

# The Role of Indigenous Mycorrhiza in Combination with Cattle Manure in Improving Maize Yield (*Zea Mays* L) on Sandy Loam of Northern Lombok, Eastern of Indonesia

Wahyu Astiko<sup>1</sup>, Ika Rochdjatun Sastrahidayat<sup>2</sup>, Syamsuddin Djauhari<sup>2</sup> and Anton Muhibuddin<sup>2</sup>

<sup>1</sup>Faculty of Agriculture, Mataram University, Jl. Majapahit No. 62 Mataram 83124, West Nusa Tenggara, Indonesia, e-mail: astiko\_mataram@yahoo.co.id or wahyuastiko@unram.ac.id

<sup>2</sup>Graduate Program, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang, Indonesia

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## ABSTRACT

A glass house study was conducted to evaluate the contribution of indigenous arbuscular mycorrhiza fungi (AMF) in improving maize yield grown on sandy loam of Northern Lombok. The package of organic fertilizers treatments were tested including: without inoculation of mycorrhiza, inoculation mycorrhiza and no added inorganic fertilizers, inoculation of mycorrhiza with cattle manure added, inoculation of mycorrhiza with rock phosphate added and inoculation mycorrhiza with inorganic fertilizers. The treatments were arranged using a Completely Randomized Design with four replications. The results of the study show that the inoculation of AMF significantly increased soil concentration of N, available-P, K and organic-C by 37.39%, 60.79%, 66.66% and 110.15% respectively observed at 60 days after sowing (DAS). The similar trend was also found at 100 DAS, where those nutrients increased by 21.48%, 69%, 43.93% and 37.07%, respectively compared to control. The improving of soil fertility status was also reflected by nutrients uptake (*i.e.* N, P, K, Ca) as well as growth and yield of maize. N, P, K and Ca uptake increased by 1,608%, 1,121%, 533% and 534%, respectively. Roots and top dry biomass at 60 DAS increased by 718.40% and 337.67%, respectively. The trend increased of the biomass was followed by observation at 100 DAS. Yield components including cobs, grain and weight of 100 grains increased by 313.60%, 411.84% and 137.54%, respectively. In addition, the inoculation of AM with F2 contributed significantly to the spore numbers and root infection.

**Keywords:** Arbuscular mycorrhiza, dryland, maize yield, soil fertility

## INTRODUCTION

The development of sustainable dryland farming system has special constrains dealing with limiting factors of crop production in particular low of soil fertility status. The soil fertility problems such as poor soil organic matter (SOM), low of essential nutrients availability as well as low of water retention have mainly considered responsible to low crop productivity in the dryland farming system and therefore it also often associated with poor farmers in the tropical semi-arid of Lombok and possibly in other tropical semi-arid region in eastern part of Indonesia.

The soil organic matter improves soil physico-chemical and biological properties. Soils with high content of soil organic matter mostly perform better growth and yield of food crops including maize, soybean and rice. However, sandy soils containing very low soil organic matter in particular often have poor soil structure and unstable aggregate. Those

soils have low nutrients and water retention capacity as well as low buffering capacity (Perner *et al.* 2007) which influence soil microbial activities (Khalvati *et al.* 2010). Soil organic matter is used by microorganisms as an energy source for their metabolism. In general, population of soil microorganisms tend to decrease along with decreasing of soil organic matter and therefore it inhibits the biochemical reaction (Hassen *et al.* 2001).

Improving soil fertility status can be conducted through several ways such as addition of phosphorous fertilizers and arbuscular mycorrhiza fungi (AMF). A substance mostly used as phosphorus sources for soil is rock phosphate. The use of rock phosphate for fertilizing soils often has better effect to the phosphorus availability compared to that of using SP-36 due to slow release of P from rock phosphate (Kabirun 2002). The positive effect of roots-AMF infection is that it can increase nutrients and water retention, nutrients uptake, growth and crops yield grown under dryland farming system (Smith *et al.* 2010). Astiko (2009) confirmed that friendly environmental fertilizing in which mycorrhiza being

inoculated on maize resulted in higher degree of infection along with number of spores, growth and yields compared to that plant without inoculation of mycorrhiza. The AM forms symbiosis with plant roots in all major terrestrial biomass (Smith *et al.* 2010). Therefore with application of mycorrhiza in soils in which cattle manure also being applied could develop symbiotic interaction and produced extensive root systems that enhanced P uptake by plant were also confirmed in this study. This possibly resulted in increasing effectiveness of in acquisition of relatively immobile nutrients such as P (Gahoonia and Nielsen 2004). In addition, production of exudates containing organic acids by cluster roots can enhance the availability of insoluble P and eventually it becomes available for plant and improves root proliferation of maize grown in Sandy soils. Mycorrhiza produces external mycelium in rhizosphere and therefore it will increase the amount of water and nutrient retention due to increase of organic matter and other soil physical properties. (Drew *et al.* 2003; Smith and Read 2008). This phenomenon occurs due to better availability of nutrients as well as better roots proliferation (Antoun and Kloepper 2001). Similar results were also reported in irrigated farming system by Sastrahidayat *et al.* (2001). However, similar reports under dryland condition seem to very limited in Indonesia.

This study was aimed to evaluate the role of indigenous mycorrhiza in combination with cattle manure in improving maize yield (*Zea mays*, L) under a glass house study using a sandy loam of dryland Northern Lombok, Eastern of Indonesia.

## MATERIALS AND METHODS

### Study Site and Design

A glass house-pot trial using soil sample of sandy loam soil from Northern Lombok was conducted in Faculty of Agriculture, Mataram University. Some package of organic fertilizers were tested as treatments. Those were involve: F<sub>0</sub> as control (without fertilizers and no AMF inoculation), F<sub>1</sub>: AMF inoculation without chemical fertilizers (CF), F<sub>2</sub>: AMF inoculation plus cattle manure (CM) added, F<sub>3</sub>: AMF inoculation plus rock phosphate (RP), and F<sub>4</sub>: AMF inoculation plus CF. The treatments were arranged using a Completely Randomized Design (CRD) with four replications.

### Inoculum and Fertilizers

Inoculation of AMF was done at the time of sowing. The inoculum of AMF (20 g per pot) was layered at 10 cm depth. The inoculum AMF was

made from residual roots mixed with spore and powder medium. Maize seeds of Bisma Variety (2 seeds per pot) were sown at 3 cm depth and after 14 days after sowing (14 DAS), there was only one plant per pot remained. Fertilizers sources used were mycorrhiza, cattle manure, rock phosphate, Urea, and SP36. Rock phosphate and cattle manure were applied at rate of 4.8 and 6.0 g per plant, respectively and inorganic fertilizers of Urea and SP-36 were applied at rate of 1.5 g and 0.6 g per plant. Those fertilizers were banded 5 cm from plant at depth of 7 cm. Harvest was conducted at 100 DAS.

### Plant and Soil Observation

Variables dealing with soil fertility status (N, P, K, organic-C and soil pH) were measured before sowing and at 60 and 100 DAS. The agronomic variables such as: top and roots dry weight biomass (60 DAS and 100 DAS), nutrients uptake (N, P, K, and Ca) at 60 DAS and components yield of maize (cobs, grain and 100 grain weight). The dry weight of the agronomic variables were measured after oven drying at 60 °C for 48 hours. Variables related to AMF activities including fungi population, roots percentage infections were also measured. Plant analysis for N was determined using Kjeldhal method, P using spectrophotometer, C-organic with colorimetric method according to Walkley and Black, K and Ca was recorded using Atomic Adsorption Spectrophotometer (AAS).

### Spore Measurement

Mycorrhiza population was observed using a wet sieving technique according to Brundrett *et al.* (1996). The last supernatant resulted from last filtering (38 µm) was added with 60% of sucrose solution and subsequently centrifused at 3000 rpm for 10 minute (Daniel and Skipper 1982). The harvested spore were stored on the Whatman paper with permanent ink marked of 0.5 × 0.5 cm. Counting of mycorrhiza population was done using stereo microscope (extended 40×). Calculation of roots percentage infections was conducted using modification of clearing and staining method (Kormanik and McGraw 1982), counted with the *Gridline Intersect* technique (Giovenetti and Mosse 1980) under stereo-microscope observation.

### Data Analysis

The effects of treatment were determined by using analysis of variance (ANOVA) and followed by further tests using Least Significant Difference (LSD) at 5% level and all were using the MStat program.

## RESULTS AND DISCUSSION

### Soil Chemical Properties

The treatment package of fertilizers containing of inoculation of mycorrhiza plus CM significantly improved soil chemical properties particularly increased concentration of soil N, available P, K, and total organic-C. The concentration of those variables were significantly different from other treatments (control, without fertilizers and no AMF F inoculation, AMF inoculation without CF, AMF inoculation plus RP and AMF inoculation plus CF) as presented in Table 1.

The highest concentration of soil nutrients (N, P, K, and C-org) were at AMF inoculation plus CM added (Table 1) and it actually suggested the better biological performance of AM compared to other treatments. It is important to note that the AMF combined with CM increased N, P, K and C-org by 37.39%, 60.79%, 66.66% and 110.15%, respectively at 60 DAS. The trend was also followed by observation at 100 DAS eventhough getting smaller than that observed at 60 DAS (21.48%, 69 %, 43.93% and 37.07% for N, P, K and C-org, respectively). Thus, the AM in combination with organic sources such as CM tended to have better positive contribution to soil fertility status.

Previous results by Warnock *et al.* (2007) showed that enrichment of AMF could be escalated using addition of organic matter and both combination had a positive effect on improving soil physico-chemical properties and therefore it was beneficial for soil microorganisms activities. This

signifies that better synergy of the inoculated AMF-treatment in combination to CM, as previously confirmed by Kato and Miura (2008). Higher soil extractable-P was found in this study was not only due to indirect contribution of CM in improving soil fertility status but also related to a positive contribution of mycorrhiza in producing phosphatase enzyme, for mineralization of organic-P in soil (Crowley and Rengel 2000; Joner and Johansen 2000) and resulted in enhancing insoluble-P in soils (Orcut and Nilsen 2000).

### Nutrient Uptake, Growth and Yield of Maize

Improvement of soil properties as presented in Table 1, particularly at AMF inoculation plus CM was also reflected by nutrient uptake data (Figure 1). The AMF in combination with CM increased nutrients uptake of N, P, K and Ca of maize by 1,608%, 1,121%, 533% and 534%, respectively. Increasing P uptake of plants due to AM inoculation had also been widely reported by previous authors (Sudova and Vosatka 2007; Angel *et al.* 2007; Deguchi *et al.* 2007). Higher P-uptake possibly was due to phosphatase activity from external hypha of AMF in rhizosphere (Widiastuti *et al.* 2003).

Increase in P-uptake will promote nutrients balance in plant and therefore it can induce plant in uptaking other essential nutrients (*i.e.* N, K, and Ca). For example, sufficient K in plant in particular which is accumulated mainly in shoots and roots will promote the cortex tissue of plant in establishing new cells, and eventually it can maintain turgor (Schweiger *et al.* 2007; Smith *et al.* 2010). Improved of soil nutrients availability as well as plant-nutrients

Table 1. Soil chemical properties (N, P, K, organic-C and soil pH) after harvesting under different treatments.

Fertilizers package treatments	N (g kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	K (cmol kg <sup>-1</sup> )	Org-C (g kg <sup>-1</sup> )	pH
60 DAS					
Control, without fertilizers	1.2 a	22.37 a	0.51 a	12.8 a	6.24 a
AM inoculation without CF	1.4 b	25.13 b	0.61 b	19.4 b	6.44 b
AM inoculation plus CM	1.6 c	35.97 c	0.85 c	26.9 c	6.04 c
AM inoculation plus RP	1.4 d	27.12 d	0.78 d	24.5 c	7.13 d
AM inoculation plus CF	1.3 e	27.75 d	0.60 b	19.4 b	6.20 e
100 DAS					
Control, without fertilizers	1.4 a	23.81 a	0.66 a	20.5 a	6.21 a
AM inoculation without CF	1.5 b	25.79 b	0.82 b	21.4 b	6.29 b
AM inoculation plus CM	1.6 c	40.24 c	0.95 c	28.1 c	6.20 a
AM inoculation plus RP	1.6 d	29.04 d	0.89 d	24.4 d	6.60 c
AM inoculation plus CF	1.5 b	25.58 b	0.83 b	22.7 e	6.20 a
Before experiment	1.1	13.82	0.57	12.1	6.25

Numbers followed by same letter within coloum are not significant different (p 0.05) by LSD test.

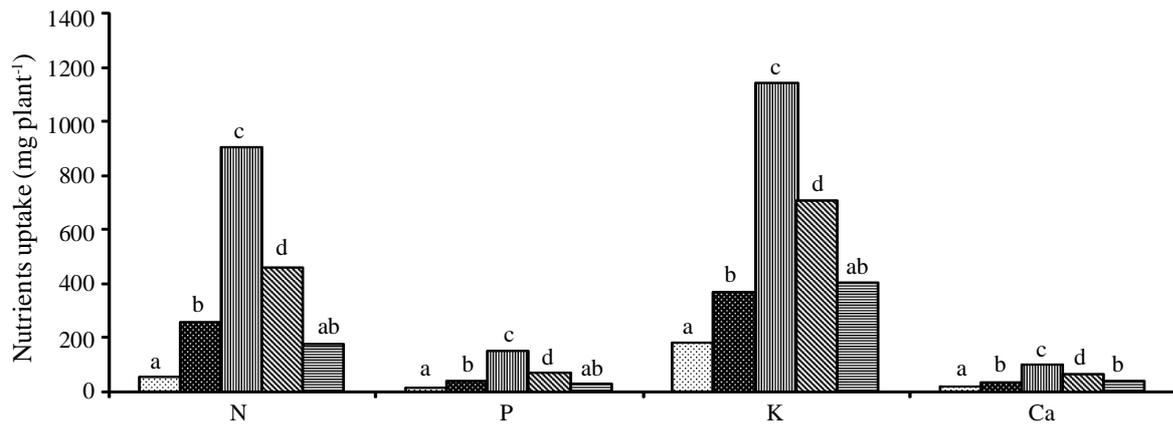


Figure 1. Nutrient uptake of N,P, K and Ca of maize at different combination package of the organic fertilizers combination on sandy loams of Nothern Lombok. □ = F0, ■ = F1, ▨ = F2, ▩ = F3, and ▪ = F4.

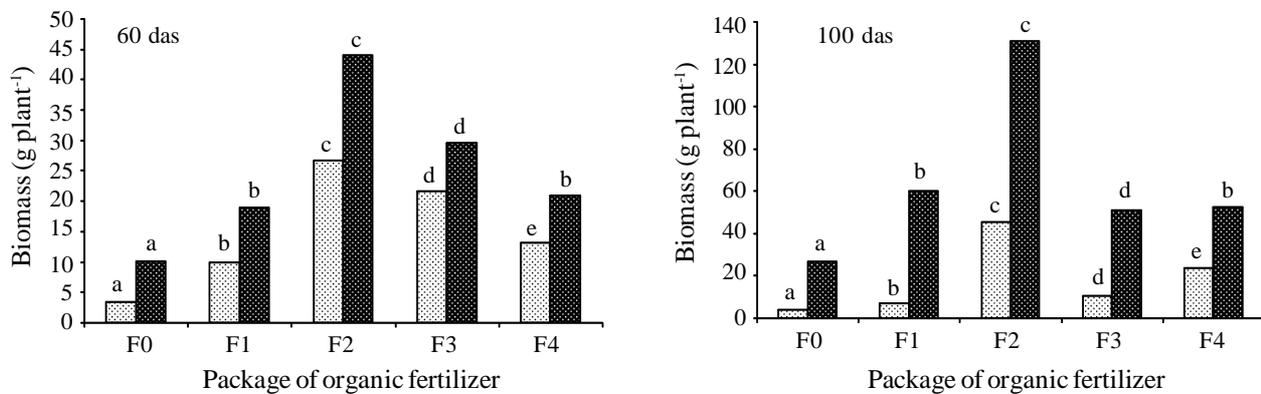


Figure 2. Roots and tops dry biomass of maize at 60 and 100 DAS under different package of organic fertilizers. □ = dry roots and ■ = dry shoot.

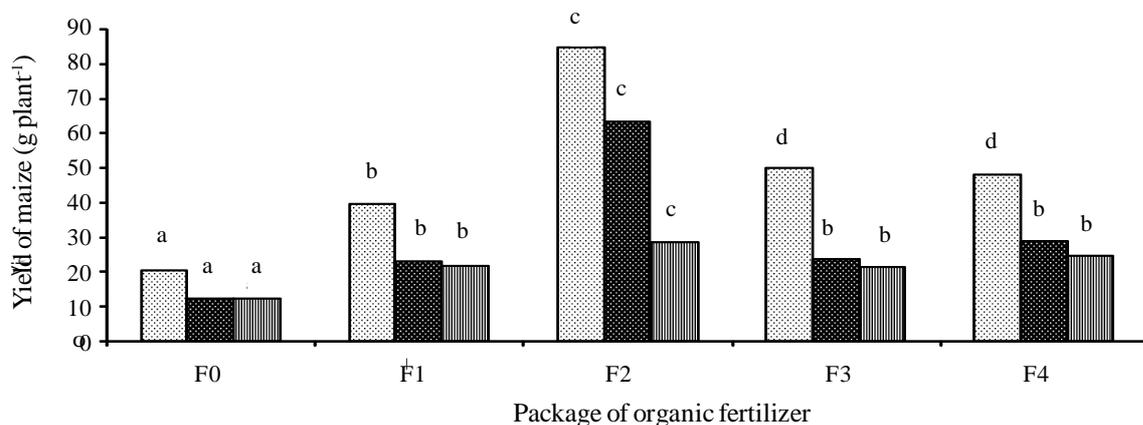


Figure 3. Component yields of maize (□ = cobs, ■ = grain and ▨ =100 grain dry weight) under different package of organic fertilizers.

uptake (data in Table 1 and Figure 2) perform better growth and yield of maize (Figure 3)

The better biological activity performed by treatment AMF inoculation plus CM was also reflected by higher increasing of roots, tops dry biomass and component yields (*i.e.* cobs, grains, and 100 grains weight). Total roots and tops dry biomass were observed at 60 DAS by 718.40% and 337.67%,

respectively. The higher trend of total dry biomass of those components compared to control were continued at 100 DAS by 1,102.11% (roots) and 390.25% (tops). Component yields including cobs, grain, and 100 grain weight resulted from different treatments are presented in Figure 3.

Component yields of cobs, grain and 100 grains weight were significantly higher at AMF inoculation

Table 2. Biological activity of mycorrhiza (number of spores and percentage of infections) under different treatments.

Fertilizers package treatments	Number of spores (spores 100 g soil <sup>-1</sup> )	Percent roots infection (%)
30 DAS		
Control, without fertilizers	512	-
AM inoculation without CF	1,169	-
AM inoculation plus CM	1,977	-
AM inoculation plus RP	1,582	-
AM inoculation plus CF	1,209	-
60 DAS		
Control, without fertilizers	1,441 a	24 a
AM inoculation without CF	2,231 b	49 b
AM inoculation plus CM	3,057 c	84 c
AM inoculation plus RP	2,443 d	64 d
AM inoculation plus CF	2,360 e	46 e
100 DAS		
Control, without fertilizers	2,344 a	-
AM inoculation without CF	2,927 b	-
AM inoculation plus CM	3,785 c	-
AM inoculation plus RP	3,262 d	-
AM inoculation plus CF	2,938 e	-
Before experiment	371	-

Numbers followed by same letter within coloum are not significant different (p 0.05) by LSD test.

plus CM treated plants compared to other treatments. Cobs, grain and 100 grains weight increased by 313.60%, 411.84% and 137.54%, respectively.

The results found in this study are in accordance with Sudova dan Vosatka (2007) who reported that mycorrhiza inoculated-plants performed higher concentration of P compared to without mycorrhiza inoculated-plants. This signifies that AMF induce P uptake. Phosphorus is an essential nutrient to support reproduction, and therefore P deficiency inhibits formation of flower, cobs, and filling grain formation (Muchane *et al.* 2010).

### **Mycorrhizal Activity**

Biological activities of AMF performed by number of spores per 100 g of soils and roots percentage infections are presented in Table 2.

Number of spores observed at treatment AMF inoculation plus CM increased by 112.14% at 60 DAS and 61.47% at 100 DAS while roots infections percentage was 250% greater compared to control. The numbers of spores was consistently getting higher along with higher growth development or increased crops age. Increased of spore as well as roots infection of maize (Clark 1997; Sylvia 2005) as observed at AM inoculation plus CM (Table 2) suggested better biological activities of AMF at rooting zone. Similar results was also previously observed by Viti *et al.* (2010). Higher roots infections in

particular found at AMF inoculation plus CM treatment was possibly due to improvement of rooting zone environment which stimulated better roots proliferation (Mouoglis and widden 1996; Nagahashi *et al.* 1996; Van der Heijden *et al.* 2001).

### **CONCLUSIONS**

The package of mycorrhiza in combination with cattle manure under maize cropping system contributed significantly to improve soil chemical properties particularly for total organic-C, N, available-P and K on sandy loam of Northern Lombok.

### **REFERENCES**

- Angel I, Ortiz-Ceballos, J Juan, Pena-Cabriaes, C Fragoso and GG Brown. 2007. Mycorrhizal colonization and nitrogen uptake by mize: combined effect of tropical earthworms and velvetbean mulch. *Biol Fertil Soils* 44: 181-186.
- Antoun H and JW Kloepper. 2001. Plnat growth promoting rhzobacteria. Brenner S and JF Miller (eds). *Encyclopedia of Genetics*, Academic Press, N.Y., pp. 1477-1480.
- Astiko W. 2009. Fertilizer application package effect on growth and yield of maize on dry land. In: *Research Center Mataram University. Proceeding of the 42<sup>th</sup> National Seminar Dies Natalis Agriculture Faculty of Mataram University, Mataram.* p 123 (in Indonesian).

- Brundrett M, N Bougher, B Dell, T Grove and N Malajczuk. 1996. Working with Mycorrhizas in Forestry and Agriculture. *Aciar Monograph* 32: 374 + x p.
- Clark RB. 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant soil* 192: 15-22.
- Crowley DE and Z Rengel. 2000. Biology and chemistry of nutrient availability in the rhizosphere. In: Z Rengel (ed). *Mineral Nutrition of Crops. Fundamental Mechanisms and implications*. The Haworth Press, Inc. NY, pp. 1-40.
- Daniels BA and HD Skipper. 1982. Methods for recovery and quantitative estimation of propagules from soil. In: NC Scenck (eds). *Methods and Principle of Mycorrhiza Research*. APS, St. Paul MN, pp. 29-36.
- Deguchi S, Y Shimazaki, S Uozumi, K Tawaraya, H Kawamoto and O Tanaka. 2007. White clover living mulch increases the yield of silage corn via arbuscular mycorrhizal fungus colonization. *Plant Soil* 291: 291-299.
- Drew EA, RS Murray and SE Smith. 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore size. *Plant Cell Environ* 251: 105-114.
- Gahoonia TS and NE Nielsen. 2004. Root traits as tools for creating phosphorus efficient crop varieties. *Plant Cell Environ* 260: 47-57.
- Giovannetti M and B Mosse. 1980. An evaluation of techniques to measure vesicular-arbuscular mycorrhiza infection in roots. *New Phytol* 84: 489-500.
- Hassen A, K Belguith, N Jedidi, A Cherif, M Cherif and A Boudabous. 2001. Microbial characterization during composting of municipal solid waste. *Bioresource Technol* 80: 217-225.
- Joner RE and A Johansen. 2000. Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. *Mycol Res* 104: 12-26.
- Kabirun S. 2002. Effect of “gogo” rice to arbuscular mycorrhizal and phosphate fertilizers in a entisol soil. *J Tanah Lingka* 3 : 49-56.
- Kato K and N Miura. 2008. Effect of matured compost as a bulking and inoculating agent on the microbial community and maturity of cattle manure compost. *Bioresource Technol* 99: 3372-3380.
- Khalvati M, B Bartha, A Dupigny and P Schroder. 2010. Arbuscular mycorrhizal association is beneficial for growth and detoxification of xenobiotics of barley under drought stress. *J Soils Sediments* 10: 54-64.
- Kormanik PP dan AC McGraw. 1982. Quantification of vesicular-arbuscular mycorrhiza in plant roots. In: NC Scenk (ed). *Methods and principles of mycorrhizal research*. The American Phytopathological Society. St. Paul. Minnesota. pp. 244.
- Moutoglis P and P Widden. 1996. Vesicular-arbuscular mycorrhizal spore populations in sugar maple (*Acersaccharum marsh L.*) forest. *Mycorrhiza* 6: 91-97.
- Muchane MN, B Jama, C Othieno, R Okalebo, D Odee, J Machua and J Jansa. 2010. Influence of improved fallow systems and phosphorus application on arbuscular mycorrhizal fungi symbiosis in maize grown in western Kenya. *Agroforest Syst* 78: 139-150.
- Nagahashi G, DD Douds Jr and GD Abney. 1996. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. *Mycorrhiza* 6: 403-408.
- Orcutt DM and ET Nilsen. 2000. *The physiology of plants under stress: Soil and biotic factors*. New York, John Wiley and Sons, Inc.
- Perner H, D Schwarz, C Bruns, P Mader and E George. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza* 17: 469-474.
- Sastrahidayat IR, ASM Subari dan M Bintoro. 2001. Effect of sludge and inoculating arbuscular mycorrhizal fungi on growth and yield of maize. *Agrivita* 22: 147-155.
- Schweiger PF, AD Robson, NJ Barrow and LK Abbott. 2007. Arbuscular mycorrhizal fungi from three genera induce two-phase plant growth response on a high P-fixing soil. *Plant Soil* 292: 181-192.
- Smith SE and DJ Read. 2008. *Mycorrhizal symbiosis*, 3<sup>rd</sup> Edition. Elsevier and Academic, New York, London, Burlington, San Diego.
- Smith SE, E Facelli, S Pope and FA Smith. 2010. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* 326: 3-20.
- Sudova R and M Vosatka. 2007. Differences in the effects of three arbuscular mycorrhizal fungal strains on P and Pb accumulation by maize plants. *Plant Soil* 296: 77-83.
- Sylvia DM. 2005. Mycorrhizal symbioses. In: DM Sylvia, JJ Fuhrmann, PG Harteland DA Zuberer(eds). *Principles and applications of soil microbiology*. Upper saddle river, New Jersey, pp. 263-282.
- Van der Heijden EW and TW Kuyper. 2001. Does origin of mycorrhizal fungus or mycorrhizal plant influence effectiveness of the mycorrhizal symbiosis? *Plant Soil* 230: 161-174.
- Viti C, E Tetti, F Dacorosi, E Lista, E Rea, M Tullio, E Sparvoli and L Giovannetti. 2010. Compost effect on plant growth-promoting rhizobacteria and mycorrhizal fungi population in maize cultivations. *Compost Sci Util* 18: 273-281.
- Warnock DD, J Lehmann, TW Kuyper and MC. Rillig. 2007. Mycorrhizal responses to biochar in soil – concepts and mechanisms. *Plant Soil* 300: 9-20.
- Widiastuti H, N Sukarno, LK Darusman, DH Goenadi, S Smith dan E Guhardja. 2003. Phosphatase activity and organic acid production in rhizosphere and hyphosphere of mycorrhizal oil palm seedling. *Menara Perkebunan* 71: 70-81 (in Indonesian).