The Use of Fungal Endophytes *Gliocladium* spp. in Different Concentration to Control of Root-Knot Nematode *Meloidogyne* spp.

Nur Amin

Department of Plant Protection, Hasanuddin University,
INDONESIA.

nuramin_62@yahoo.com

ABSTRACT

Root-knot nematodes Meloidogyne spp are serious pests of many cultivated crops around the world and is estimated economic losses around US $ 157 billion annually. The purpose of the present investigation was to see of efication of endophytic fungi Gliocladium spp.in different concentration towards root-knot nematode Meloidogyne spp. in greenhouse. All of the treatment concentration of endphytic fungi Gliocladium spp. in term of intensity damaged and population density of meloidogyne-J2 statistically different to control.

Keywords: Fungal Endophyte,Gliocladium spp., Root-Knot Nematode, Meloidogyne

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an eudicotiledonous plant that belongs to the family Solanaceae together with other economically important crops such as pepper, eggplant and potato. It is the most important grown fresh market vegetable worldwide with more than 5 million hectares harvested in China, United States of America, India, Turkey and Egypt as the five first producers, respectively.

Root-knot nematodes of the genus Meloidogyne are more widely distributed throughout the world than any other major group of plant-parasitic nematodes. The damages to global agricultural crops due to root knot nematodes is estimated around US$ 157 billion annually (Abad et al, 2008).Infected plants show reduced growth, swollen roots which develop into the typical root-knot galls, are two, or three times larger in diameter as healthy root. Root-knot nematodes are very difficult to control because they are polyphagous, where its over 2000 plants species is a highly specialized and complex feeding relationship with their host (Hussey and Janssen, 2002). The life cycle is almost completely confined inside the host plant and high reproductive capacity. Although chemical control is still a common method for reducing nematode population, there is a considerable public pressure to limit or even ban the use of nematicides. Many nematicides are highly toxic and sometimes very mobile in the soil because of their solubility in water. Concern over these chemicals has led to an increased interest in biological control in order to achieve more environmentally friendly methods of reducing nematode damage.

By definition, an endophytic funguslives in mycelial form in biological association with the living plant, at least for some time. Therefore the minimal requirement before a fungus is termed an‘endophyte’ should be the demonstrationof its hyphae in living tissue. Sathe andRaghukumar used a bleaching and a staining technique for demonstrating intracellular hyphae in seagrass and this technique may be generally applicable (Sathe and Raghukumar, 1991). In the last few years fungal endophytes have been detected in hundreds of plants including such important agricultural commodities as bananas (Nur Amin, 1994), maize (Nur Amin, 2013a); tree palm oil (Nur Amin et al., 2008) and cocoa plant (Nur Amin et al., 2014). A number of authors have documented that the presence of endophytic fungi provide a

Copyright © 2014 SAVAP International

ISSN: 2223-9944, eISSN: 2223-9553

protection of the plant hosts against plant parasitic nematodes (Nur Amin, 1994; 2013b); (Elmi et al., 2000) and (Hallmann, 1994a). Fungal endophyte *Gliocladium* sp. have been isolated from different host plant such as maize (Nur Amin, 2013a), cocoa (Nur Amin et al., 2014), tree palm oil (Nur Amin et al., 2008). The purpose of the present investigation was to see of the eficacy of endophytic fungi *Gliocladium* spp.in different concentration towards root-knot nematode *Meloidogyne* spp. in greenhouse

MATERIAL AND METHODS

Source of Endophytic Fungi *Gliocladium* sp

Endophytic fungi *Gliocladium*sp was originally isolated from cortical tissue of surface sterilized root of tomato plant *Solanum lycopersicum.*

Source of *Meloidogyne* spp

The root-knot nematode *Meloidogyne* spp.was originally isolated from an infested field on tomato plant in distric Barombong, south Sulawesi, Indonesia. The extraction to obtained Meloidogyne-J2 by using the modified extraction technique of Hooper et al (2005). Roots were washed free from soil under tap water, cut into 1 cm pieces and macerated in a warring blender at high speed for 20 s and collected in a glass bottle. Sodium hypochloride (NaOCl) was added to obtain a final concentration of 1.5% active Chlorine and the bottle was shaken vigorously for 3 min. The suspension was then thoroughly washed with tap water through a sieve combination 250, 100, 45 and 25 µm mesh to remove the NaOCl. Eggs were collected on the 25 µm sieve and then transferred to a glass bottle. The egg suspension was supplied with oxygen from an aquarium pump over 10 days to induce juvenile hatching. To separate active J2 from unhatched eggs or dead J2, a modified Baermann technique over 24 hours was used. The collected active J2 were adjusted to 1000 J2 5 ml-1 and used immediately as inoculum.

The Efficacy of Endophytic Fungi *Gliocladium* sp. In Different Concentration against Root-Knot Nematode *Meloidogyne* spp. In Greenhouse

Tomato seeds (Lycopersicon esculentum) Var. Marglobe were surface sterilized by first shaking them in a 75% Ethanol solution for 1 min and then in a 1.5% Sodium hypochloride (NaOCl) solution for 3 min. The seeds were washed with tap water and transferred to sterile sand for germination at greenhouse conditions.

The tomato seedlings were transplanted into 1000-ml polybag (20 x 30cm) containing a sterilized of a mixture of soil-sand-cow manure with the ratio (2:1:1). At the time of transplanting a 10 ml conidia suspencion of endophytic fungi *Gliocladium* sp. in different concentration was applied as a soil drench around the stem where as the control was treated with 10 ml sterile aquadest. The conidia suspencion of endophytic fungi *Gliocladium* sp. was repeated one week later. Simultaneously 1000 J2 of *Meloidogyne* spp. were added in 10 ml of tap water in three holes around the stem.

The investigation is based on the completely randomized design (CRD) with 4 treatments and 4 replications in which each treatment plant using 3 samples, that contained 48 units of the plant . The treatments used were:

P0 = Control (Tomato Plant + 1000 *Meloidogyne*-J2)

P1 = (Tomato Plant + Gliocladium 10^4 ml^{-1}.+ 1000 *Meloidogyne*-J2)

P2 = (Tomato Plant + Gliocladium 10^5 ml^{-1}.+ 1000 *Meloidogyne*-J2)

P3 = (Tomato Plant + Gliocladium 10^6 ml^{-1}.+ 1000 *Meloidogyne*-J2)

Copyright © 2014 SAVAP International

ISSN: 2223-9944, eISSN: 2223-9553

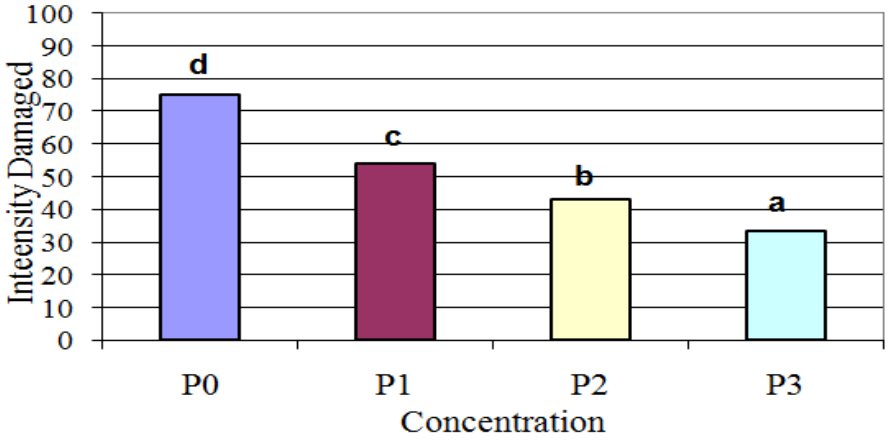
The investigation was terminated 21 days after nematode inoculation at which time intensity damaged, means of population nematode on the root and means of population nematodes in the soil were measured.

DATA ANALYSIS

ANOVA was also performed to determine the effects of concentrations of endophytic fungi to intensity damaged, means of population nematode on the root and means of populations nematodes in the soil. The percent data were arcsine-transformed before being subjected to ANOVA. When significant differences were detected, means were separated using Tukey's test at 5% probability level.

RESULT AND DISCUSSION

Significant differences in intensity damaged and population density of *Meloidogyne*-J2 on soil gram^{-100} and the root of tomato gram^{-1} in different of 3 concentration of conidia suspension of endophytic fungi *Gliocladium* sp.were observed. Concentration of conidia suspension of endophytic fungi *Gliocladium* 10^6 ml^{-1} decreased significantly different of intensity damaged 33 % to control 75 % (Figure 1).



The population density of of *Meloidogyne*-J2 on soil gram^{-100} and the root of tomato gram^{-1} in the treatment of conidia suspension of endophytic fungi *Gliocladium* sp.were greatlyreduced in all treatments over the control. However, there were no differences between the different concentra-tion of conidia suspension of endophytic fungi *Gliocladium* sp. on the observation of *Meloidogyne*-J2 (Table 1).

Table 1. Means of Population Density of *Meloidogyne*-J2 onSoil Gram^{-100} and the Rootof Tomato Gram^{-1} in Different of 3 Concentration of Conidia Suspension of Endophytic Fungi *Gliocladium* sp

Concentration	*Means of Meloidogyne-J2 on*	
	Soil Gram^{-100}	*Root of Tomato Gram^{-1}*
P0	223 b	135 d
P1	145 a	98 c
P2	135 a	80 b
P3	111 a	49 a

Copyright © 2014 SAVAP International

ISSN: 2223-9944, eISSN: 2223-9553

The intensity damaged and the population density of *Meloidogyne*-J2 reduced on the treatment of conidia suspension of endophytic fungi *Gliocladium* sp. The actuality condition is may be related to nematode penetration, in this case due to the influence of fungal colonization. Many researchers have shown that the endophytic fungi can reduce nematode penetration in different crops (Hallmann and Sikora, 1994a, b; Niere et al., 1999; Pocasangre, 2000; Sankaranarayana et al., 2002; Dieuhiou et al., 2003; zum Felde et al., 2004; Vu, 2005; Dababat, 2007). For instance, the mutualistic endophyte *Fusarium oxysporum* Fo162 reduced penetration of *Meloidogyne incognita* in tomato and *Radopholus similis* in banana by 28% to 41% respectively (Vu, 2005; Dababat, 2007). Many studies have elucidated that fungal endophytes may alter chemical or physical properties of the root exudates or interact with the plants to produce chemical or hormone complex compounds which repel or interfere with nematode attraction (Diez and Dusenbury, 1989; Dababat and Sikora, 2007).

The other mechanisms to reduce of intensity damaged and the population density is related to induce systemic resistance. Induced systemic resistance (ISR) is commonly defined as "a phenomenon whereby resistance to infectious disease is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds. The activity of the inducing agents is not due to antimicrobial activity per se or their ability to be transformed into antimicrobial agents. However, antimicrobial agents can induce resistance, and they provide protection from the time of application until ISR is fully expressed".

REFERENCES

[1] Abad, et al., (2008). Genome sequence of the metazoan plant parasitic nematode Meloidogyneincognita. *Nature Biotechnology, 26*, 909 – 915.

[2] Bacon, C. W. & White, J. F. (2000). *Microbial Endophytes.* NewYork: Marcel Dekker, Inc.

[3] Dababat, A. & Sikora, R. A. (2007). Important of inoculation time and inoculum density of *Fusarium oxysporum* 162 for biological control of *Meloidogyne incognita* ontomato. *Nematropica, 37*(2), 267-276.

[4] Dababat, A. A. (2007). *Importance of the mutualistic endophyte Fusarium oxysporum 162for enhancement of tomato transplants and the biological control of the root-knot nematode Meloidogyne incognita, with particular reference to mode-of-action*. PhD thesis, University of Bonn, Germany, 104 pp.

[5] Diedhiou,et al., (2003). Effect of arbuscularmycorrhiza fungi and a non-pathogenic *F. oxysporum* on *M. incognita*infestation of tomato. *Mycorrhiza, 13*, 199-204.

[6] Diez, J. A. & Dusenbury, D. B (1989). Repellent of root-knot nematodes from rootexudates of root hosts. *Journal of Chemical Ecology, 15*(10), 2445-2455.

[7] Elmi, et al., (2000). Endophyte effects on reproduction of a root-knot nematode (*Meloidogyne marylandi*) and osmotic adjustment in tall fescue. *Grass and Forage Science, 55*(2), 166–172.

[8] Hallmann, J. & Sikora, R. A. (1994a). Influence of Fusarium oxysporum, a mutualisticfungal endophyte, on Meloidogyne incognita infection of tomato. *Journal of Plant Diseases and Protection, 101*, 475-481.

[9] Hallmann, J. & Sikora, R. A. (1994b). Occurrence of plant parasitic nematodes andnonpathogenic species of *Fusarium* in tomatoe plants in Kenya and their role

Copyright © 2014 SAVAP International

ISSN: 2223-9944, eISSN: 2223-9553

asmutualistic synergists for biological control of root knot nematodes. *International Journal of Pest Management, 40*, 321-325.

[10] Hooper, (2005). Methods for Extraction, Processing and Detection of Plant and Soil Nematodes. In Luc, M., Sikora, R.A. and Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical Agriculture* (pp. 53-86). Wallingford: CAB International.

[11] Hussey, R. S. & Janssen, G. J. W. (2002). Root- knot nematodes: *Meloidogyne* species. In: Starr, J.L., Kerry, B.R. (1990). An assessment of progress toward microbial control of plant parasitic nematodes. *Journal of Nematology, 22*, 621-631.

[12] Niere,et al., (1999). A novel approach to the biological controlof banana nematodes. *Deutscher Tropentag, 1999*.

[13] Nur, A. (1994). *Untersuchungen über die Bedeutung endophytischer Pilze für die biologische Bekämpfung des wandernden Endoparasiten Radopholus similis (Cobb) Thorne an Bananen.* PhD-Thesis, 112 p. Bonn University.

[14] Nur, A, La, D. & Nasruddin, A. (2008). The use of endophytic fungi to control of pest leaf borer *Oryctes rhinoceros* and *Sexava* sp. on oil palm plant. *Research Report, Indonesian Ministry of Research and Technology* (In Indonesian Language)

[15] Nur, A. (2013 a). Diversity of endophytic fungi from root of Maize var. Pulut (waxy corn local variety of South Sulawesi, Indonesia). *International Journal of Current Microbiology and Applied Science, 2*(8), 148-154.

[16] Nur, A. (2013b). Investigation of Culture Filtrate of Endophytic Fungi *Nigrospora* sp. Isolate RS10 in Different Concentrations towards Root-Knot Nematode *Meloidogyne* spp. *Indian Journal of Science and Technology, 6*(9), 5177 – 5181.

[17] Nur, A., Salam, M., Junaid, M., Asman & Baco, M. S. (2014). Isolation and Identification of Endophytic Fungi from Cocoa Plant Resistante VSD M.05 and Cocoa Plant Suscebtible VSD M.01 in South Sulawesi, Indonesia. *International Journal of Current Microbiology and Applied Science* (In Press).

[18] Pocasangre, L. E. (2000). *Biological enhancement of banana tissue culture plantlets withendophytic fungi for the control of the burrowing nematode Radopholus similisand the Panama disease* (*Fusarium oxysporum* f.sp cubense). PhD thesis,University of Bonn, Germany, 107 pp.

[19] Sankaranarayanan,et al., (2002). Parasitism of *Meloidogyne incognita* eggs by *Fusarium oxysporum* and otherfungi. *Indian Journal of Nematology 32*(1), 33-36.

[20] Sathe, V. & Raghukumar, S. (1991). Fungi and their biomass in detritus of the seagrass *Thalassia hemprichii* (Ehrenberg) Ascherson. *Botanica Marina, 34*, 271–277.

[21] Vu, T. T. T (2005). *Mode of action of non-pathogenic Fusarium oxysporum endophytes forbio-enhancement of banana toward Radopholus similis*. PhD thesis, University of Bonn, Germany, 101 pp.

[22] Zum, F. A., Pocasangre, L. E. & Sikora, R. A. (2004). Use of microbial communities inside suppressive banana plants to increase biocontrol of the burrowing nematode Radopholus similis. In: Turner DW abd Rosales FE (Eds). In *Proceedings of an international symposium on Banana Root Systems: towards abetter understanding for its productive management, 3-5 Nov. 2003. San Jose,Costa Rice.*

Copyright © 2014 SAVAP International

ISSN: 2223-9944, eISSN: 2223-9553