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Potency of local isolate *Paecilomyces* from West Sumatera for control of root-knot nematodes (*Meloidogyne* spp) on vegetables

Winarto, Darnetty and Yenny Liswarni

ABSTRACT

Paecilomyces is an antagonistic fungus that can be used as a base material for the manufacture of bionematicides for the control of root knot nematodes (*Meloidogyne* spp) because it has antagonistic activity as a nematode egg parasite. Utilization of Paecilomyces local isolates has good potentials for the control of parasitic nematodes, especially root knot nematodes. The environmental conditions of fungal isolates affect the ability of fungi in parasitizing nematode eggs. The objective of the study was to obtain local isolate of Paecilomyces fungi which have high potential to control root knot nematodes. Fungal samples were from soil rhizosphere of tomato plants infected by root knot nematodes (*Meloidogyne* spp) from several locations of vegetable production centers of West Sumatra, namely Alahan Panjang, Agam and Tanah Datar. As results of the research, 10 isolates of Paecilomyces fungi were obtained, 3 isolates from Alahan Panjang (PAP1, PAP2, and PAP3), 3 isolates from Agam (PA1, PA2, and PA3) and 4 isolates from Tanah Datar (PTD1, PTD2. PTD3 and PTD4). All Paecilomyces isolates had potency to control root knot nematode (*Meloidogyne* spp) with suppression percentage ranged from 55% - 70.9%. The PAP 2 (isolate from Alahan Panjang) had the best ability to control root knot nematodes with suppression percentage of 70.90%.

Keywords: Bionematicides, parasitic nematodes, nematode egg parasite

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INTRODUCTION

One of the obstacles in increasing the production of vegetable crops, especially tomato plants is the plant parasitic nematodes. Among the most important and harmful plant parasitic nematodes are root knot nematodes (RKN), *Meloidogyne* spp. (Sasser and Freckman, 1987). Root-knot nematodes are distributed worldwide, are obligate parasites attacking more than 2000 species of cultivated plants in food crops, horticulture and plantations and ornamental plants with varying degrees of attack and they cause approximately 5% of global crop loss (Moens *et al*., 2009). Results of survey in several vegetable production centers in West Sumatera namely Solo, Agama and Tanah Data districts indicated that root knot nematodes caused by *Meloidogyne* spp had spread causing decreased the production of vegetable crops. Also, results of interview with farmers showed root knot nematodes had reduced vegetable production about 40-50%.

Control of plant parasitic nematodes is generally done by using nematicides (Talavera *et al.*, 2012). Usage of pesticide continuously for control nematodes can lead to several negative impacts such as environmental pollution, resurgence, and resistance due to natural enemy death. To avoid the negative impacts, it is necessary to look for an environmentally friendly alternative control, one of which is the utilization of natural enemies. According to Baker and Cook (1974), soils with high antagonistic potential cause the suppression of soil borne pathogens in which pathogens cannot establish and persist, or cause little or no damage. Utilization of natural enemies and other biological potentials has an important role in controlling parasite nematode populations and

maintaining ecosystem balance, therefore natural enemies already presented in local ecosystems need to be preserved, increasing their role in the control of plant parasitic nematodes.

Root knot nematodes have many potential natural enemies as controlling agents such as antagonistic fungi. One of the antagonistic fungi is Paecilomyces which is as an egg parasite (Atkins *et al.*, 2005). The antagonistic mechanism of *P. lilacinus* is a direct infection of eggs (Stirling, 1991). The chitinase and protease enzymes produced by the fungi serve to soften the shell or nematode egg shell making it easier penetration, and is the key to the mechanism of antagonists (Khan *et al.*, 2004). The utilization of *Paecilomyces* for control of plant parasitic nematodes, especially the root knot nematode is a potential alternative to be developed in Indonesia because the source of isolates is easy to obtain, the multiplication is easy to do on cheap materials and its application in the field can coincide with the composting or manure and can survive inside soil as a saprophyte.

The environment of fungal isolates abanillas *et al.* (1989) will affect the physiological character and pathogenicity of the fungus on nematodes. Isolates taken from different places will have different pathogenicity. In addition to pathogenicity, adaptability to new environmental conditions greatly determines the success of Paecilomyces isolates in controlling parasitic nematodes. The purpose of this study was to obtain isolates of Paecilomyces that have high pathogenicity for control of root knot Nematodes (*Meloidogyne* spp.)

METRIALS AND METHODS

The research was conducted in the laboratory of Biological Control of Department of Plant Pest and Disease and in the Greenhouse of Faculty of Agriculture, Andalas University, and Padang. The study was conducted from May to October 2017.

Soil Sampling and Paecilomyces Isolation

The soil samples (500 g) were taken from tomato rhizosphere infected by root knot nematodes at a depth of between 10-15 cm in the tomato production center of West Sumatra, Agam, Solok and Alahan Panjang and were transferred in plastic bags and taken to the laboratory.

The fungal isolation was carried out by taking 10 grams of soil samples in 90 mL of distilled water in Erlenmeyer and shaking in a Shaker for 30 minutes. The soil suspension was diluted to 10-2, 10-3 and 10-4. One milliliter of the suspension from each dilution was inserted into a sterile Petridish, then poured approximately 10 mL of Potato Dextrose Agar (PDA). The cultures were incubated at room temperature for 3-5 days. Each fungal colony appearing was isolated on the PDA in a Petri dish until a pure culture was obtained.

Fungal isolation was also carried out from the same location of soil sampling from a group of nematode eggs extracted from the roots of tomato plants showing root knot symptoms. Egg mass were washed and surface sterilized by using alcohol 70%. One group of infected nematode eggs was inoculated in to PDA and incubated for 3 days at 25ºC. Observations including identification were made on the fungi that grew on the egg mass.

Identification of fungi isolates

To determine Paecilomyces fungi, all fungal isolates were identified morphologically by observing macroscopic and microscopic characteristics. Identification was based on key identification of Barnet and Hunter (1972) and Watanabe (2002).

Paecilomyces against Nematode Eggs

This test was conducted to determine the ability of fungi in parasitizing root knot nematode eggs (*Meloidogyne spp*.). The method used was from Olivares-Bernabeu and Lopes-Liorca (2002) by spreading 50 eggs of nematodes in 1% water agar in the convex glass object and then 10 µL of conidial suspension with a concentration of 106 conidia / ml was dripped on each fungus. Each was made 3 replicates and then incubated at 25°C in at dark. The fungus that colonized nematode eggs was regarded as fungal egg parasite of root knot nematode (*Meloidogyne spp.*).

Observation of Fungal Characteristics

The physiological characteristics of antagonistic fungi observed were colony area and sporulation. The colony area observation was done by taking the pieces of Paecilomyce isolates by using cork borer (dia: 0.7 cm) then inoculating in the middle of PDA in Petri dish and incubating at 25°C. The colony diameter of each fungus was measured daily until day 15.

Sporulation calculation of each antagonistic fungi from various sampling areas was done by preparing a conidia suspension with a concentration of 105 conidia / ml. For each fungus, 0.1 ml of conidial suspension is inserted in a Petri dish filled with SDAY media. Cultures were incubated for 15 days at 25°C. After 15 days, cultures in petridishes were put into Erlenmeyer flasks and 50 mL of sterile distilled water was added. Then, cultures were vortexed for 5 minutes, filtered and diluted up to 4 times. The conidial concentration of the suspension was calculated by Haemocytometer.

Paecilomyces against root knot nematodes

Tests were performed in a greenhouse by using a complete randomized design (CRD) with 10 treatments and 3 replications. The treatment consisted of conidia suspension derived from 10 Paecilomyces isolates with a concentration of 106 conidia / ml. Five ml conidia suspension of Paecilomyces were cultured in 10 grams rice bran medium and incubated for 14 days, then applied to a sterile planting medium consisting of a mixture of soil, manure and sand (1: 1: 1 v / v / v) in previously inoculated polybags with 500 eggs Meloidogyne spp and incubated for 1 week. Furthermore tomato seeds (21 days) were planted. Observations were made 45 days after inoculation of nematode eggs and the parameters observed were the number of root knot, egg and egg mass in the egg group.

RESULTS

Isolation and Identification

Based on the results of isolation and identification, 10 isolates of Paecilomyces were obtained from 3 locations in West Sumatera, Alahan Panjang 3 isolates (PAP1, PAP2 and PAP3), Tanah Datar 4 isolates (PTD1, PTD2, PTD3 and PTD4) and Agam 3 isolates (PA1, PA2 and PA3) as showed in Table 1. Paecilomyces identification was based on morphological characteristics, and the results are showed in Fig. 1

Parasitism Test

The results of parasitism test showed that 10 isolates of Paecilomyces obtained could parasitize the root knot nematode (*Meloidogyne* sp.) eggs as showed in Figure 2.

Colony Area and Conidial Producton

The colony area and the conidial production of each isolate were calculated after a 14- day-old culture. The PAP3 isolate had the widest colony while the largest conidial production was produced by PAP2 isolate as shown in Table 2.

Pathogenicity of Each Paecilomyces isolate

All Paecilomyces isolates obtained indicated a high enough ability to suppress the formation of root knot, egg mass and the number of eggs in each egg group in tomato plants with

Table 1. Paecilomyces isolates from 3 locations in West Sumatera

Isolates origin	Paecilomyces isolate code				Number of isolates
Alahan Panjang	PAP1	PAP2	PAP3		3
Tabah Datar	PTD1	PTD2	PTD2	PTD3	4
Agam	PA1	PA2	PA3		3

Tabel 2. Colony area and spore production by each Paecilomyces isolate of 14-day-old

Paecilomyces isolate	Colony area (cm^2)	Number of conidia/ml
PAP1	45.78	6.0×10^5
PAP2	42.25	9.5×10^5
PAP3	61.84	5.5×10^5
PTD1	16.91	6.0×10^5
PTD2	32.05	4.0×10^5
PTD3	27.44	3.0×10^5
PTD4	45.80	5.5×10^5
PA1	48.37	7.0×10^5
PA1	18.84	4.0×10^5
PA1	48.51	4.5×10^5

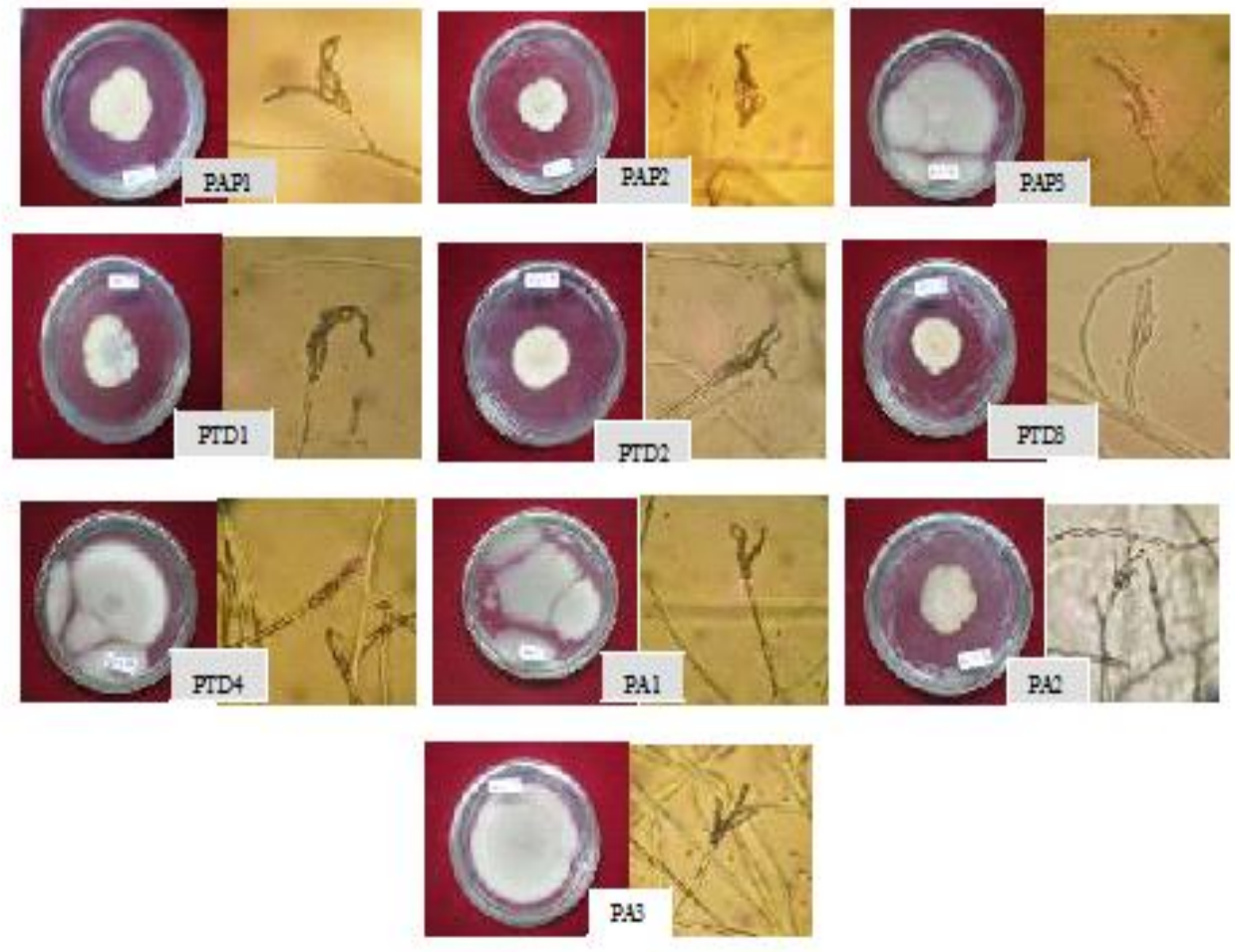

Figure 1. Macroscopic and microscopis characteristics of *Paecilomyces* isolates from different locations

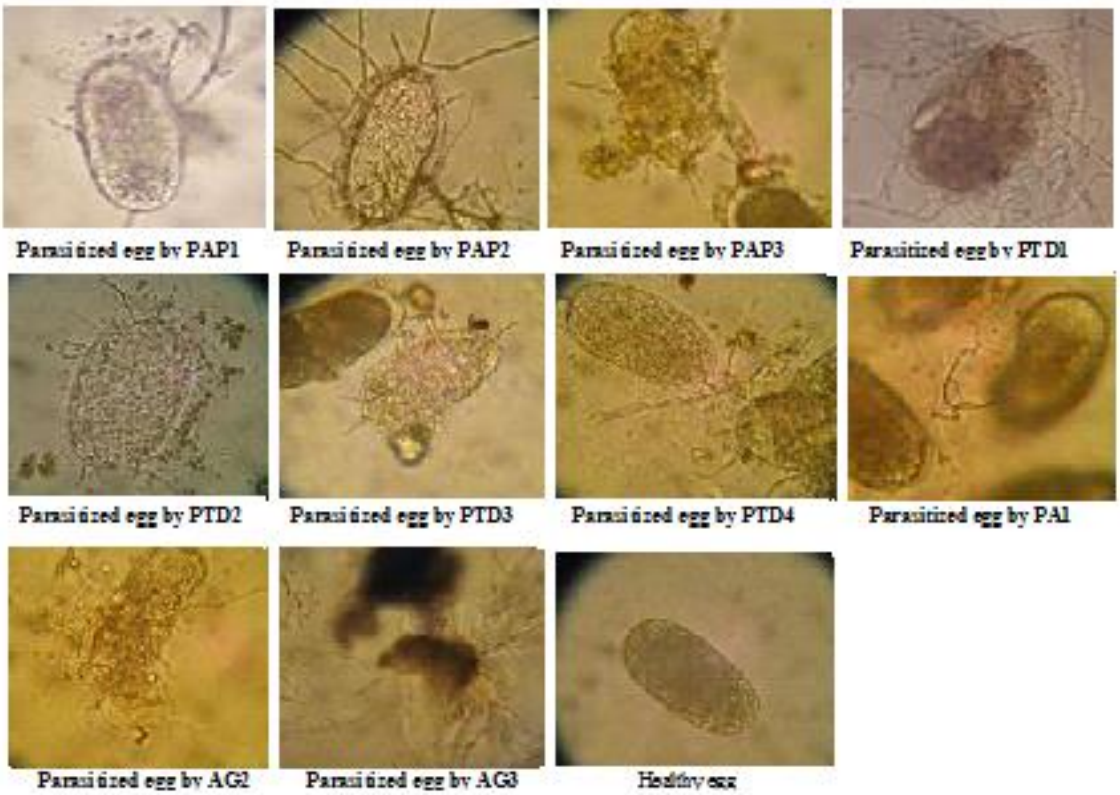

Figure 2. Meloidogyne eggs parasitized by 10 Paecilomyces isolates and healthy egg

Table 3. Effect of each Paecilomyces isolate treatment on the formation of root knot, egg mass and egg in the egg mass

Paecilomyces Isolates	Root knot / plant	Percentage of suppression	Egg mass /plant	Effectivity (%)	Egg /egg mass	Effectivity (%)	Effectivity average (%)
PAP1	29.33 b	62,55	10.33 df	70,12	34.33 b	54,62	62,43
PAP2	26.00 a	66,80	6.66 a	80,78	26.33 a	65,19	70,92
PAP3	32.66 bc	58,30	11.00 f	68,26	37.00 bcd	51,09	59,22
PTD1	34.33 cd	56,17	8.33 b	75,97	36.00 bc	52,42	61,52
PTD2	36.33 d	53,62	9.66 cd	72,13	38.00 cd	49,80	58,52
PTD3	35.66 d	54,47	9.00 bc	74,03	37.33 bcd	50,66	59,72
PTD4	37.00 d	52,75	9.66 cd	72,13	40.33 de	46,69	57,19
PA1	34.33 cd	56,17	9.00 bc	74,03	42.33 e	44,05	58,08
PA2	36.33 d	53,62	11.00 f	68,26	42.33 e	44,05	55,31
PA3	36.33 d	53,33	8.66 bc	75,01	46.66 f	38,32	55.55
Control	78.33 e	0,0	34.66 g	0,00	75.66 g	0,0	0,0

A number in the same column followed by the same letter shows no real difference in the DNMRT test at a 95% confidence level

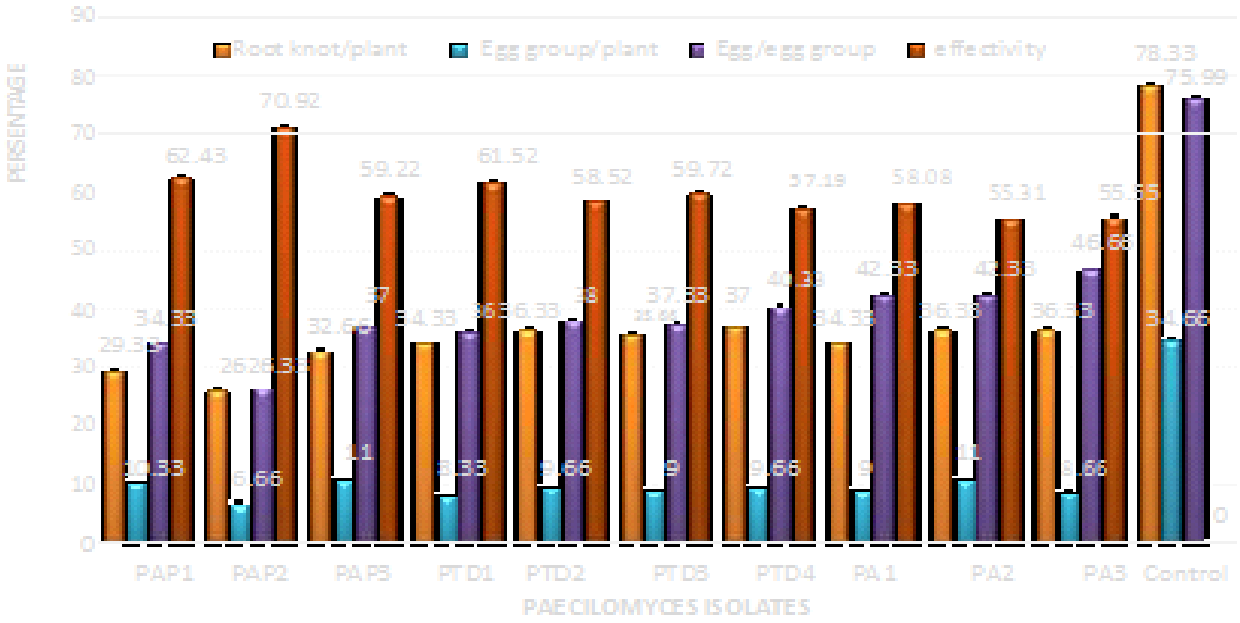

Figure 3. Chart of effect of each Paecilomyces isolate on the formation of root knot, egg mass and egg in the egg mass.

different levels of ability. Effectiveness of suppression ranged from 55.31% - 70.92%.

The highest ability was shown by PAP2 isolates with the effectiveness of 70.92% shown in Table 3 and Figure 3

DISCUSSION

All Paecilomyces isolates obtained had ability to control the development of root knot nematode (*Meloidogyne* spp.). This is due to all Paecilomyces isolates were able to paratisize nematodes eggs as shown in Fig. 2. The parasitized eggs could not develop into a larva that will infect the roots of the plant so that the root knots were not formed. It is indicated that all Paecilomyces isolates are egg-nematode parasites. According to Gortari *et al.* (2008) in Indarti and Rahayu (2014), biological control by using egg parasitic fungi has a high success rate to be applied in the field on a wide scale especially for endoparasitic nematodes that are sedentary in roots. Factors that support the success rate of egg parasitic fungi are the ability of fungi to colonize and damage the eggs and other stadia resistant in the life cycle of nematodes. Chen and Dickson (2004) also stated that the fungus Paecilomyces is a group of fungi that produce toxic substances or antibiotics that cause eggs cannot hatch, inhibit the mobility of larvae stage II or also have activity as nematicides. Paecilomyces isolates had different pathogenicity to root knot nematodes that were to be seen from suppression of the formation of root knot, the egg mass and the number of eggs produced. Their pathogenicity was different because the isolates were collected from different areas so that each area has different environmental conditions that will affect the pathogenicity of each fungal isolate. As stated by Devide and Zorilla (1985) that fungal isolates obtained from different sampling sites will have different pathogenicity in controlling plant parasitic nematodes Isolate PAP1 from Alahan Panjang had the highest ability to control root knot nematodes compared with other isolates. This is because PAP1 isolates have higher pathogenicity with ability to adapt to new environmental conditions to grow and develop rapidly so that their ability is higher in controlling root knot nematodes. According to Ganaie and Khan (2010), fungal isolates that have the ability to adapt to various environmental conditions will be able to control plant parasitic nematodes with a higher success rate. Some fungi of Paecilomyces group, Trichoderma and Gliocladium can suppress the development of root knot

nematodes. Paecilomyces lilacinus fungus is able to adapt to various agroclimate conditions. Esfahani and Pour (2006) suggest that *Paecilomyces lilacinus* has the ability to adapt to various physical environmental conditions and is able to control root knot nematodes properly. According to Cabanillas *et al*. (1989) temperatures affect the ability of fungal isolates *Paecilomyces lilacinus* in controlling the development of root knot nematodes (*Meloidogyne spp*.). Differences in temperature of the origin of the isolates to the temperature of the field where the application affects the development of the fungus. From this study, we made conclusion that all Paecilomyces isolates obtained could parasitize root knot nematode egg (*Meloidogyne spp.*), The largest colony area was found on PAP3 isolate and the highest conidial production on PAP2 isolate. The highest ability in controlling root knot nematode (*Meloidogyne* spp.) was PAP2 isolate from Alahan Panjang.

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