while between combination with PSB (+b) and without PSB (- b) was significantly difference on available P, total P, pH, and exchangeable Al (Table 3). The

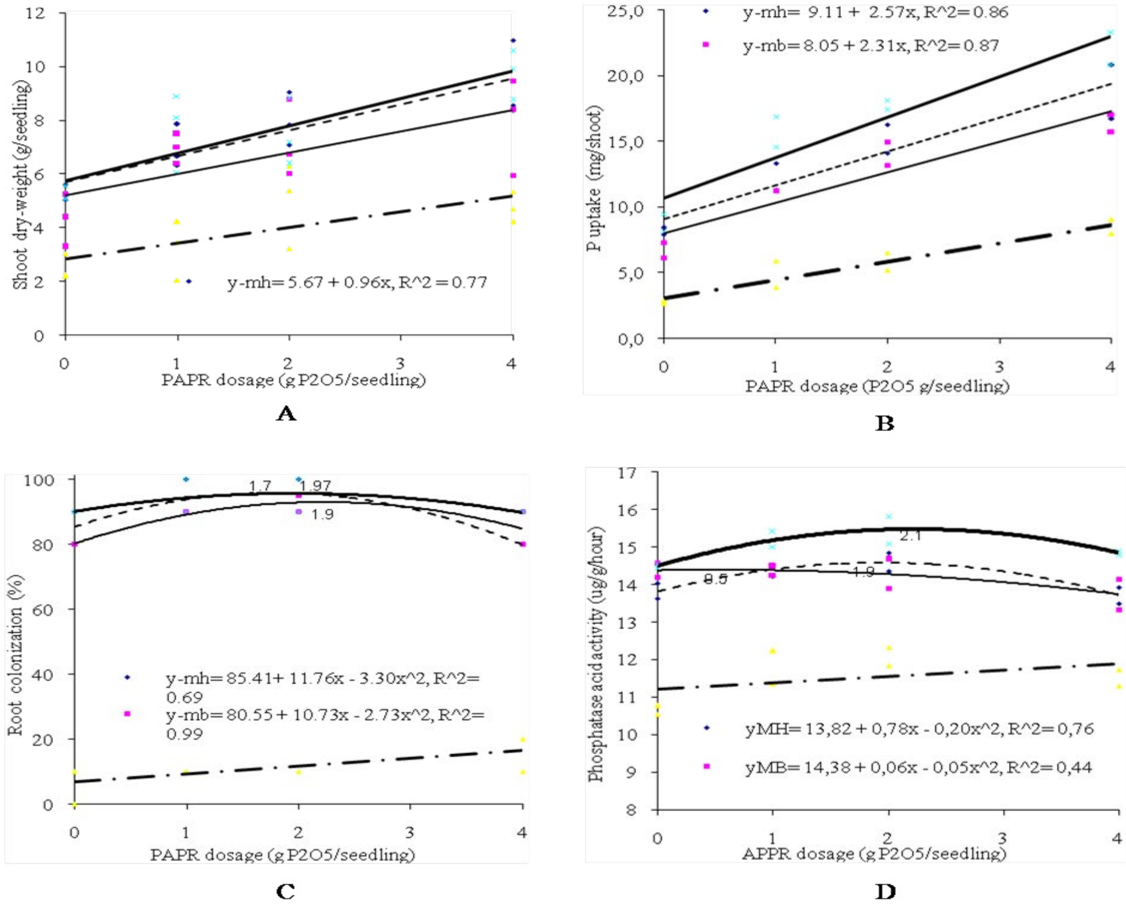


Figure 1. Interaction curve response of shoot dry-weight (a), shoot P uptake (b), root colonization (c), and acid phosphatase activity (d) to the levels of PAPR dosage with several combinations.

application of humic acid (+h) and without humic acid (-h) showed no differences to available P. However, the role of AMF inoculation in combination treatment was more effective than PSB and humic acid. AMF inoculation increased available P 22.02%, total P 20.74%, and pH media 1.34%. AMF was also effective in decreasing exchangeable Al of the media by 12.60%.

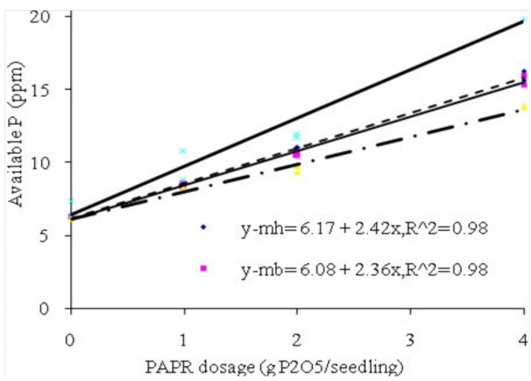
Medina *et al.* (2005) asserted that the use of PSB combination and AMF inoculation would release more available P to be absorbed by plants. Phosphate solubilizing bacteria with its organic acids content were known as external mechanism in solubilizing P both from ARP and P bound by Fe and Al in acid soil, and hence it became available for the plant (Xu *et al.*, 2004). At all phosphate rock dosage levels, the availability of P and total P content of root (Figure 2) were increased by the combination of AMF, PSB, and humic acid (mbh). Mayhew (2003) reported that nutrients released from phosphate rock to plants through complex geo-biology interaction occurred in the rhizosphere microclimate of plants and at the zone between bacteria and phosphate rock mineral. When plants, bacteria, and fungi dissolved the phosphate rock with different rates, the releasing of organic acids by different organisms influenced the synergy, and hence nutrients released would be sustainable. The low value of exchangeable Al in the combination treatment of AMF, PSB, and

Humic acid (mbh) showed an indication of a synergism among the structure components of treatment combination by the availability of organic acid exudates and the role of humic acid in heavy metal adsorption and also phosphate rock solubility. Carboxylate acid mobilized both an-organic P and organic P which chelating metal ion that bound P with 'ligand' change (Lambers *et*

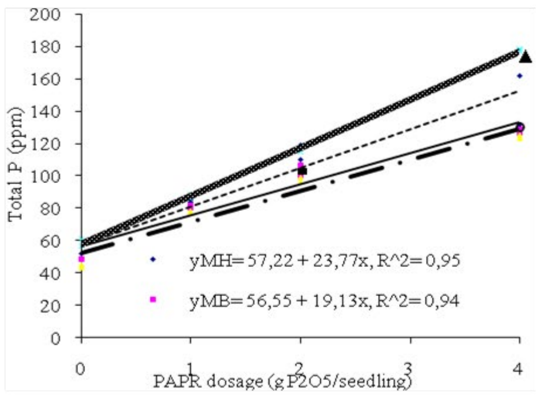
Table 3. Effect of several treatment combinations on available P, pH, total P, exchangeable Al evaluated with Contrast Orthogonal test

Treatment	Available P (ppm)	Total P (ppm)	Media pH	Exchangeable Al (me/100 g)
Combination :				
mh	11.57	108.25	4.50	15.16
mb	11.34	100.05	4.56	15.73
mh	10.22	95.60	4.47	17.22
mbh	12.47	115.43	4.53	15.05
Contrast Orthogonal test				
+ m vs – m	**	ns	ns	**
+ b vs – b	**	*	**	**
+ h vs – h	ns	**	*	**
mbh vs others	**	**	ns	**
Effectiveness (%):				
mbh vs mh (b)	7.78	6.63	0.01	-0.01
mbh vs mb (h)	9.96	15.37	-0.01	-4.32
mbh vs bh (m)	22.02	20.74	1.34	-12.60

Note: **m**: Mycofer AMF; **b**: PSB isolate FT.3.2; **h**: humic acid (3.10^{-3} mL); (+): increase; (-): decrease, *: significant, **: highly significant, ns: non-significant.



A



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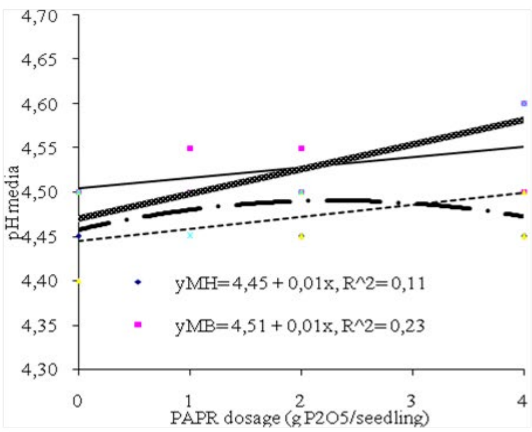

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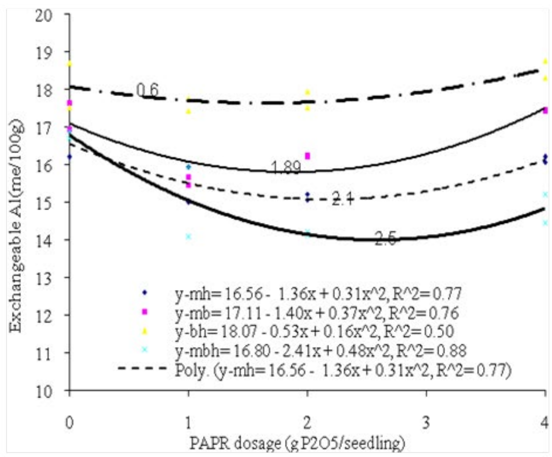

D

Figure 2. Interaction curve response of available P (a), total P (b), pH media (c), and exchangeable Al (d) to levels of PAPR dosage with several treatment combinations

al., 2006). Jones (1998) stated that organic acids anion with oxygen including hydroxyl cluster and carboxyl had the ability to form stable complex with cations such as Fe^{3+} and Al^{3+}. Denny & Ridge (1992) asserted that AMF decreased heavy metal poison in plants beside helping for P uptake in plants.

Tan (2003) argued that nutrients released from phosphate rock was also caused by biological activity, which was increased by the availability of humic compound. But, this experiment showed that the treatment of PSB and humic acid combination gave low influence compared to other combinations. It seemed that inoculated PSB was not able to optimally dissolve P from phosphate rock; therefore the growth of cocoa seedling was depressed.

CONCLUSION

Mycofer AMF inoculation, PSB isolate FT.3.2, and humic acid application at 3.10^{-3} mL/seedling were the best combi- nation treatment which produced an average seedling's height of 44.43 cm, shoot dry-weight 7.62 g/seedling, and P uptake 16.09 mg/shoot. Decreasing of exchangeable Al only occured at dosage level 2.0 g P_2O_5/seedling of Ayamaru phosphate rock followed by the increasing of root colonization and acid phosphatase activity. Increasing PAPR dosage up to 4.0 g P_2O_5/seedling increased exchangeable Al followed by the decreasing of root colonization and acid phosphatase activity. Mycofer AMF inoculation in cocoa seedling was more effective in increasing Ayamaru phosphate rock efficiency (104.29%) than the phosphate solubilizing bacteria (4.38%) and humic acid (4.24%).

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