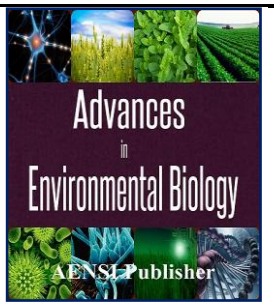
Advances in Environmental Biology, 9(23) October 2015, Pages: 22-26

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/



Synergistic Effectivity of Arbuculus Mychorrizal Fungi and Mychorriza Helper Bacteria againts Pratylenchus Coffeae

[1]Iis Nur Asyiah, [2]Soekadar Wiryadiputra, [3]Rita Harni, [1]Nur Rohmah Heny Handayani and [1]Erlia Narulita

[1] Study Program of Biology Education, University of Jember, Indonesia
[2] Indonesian Coffee and Cocoa Research Institute
[3]Balai Penelitian Tanaman Industri dan Penyegar, Litbang Deptan, Indonesia

ARTICLE INFO

Article history:
Received 28 September 2015
Accepted 15 November 2015
Available online 24 November 2015

Keywords:
Pratylenchus coffeae, Glomus, Mychorriza helper bacteria [MHB], arabica, coffeae

ABSTRACT

Background: *P. coffeae* is major parasitic nematodes of coffee plants that need to be controlled. Method of control in accordance with *Green Economy* concept is biological control. One of biological control agents that potentially useful in controlling nematodes is mycorrhiza. Increasing the effectiveness of mycorrhiza can be obtained through the addition of *Mychorriza Helper Bacteria* [MHB]. **Objectives:** to examine the synergistic effect of arbuculus mycorrhizal [AM] fungi and MHB [*B. subtillis* and *P. diminuta*] in controlling *P. coffeae* in the Arabica coffee seedlings and analyze its mode of action. **Results:** The results showed that addition of both *B. subtillis* and *P. diminuta* were able to improve the ability of AM fungi *Glomus* spp. in reducing populations of *P. coffeae*, canopy damage scores, and root damage scores. *P. coffeae* population declined about 87.4% to 97.02% compared to controls. Applications those MHB also improved plant peroxidase activity. **Conclusion:** MHB was able to synergize with AM fungi in reducing populations of *P. coffeae* while enhancing the growth of Arabica coffee seedlings.

To Cite This Article: Iis Nur Asyiah, Soekadar Wiryadiputra, Rita Harni, Nur Rohmah Heny Handayani and Erlia Narulita., Synergistic Effectivity of Arbuculus Mychorrizal Fungi and Mychorriza Helper Bacteria againts Pratylenchus Coffeae. ***Adv. Environ. Biol., 9(23),*** *22-26, 2015*

INTRODUCTION

Indonesia is the largest coffee producer in Southeast Asia and the third largest in the world after Brazil and Vietnam. Heretofore, *P. coffeae* is the most common nematodes and endanger the coffee plant in Indonesia. This is due to nematodes widespreade in almost all coffee producing provinces, at an altitude unto more than 1,000 m above sea level. *P. coffeae* causing yield losses in Robusta coffee and Arabica coffee plantations up to 78% and 57%, respectively [1]. Therefor, controlling of *P. coffeae* is absolutely necessary. *P. coffeae* control must be in line and support the research priorities in the Center for Agribusiness Coffee Plantations, especially directed forward on a national green economy in order to meet the demands of the international market which requires food security, environmental conservation and improving the welfare of farmers. One way of controlling plant intruder organism, which is in line with the concept of green economy is biological control.

A few study have shown about the inhibition of penetration and development of the nematode by mycorrhizal fungi inoculation [2,3,4,5,6,7]. Mycorrhizal fungi symbiosis is not only considered as an interaction between plants and fungi, but also should include supporting organism. Supporters and mycorrhizal organisms are known to give each other mutual influence, which then produce what is referred to as "mycorrhizosphere" [8,9,10]. Mycorrhizosphere composed of mycorrhiza, the external mycelium, and supporting organisms [11]. Mycorrhizosphere lead to improve nutrition, growth, and plant disease resistance [10,12].

The role of mycorrhizal and supporters organisms [bacteria] has the potential to be applied as biofertiliser. Bacteria are able to enhance the development of mycorrhiza named *Mycorrhiza Helper Bacteria* [MHB] [13]. Nowadays, numerous bacterial strains from a wide range of major clades have been shown to have MHB-type functions in both arbuscular and ectomycorrhizal symbioses [15]. Some researchers found that the bacteria isolated from mycorrhizal fungi can stimulate mycorrhizal infection, spore production and also resistance against plant pathogens [14, 15, 16]. It is well known that mycorrhizae can improve coffee plant growth and

Corresponding Author: Iis Nur Aisyah, Study Program of Biology Education, University of Jember, Jl. Kalimantan 37 Jember, Indonesia
E-mail: iisnaza@gmail.com

controlling nematodes *P. coffeae*, but the density of mycorrhizae in the soil decreased significantly with the presence of nematodes [2]. Mycorrhizal density can be increased by using *Mycorrhiza Helper Bacteria* [MHB] so that the role of mycorrhizal in nematodes suppress can be increased. Thus, we suggests that MHB and AM fungi have potentially synergism in controlling *P. coffeae*.

Methodology:

Coffee seeds multiplication:

Arabica coffee seedlings were originally obtained from plantations on Banyuwangi. Coffee seeds sterilized beforehand with alcohol and sublimat, then placed in sterile sand seedling media. Seedlings were treated at the age of 3 months or has had four leaves.

Propagation of nematodes:

Nematodes *P. coffeae* were collected from the extraction of the coffee plant roots that attacked by *P. coffeae*. Extraction was performed using a modified Baermann method [17]. The number of nematodes were inoculated is 50 heads per pot.

Preparation of MHB isolates:

MHB *P.diminuta* [author collection] and *B. subtilis* [collection of soil biology laboratory, UNPAD] were used in this study. *P. diminuta* and *B. subtilis* were cultured on medium NA slant for 2 x 24 hours. One loop of inoculum was transferred into 100 ml Nutrient Broth medium in 250 ml Erlenmeyer and incubated in a shaker at room temperature [28 ± 2 ° C] with a speed of 110 rpm for 48 hours. After incubation for 48 hours, the liquid medium is ready to be tested. Concentration used was 108 cfu/ml.

Preparation of AM fungi:

Glomus spp. was acquired from Mycological Laboratory of Faculty of Agriculture UGM, which consists of five species of *Glomus* spp. selected from various regions in Indonesia. *Glomus* spp. propagated in zeolites media with host plants such as corn. Spores used in the study was 100 spores.

Impact of MHB and mycorrhizal against P. Coffeae:

This study consisted of 8 treatments, 5 repetitions and each repetition consisted of 2 samples Arabica coffee seeds. Latosol soil and manure were used as planting medium in the ratio 1: 1 up to 1500g in plastic pots. Application of treatment [Figure 1] was made 2 weeks after seedlings transplanting. Eight treatments were: positive control [K +] without mycorrhiza, nematodes and bacteria [A], a negative control [K] by using nematodes alone [B], spores *Glomus* spp. and *B. subtilis* bacteria density 108cfu / ml [C], 100 spores of *Glomus* spp. and the bacteria *B. subtilis* with 2x the density of 108 cfu / ml [D], 100 spores of *Glomus* spp. and P. diminuta bacterial density 108 cfu / ml [E], spores *Glomus* spp. and *P. diminuta* bacterial density 2 x108 cfu / ml [F], 100 spores of *Glomus* spp. [G], and nematicides carbofuran at a dose of 5g / pot [H]. Population of nematodes in the roots and the soil, the degree of mycorrhizal infection [18], and scores of root damage were determined on sixteen weeks after treatment.

Peroxidase Activity Assay:

Peroxidase activity assay was performed according to [19] with some modifications. MHB bacteria [OD_{420}=1] were poured onto three months-old coffee seed. Twelve days after treatment, the coffee plant was dismantled to measure peroxidase activity in plants. Peroxidase activity was measured by direct absorbance measurement method using a spectrophotometer. 1g roots was crushed in a 0.01M phosphate buffer [pH=6.0] with a ratio of 1: 4. Root extract centrifuged at 5000 rpm for 30 min at 4°C then filtered using Whatman filter paper. Supernatant obtained was used as an enzyme preparation. 0.2 ml of enzyme preparation diluted 1: 3 with 0.01 M phosphate buffer [pH=6.0] and it was added by 5mL pyrogallol 0.5 M and 0, 5 ml H_2O_2 1%. The suspension solution was homogenized for 5-10 seconds and the absorbance values were calculated at a wavelength of 420nm with intervals every 30 seconds for 150 seconds.

RESULTS AND DISCUSSIONS

The aim of this study was to check whether the synergism of AM fungi and MHB [*B. subtillis* and *P. diminuta*] is beneficial in controlling *P. coffeae* in the Arabica coffee seedlings and to analyze its mode of action. Co-inoculation of MHB and *Glomus* spp. reduced *P. coffeae* population both in roots and soil significantly [Table 1]. Population of *P. coffeae* was decreased with MHB and *Glomus* spp. on treatment D and F as compared to nematicide carbofuran [H] treatment.

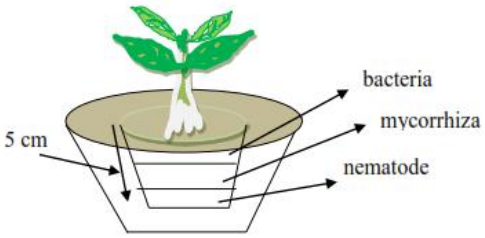


Fig. 1: Diagram of placement of mycorrhizal inoculants, bacteria and nematodes in pot.

Table 1: Effect of inoculation MHB [*P. diminuta* and *B. subtilis]* and Glomus spp. to the population of nematodes *P. coffeae* on the roots and soil.

Treatments	Population *P. coffeae*		
	Roots	Soil	Total
[A] Positive control [K+]	0 ± 0,00^{a}	0 ± 0,00^{a}	0 ± 0,00^{a}
[B] Inoculated by *P.coffeae* [K-]	626 ± 0,05^{e}	382 ± 0,10^{d}	1008 ± 0,06^{e}
[C] *Glomus* spp.+ *Pc* + *B.subtilis* 10^{8}	76 ± 0,36^{d}	51 ± 0,27^{c}	127 ± 0,30^{d}
[D] *Glomus* spp.+*Pc*+ *B.subtilis* $2x10^{8}$	22 ± 0,65^{bc}	10 ± 0,68^{b}	32 ± 0,72^{bc}
[E] *Glomus* spp. + *Pc*+*P. diminuta* 10^{8}	65 ± 0,20^{d}	45 ± 0,11^{c}	110 ± 0,12^{d}
[F] *Glomus* spp.+*Pc*+ *P. diminuta* $2x10^{8}$	22 ± 0,86^{b}	8 ± 0,63^{b}	30 ± 0,93^{b}
[G] *Glomus* spp.100+*Pc.*	134 ± 0,07^{d}	60 ± 0,08^{c}	194 ± 0,04^{d}
[H] *Carbofuran* 5gr/pot+*Pc*	41 ± 0,09^{cd}	23 ± 0,08^{c}	64 ± 0,09^{cd}

Description: - Figures average followed by the same letter in the same column indicates not significantly different by Duncan test using α 5%. Pc= *P. coffeae.*

Co-inoculation of *Glomus* spp. and MHB were able to decrement the nematode population significantly compared to mono-inoculation as well as treatment G [Table 2]. *P. coffeae* population drastically diminution of 80.75% -96.8% compared to negative control. Effectiveness of co-inoculation role in controlling nematodes *P. coffeae* associated with degrees of mycorrhizal infection to the root tissues. This co-inoculation potentially raised mycorrhizal infection as in Table 2.

Table 2: The effect of inoculation of *Glomus* spp. and MHB [*P. diminuta* and *B. subtilis*] to the degree of infection of *Glomus* spp.

Treatments	Degree of mycorrhizal infection [%] ± Std.	Score of root damage [%] ± Std.
[A] Positive control [K+]	0 ± 0,00^{a}	0 ± 0,00^{a}
[B] Inoculated by *P.coffeae* [K-]	0 ± 0,00^{a}	67 ± 0,058^{e}
[C] *Glomus* spp.+ *Pc* + *B.subtilis* 10^{8}	89,6 ± 0,031^{c}	13 ± 0,089^{cd}
[D] *Glomus* spp.+*Pc*+ *B.subtilis* $2x10^{8}$	97,2 ± 0,019^{d}	8 ± 0,457^{bc}
[E] *Glomus* spp. + *Pc*+*P. diminuta* 10^{8}	93,6 ± 0,017^{cd}	14 ± 0,121^{cd}
[F] *Glomus* spp.+*Pc*+ *P. diminuta* $2x10^{8}$	98,4 ± 0,009^{d}	5 ± 0,533^{b}
[G] *Glomus* spp.100+*Pc.*	78,8 ± 0,018^{b}	27 ± 0,107^{d}
[H] *Carbofuran* 5gr/pot+*Pc*	0 ± 0,00^{a}	39 ± 0,089^{cd}

Description: - Figures average followed by the same letter in the same column indicates not significantly different by Duncan test using α 5%. Pc= P. coffeae.

Several authors have characterized the symptoms caused by *Pratylenchus* spp. in coffee plants, under controlled conditions [20, 21]. Symptoms observations of infected plants in this study conducted by scoring damage of roots and plant canopy. The results showed that the score of roots and plant canopy damage is directly proportional to the effectiveness of mycorrhizal infection. Co-inoculation was able to diminish the score of roots and canopy damage compared to the negative control [Table 2]. Degression of *P. coffae* population due to co-inoculation treatment affect to damage reduction. Co-inoculation of MHB and *Glomus* spp. also influenced increasing of Arabica coffee plants growth [Suplemental Table 1]. The treatment of either mono-inoculation or co-inoculation significantly enlarged plant height, stem diameter, and number of leaves compared to the control.

To determine the mode of action of MHB in reducing nematode populations, we performed peroxidase activity assay. Peroxidase induce plant to produce resistance genes to against pests or diseases including nematodes. Peroxidase assay results indicated that *P. diminuta* and *B. subtilis* have a higher peroxidase activity compared to control [Table 3], with the higest level occuring in *P. diminuta*. Higher peroxidase activities are closely connected with plants growth [22].

Peroxidase enzyme typically involved in lignin and chitin biosynthesis as well as some cell wall constituent compounds forming plant defense mechanism [23]. Peroxidase mechanism in controlling nematodes through induced hypersensitivity reaction [HR], which is characterized by necrotic lesions resulting from localized host cell death at the site of infection. Its prevent growth and the spread of the pathogen into healthy tissues [24, 25].

Table 3: Peroxidase activity assay.

Treatment	Peroxidase activity [U/g sample]
P. diminuta 10^8 cfu/ml	8,4547
B. subtilis 10^8 cfu/ml	3,6924
Control	3,1425

As we mentioned above, MHB bacteria secrete certain hydrolitic enzymes that advantageous for mycorrhizal symbiosis. In this study, the hydrolytic enzymes that produced by both *P. diminuta* and *B. subtilis* and play an important role in controlling nematode population was chitinase. Chitinase is glycosyl hydrolases that catalyzes the degradation of chitin, a linear polymer composed of monomer β-1,4-N-acetyl-D-glucosamine [GlcNAc] which is widely distributed in nature. Chitinase has ability to degrade chitin directly to low molecular weight chitooligomers and to cut off non-reducing ends of chitin [26]. Biological control of some nematodes diseases has been associated with chitinase production. Chitinase controled parasitic *P. coffeae* by breaking down its body wall that contain chitin, resulting in body destruction of nematodes. Thereunto, chitinase also hydrolyzed chitin as component of eggshell of nematodes and could be deleterious to the embryogenesis of eggs.

Conclusion:

MHB was able to synergize with AM fungi in reducing populations of *P. coffeae* while enhancing the growth of Arabica coffee seedlings. Nematode population declines up to 80.75%-96.8% compared to the control. Potency of MHB and mycorrhizal should be followed up by making applicable and inexpensive formula.

ACKNOWLEDGEMENT

The author would like to thank the Ministry of Agriculture of Republic of Indonesia for supporting research funding through KKP3N 2014, Mycological Laboratory of Faculty of Agriculture UGM for Glomus spp. collection and Soil Biology Lab of Faculty of Agriculture UNPAD for the collection of *B. subtillis* isolates

REFERENCES

[1] Wiryadiputra, S. and L.K. Tran. 2008. Indonesia and Vietnam. *In:* Souza, R.,M [Eds]. Plant-Parasitic Nematodes of Coffee: Springer Science+Business Media B.V., 277-292.
[2] Baon, J.B. and S. Wiryadiputra, 1994. Parasitic nematode development on robusta coffea inoculated by VAM fungi. *In:* Soetisna, U.; Tappa, B.; Sukara, E.; Sukiman, H.I.; Widyastuti, Y.; Ermayanti, T.M.; Imelda, M.; Prayitno, N.R.; Loedin, I.H.S. [Eds.]. Proceeding of Second Conference of Biotechnology Research and Development, Bogor, 6-7.
[3] De la Peña, E., Susana Rodríguez Echeverría, Wim H. van der Putten, Helena Freitas dan Maurice Moens, 2006. Mechanism of control of root-feeding nematodes by Blackwell Publishing Ltd mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist*, 169: 829-840.
[4] Shanthi, A. dan, G. Rajendran, 2006. Biological Control Of Lesion Nematodes In Banana. *Nematol. Medit.*, 34: 69-75.
[5] Jumjunidang, 2009. Efficacy of Arbuscula Mycorrhyzal Fungi Isolate *Indigenous* Banana to Nematode *Radopholus similis* in Pisang Ambon Hijau. *J. Hort.*, 19(2):186-191.
[6] Serfoji, P., Rajeshkumar, S. dan Selvaraj, T. 2010. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans. Journal of Agricultural Technology* Vol.6[1]: 37-45.
[7] Asyiah, N.I., Soekarto, M. Husain, 2012. Potensi MYCOFER® dalam Pengendalian Nematoda Sista Kentang [*Globodera rostochiensis*], 401-406. *in* Rudi H.M., Tri Joko, Erlina A, Didik I, Nasih W, Eko H, Subejo, Jamhari [eds]. *Proceeding of National Conference on Agricultural and Fisheries Research*.
[8] Foster, Marks, 1966. The Fine Structure of The Mycorrhizas of *Pinus Radiata*. *Australian J Biol Sci.*, 19: 1027-1038.
[9] Meyer, J.R., R.G. Lindermann, 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum. Soil Biol Bioch*, 18: 191-196.
[10] Frey-Klet and J. Garbaye, 2005. Mychorrhiza helper bacteria: a Promising Model for the Genomic Analysis of Fungal – Bacterial Interactions. *New Phytol.*, 168-48.
[11] Barea, J.M., M.J. Pozo, R. Azcon, C. Azcon-Aguilar, 2005. Microbial co-operation in the rhizosphere. *J Exp Bot* 56:1761-1778.
[12] Linderman, R.G., 1988. Mychorrizal Interaction With the Rhizosphere microflorathe Mycorirhizosphere effect. *Phytopatology*, 78: 366-371.

[13] Frey-Klett P., J. Garbaye, and M. Tarkka, 2007. The mycorrhiza helper bacteria revisited. *New Phytol.,* 176: 22–36.
[14] Garbaye, J., 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol.,* 128: 197-210.
[15] Von, A.H., A. Lindermann, F. Schonbeck, 1993. Stimulation of vesicular arbuscular mycorrhiza fungi by fungicide or rhizospere bacteria. *Mycorrhizae*, 2: 167173.
[16] Barea, J.M., G. Andrade, V. Biancioto, D. Dowling, S. Lohkre, P. Bonfante, F. O'Gara, C. Azcon-Aguilar, 1998. Impact on arbuscular mycorrhiza formation on Pseudomonands strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. *App Envir Microbiol.,* 64: 2304-2307.
[17] Sulistyowati, E., D.S. Rahayu, F.N. Aini, 2012. Aplication of *Paecilomyces lillacinus* to Induce Resistance of Coffe Plant against *Pratylenchus coffeae*: Efectivity of *Paecilomyces lillacinus* strain 251 to *Pratyelnchus coffeae*. Proceeding of InSINas, PG-145.
[18] Giovanneti, M. and B. Mosse, 1980. An Evaluation of Techniques For Measuring Vesicular Arbuscular Mychorrizal Infection in Roots. New Phytol., 84: 489-500.
[19] Chance, B. and A.C. Maehly, 1955. *Methods in enzymology*, II: 773-775.
[20] Inomoto M.M., C.M.G. Oliveira and P. Mazzafera, 1998. Effects of *Pratylenchus brachyurus* and *P. coffeae* on seedlings of *Coffeae arabica*. J. Nematol, 30: 362–387.
[21] Kubo, R.K., R.A. Silva, M.D. Tomazini, 2003. Patogenicidade de *Pratylenchus coffeae* emplântulas de cafeeiro cv. Mundo Novo. Fitopatol Bras., 28: 41–48.
[22] Zheng, X. and R.B. Van Huystee, 1992. Peroxidase regulated elongation of segments from peanut hypocotyls. *Plant Sci*., 81: 47-56.
[23] Hallmann, J., 2001. Plant interaction with endophyticbacteria. *in*: Jeger,M.J. and N.J.Spence[eds.]. Biotic Interactionin Plant-Pathogen Associations.CAB International.
[24] Goodman, R.N., A.J. Novacky, 1996. The Hypersensitive Reaction in Plants to Pathogens: A Resistance Phenomenon, 2nd ed. [St. Paul, MN: American Phytopathological Society].
[25] Liharska,T. and V.M. Williamson, 1997. Resistance to root knot nematodas in tomato. in: Fenoll, C., F. M. W. Grundler, and S.A.Ohl.[eds]. Celluler and Moleculer Aspects of Plant Nematoda Interaction. The Netherland: Kluwer Academic Publishers, 191-200.
[26] Haliza, W., M.T. Suhartono, 2012. Characteristic of Chitinase of Microbes. *Buletin Teknologi Pascapanen Pertanian*, 8(1): 1-14.