Morpho-molecular identification and pathogenicity test on fungal parasites of guava root-knot nematode eggs in Lampung, Indonesia

By I GEDE SWIBAWA; YUYUN FITRIANA; SOLIKHIN; RADIX SUHARJO; F.X. SUSILO; EKA RANI; MEI SRI HARYANI; RACHMANSYAH A. WARDANA

WORD COUNT 5704
TIME SUBMITTED 14-AUG-2020 02:21PM
PAPER ID 62166668

BIODIVERSITAS
Volume 21, Number 3, March 2020
Pages: 1108-1115

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210334

Morpho-molecular identification and pathogenicity test on fungal parasites of guava root-knot nematode eggs in Lampung, Indonesia

I GEDE SWIBAWA[1,♥], YUYUN FITRIANA[1], SOLIKHIN[1], RADIX SUHARJO[1], F.X. SUSILO[1], EKA RANI[2], MEI SRI HARYANI[2], RACHMANSYAH A. WARDANA[2]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Lampung. Jl. Sumantri Brojonegoro No. 1, Bandar Lampung 35141, Lampung, Indonesia. Tel.: +62-721-787029, ♥email: igswibawa@yahoo.com; igede.swibawa@fp.unila.ac.id
[2]PT NTF Way Kambas, East Lampung 34375, Lampung, Indonesia

Manuscript received: 11 December 2019. Revision accepted: 18 February 2020.

Abstract. *Swibawa IG, Fitriana Y, Solikhin, Suharjo R, Susilo FX, Rani E, Haryani MS, Wardana RA. 2020. Morpho-molecular identification and pathogenicity test on fungal parasites of guava root-knot nematode eggs in Lampung, Indonesia. Biodiversitas 21: 1108-1115.* This study aimed to obtain and discover the identity of the species of fungal egg parasites of root-knot nematodes (RKN), which have a high pathogenic ability causing major losses in vegetable crops. The exploration of the fungi was carried out in 2016 and 2018 from Crystal guava plantations in East Lampung, Central Lampung, Tanggamus, and NirAma, a commercial product that has been used for controlling *Meloidogyne* sp. in Indonesia. Identification was carried out based on morphological characteristics and molecular-based gene sequential analysis of Intergenic Transcribed Spacer (ITS) 1 and ITS 4. A pathogenicity test was carried out in vitro and in a greenhouse using tomato plants as indicator plants. In the in vitro test, observations were made on the percentage of infected RKN eggs. The observations in the greenhouse test were carried out on RKN populations in the soil and roots of tomato plants, root damage (root knots), and damage intensity due to RKN infection. The exploration resulted in five isolates of fungal egg parasites of RKN from the guava plantations in East Lampung (2), Central Lampung (1), Tanggamus (1), and from the isolation results of commercial products (1). The isolates were given codes as B4120X (PT GGP PG1), B3010 (PT GGP PG4), B412G (PT GGP PG 4), B01TG (Tanggamus), and BioP (Commercial products). Based on their morphological characteristics, the isolates were classified into the genus of *Paecilomyces*. The results of molecular identification showed that the discovered fungi were *Purpureocillium lilacinum* (Thom.) Luangsa Ard. *(Syn. Paecilomyces lilacinus* (Thom.) Samson.). Based on the in vitro tests, the five fungal isolates were able to parasitize RKN eggs at 86.4-100%. In the greenhouse test, all isolates significantly suppressed nematode populations in the soil and tomato roots, inhibited the formation of root knots, and produced lower damage intensity compared to controls. Among all the isolates tested, B01TG had the best ability to infect nematode eggs (99.5%), suppressing the formation of root knots, nematode population in the soil and the roots of tomato plants, and the damage intensity compared to other isolates.

Keywords: Guava, pathogenicity test, *Purpureocillium lilacinum*, root-knot nematode

INTRODUCTION

Crystal guava plants in Lampung have been reported to be infected by root-knot nematode (RKN), *Meloidogyne* spp. (Swibawa et al. 2017). Three reported species of root-knot nematodes attacking guava are *Meloidogyne incognita* (Razak and Lim 1987), *M. Javanica* (Dawabah et al. 2019) and *M. enterolobii* (Kumar and Rawat 2018). According to Swibawa et al. (2017), all of the RKN attack rates is severe with a second-stage juvenile (J-2) population more than 1000 individuals per 5 g of roots. The problem of RKN in guava Crystal in Lampung is getting more serious because this nematode is difficult to control. Besides, its population is accumulative and continues to increase.

The use of chemical nematicides for controlling RKN in Crystal guava is not quite appropriate because the residues of the substances will harm the environment and can accumulate on the fruit, thus endangering the fresh fruit consumers. Therefore, the use of biological control agents such as fungal egg parasites of nematode for environmentally friendly and safe biological control programs for consumer health is highly recommended. One type of fungal agent for controlling nematode is *Purpureocillium lilacinum* (Thom.) Luangsa Ard. (Syn. *Paecilomyces lilacinus* (Thom) Samson). This fungus is also known as the fungal egg parasites of root-knot nematode, *Meloidogyne* (Jatala 1986).

Purpureocillium. lilacinum has been used as a biological control agent for nematode in various countries and has shown positive results (Stirling 2014). Besides effectively controlling root-knot nematodes, the fungus is also reported to be effective for controlling burrowing nematodes (*Radopholus similis*) in banana plants (Khan et al. 2006), root-knot nematodes in tomato plants (Singh et al. 2013), and root-lesion nematodes (*Pratylenchus thornei*) in wheat cropping in Turkey (Kepenekci et al. 2018). *P. lilacinum* is also reported to be able to promote plant growth. The application of *P. lilacinum* was proven to be able increasing the amount and weight of fresh grains of wheat (Hernadez-Leal et al. 2016).

Purpureocillium lilacinum is easy to find, isolate, and reproduce. Prabu et al. (2009) produced *P. lilacinum* using agar media and Sundararaju and Cannayane (2002) using rice, rice bran, and banana media, while Brand et al. (2009)

reproduced this fungus using solid form fermentation. *P. lilacinum* has been used as an active ingredient of bionematicides and is marketed under various trade names, such as Bio-Nematon, a bionematicide in the form of liquid and solid (Stanes and Company Limited 2017). Many commercial bionematicides contain active ingredients of *P. lilacinum*, such as Myco-Nematocure, Ukarsh Nematoz P, and Ecoman-R Bio-nematicide (Ecoman Biotch. Co., Ltd. 2014). Hore et al. (2018) mentioned that Bio-Nematon (*P. lilacinum* 1.15% WP) bionematicide was effective in controlling root-knot nematodes (*M. incognita*) in tomato plants.

The mass of RKN eggs attacking the guava plantations in Lampung was found to be parasitized by fungi. Swibawa et al. (2017) reported that the fungus that parasitized the mass of RKN eggs attacking the guava plantations in East Lampung was *P. lilacinum* with a parasitization rate of 16-26%. The discovery of this fungus indicates that there are fungal parasites of nematodes that have the potential to be explored and used as active ingredients of bionematicides. The application of indigenous (local biological agent) fungi to control nematodes is likely to be more effective than the use of exotic ones. More accurate identification to determine the identity of indigenous fungi is needed to study the biological characteristics in its use as a bionematicide active ingredient. This research aimed to identify and reveal the pathogenicity of fungal egg parasites of RKN from the exploration at the guava plantation in Lampung.

MATERIALS AND METHODS

Exploration of fungal egg parasites of root-knot nematodes

The exploration of fungal egg parasites of RKN was carried out twice, June-October 2016 and April-September 2018, in Lampung Province, Indonesia. Exploration in 2016 was carried out in the Crystal guava plantations of PT. Great Giant Plantation (GGP) Plantation Group (PG4) in Central Lampung (4.9°S and 105°E) and PT. GGP PG4 East Lampung (05°00 '-05°15'S and 105°30'-105°45'E). Exploration was also carried out from NirAma, a commercial product that has been widely used for controlling *Meloidogyne* sp. in Indonesia. Exploration in 2018 was carried out in the Crystal guava plantations of PT. GGP PG4 (05°33'55.7964'S) and in Sinar Betung Village, Gunung Alip Sub-district, Tanggamus District (104°32 '21,516' E) (Figure 1).

The samples of infected roots were collected from the Crystal guava plantations attacked by RKN. At each purposively selected plant, root samples were taken using a sucker in the root zone at 20-40 cm of distance from the base of the stem with 0-20 cm of soil depth at four cardinal points following the direction of the compass. Root samples from each plant were compiled and labeled for laboratory analysis. After being washed and dried, the roots were observed under a binocular stereomicroscope (Leica EZ4HD, Switzerland) at 40-60X magnification to find the mass of RKN eggs infected by fungi. The mass of RKN eggs infected by fungi (Figure 2) was separated for isolation purposes. Before isolation, the root samples containing the mass of RKN eggs were dipped in a 1% NaOCl solution for 30 seconds. The fungi infecting the egg mass of RKN were taken using a sterile needle and placed in the middle of the sterile Potato Sucrose Agar (PSA) medium that put on the sterile plastic petri dish (diameter 9 cm) under a sterile condition in Laminar Air Flow.

Identification of fungal egg parasites of root-knot nematodes

Morphological identification.

Fungal growth and development on PSA media were observed for morphological identification. The fungal morphological characteristics observed were the shape and color of the colony, as well as mycelium and spores. The shape and color of fungal colonies were observed directly, while the morphology of mycelia and spores was observed using a compound microscope (Leica DM500, Switzerland) at a magnification of 400-1000 X. The observed fungal morphological characteristics were confirmed with the fungi identification key (Barnett and Hunter 1998).

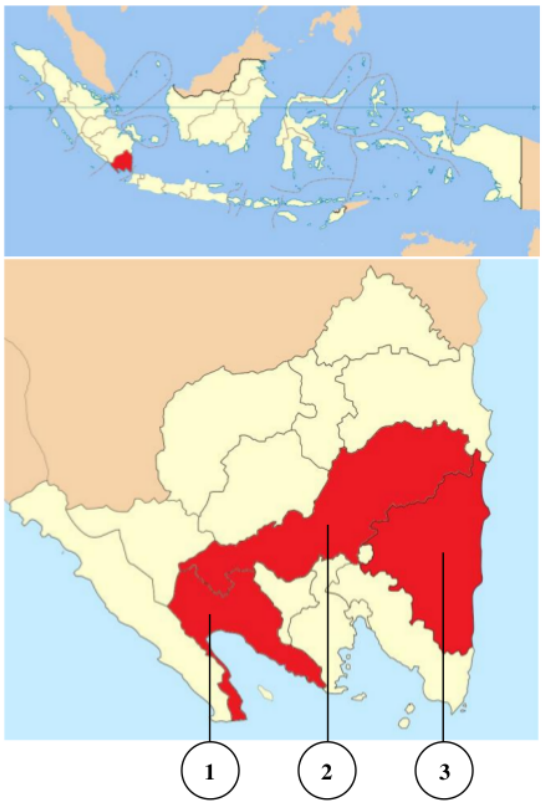

Figure 1. Sampling locations in Lampung Province, Indonesia: 1. Tanggamus, 2. Central Lampung, 3. East Lampung

Molecular identification

DNA extraction

The DNA extraction was carried out from fungal cultures aged three weeks. The fungi collected from the petri dish was suspended with 10 mL of sterile water in a 30 mL centrifuge tube, then centrifuged at 14,000 rpm for 10 minutes, then added with 1 mL of 70% chilled ethanol and centrifuged for 10 minutes at 14,000 rpm. The supernatant was removed, and the pellet was added with 1 mL extraction buffer (0.5 mL Tris HCl, 1 mL SDS 1% + 2.8 mL NaCl, 0.2 mL Mercaptho Ethanol, 2 mL EDTA, 3.5 mL sterile water). The pellet was moved into a mortar and incubated at-38°C for 24 hours, then crushed until pulverized. As much as 500 µL pellet suspension was put into a 1.5 mL tube, and 400 µL of 2% cetyltrimethylammonium bromide (CTAB) was added, homogenized and heated at 65°C for 1 hour using a water bath (Brookfield TC 550 MX-230, USA). After that, 500 µL of Phenol Chloroform Isoamyl (PCI) alcohol solution (25: 24: 1) was added, homogenized, and centrifuged at 14,000 rpm for 10 minutes. A total of 600 µL supernatant was then transferred to a new 1.5 mL tube, and 600 µL Chloroform Isoamyl Alcohol (24:1) was added, homogenized, and centrifuged again at 14,000 rpm for 10 minutes. A total of 400 µL supernatant was put into a new 1.5 mL tube, and 400 µL cold isopropanol was added, homogenized slowly by hand, and incubated at-40°C for 20 minutes. The suspension was centrifuged again at a speed of 14,000 rpm for 15 minutes. The supernatant was removed, and the pellet obtained was then added with 500 µL of cool 70% ethanol. After that, it was then centrifuged again at a speed of 14,000 rpm for 5 minutes. The supernatant was removed, and the pellet obtained was incubated at room temperature for 24 hours to dry. After dry, the pellet was added with 50 µL 1x Tris-HCL EDTA (TE) pH 8.0 (Invitrogen). All centrifugation processes were performed using a centrifuge (Microspin12, Biosan, Latvia).

PCR Amplification

Amplification was carried out with a CFX Connect Real-Time PCR (Bio-RAD) machine by using 12.5 µL Master Mix (Red Mix) (Bioline), 1 µL ITS 1 (5'TCC GTA GGT GAA CCT TGC GG 3 '), 1 µL ITS 4 (5'TCC TCC GCT TAT TGA TAT GC 3 '), 2 µL DNA template and 8.5 µL sterile distilled water. DNA amplification using PCR consisted of one cycle of the initiation stage at a temperature of 95°C for 5 minutes, 30 cycles of denaturation stages at a temperature of 95°C for 1 minute, annealing at 44-56°C for 1 minute, extension at 72°C for 1 minute, and one cycle of elongation at 72°C for 5 minutes. The PCR results were then electrophoresed using 0.5% agarose gel in 20 mL 1x buffer Tris-Boric Acid-EDTA (TBE) (Invitrogen) containing 1 µL Ethidium Bromide (EtBr 10 mg/mL). The electrophoresis was carried out using 1x TBE buffer at a voltage of 50 volts for 70 minutes. The results were visualized using DigiDoc UV transilluminator (UVP, USA).

Sequencing and phylogenetic tree construction

The PCR results were then sent to 1st Base Malaysia for the sequencing process. Sequencing results obtained were analyzed using the BioEdit program for windows ver. 7.2.6 (Hall 1999). The phylogenetic tree was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method with the MEGA 7 for Windows program (Kumar et al. 2016). The sequence data of ITS1 and ITS4 reference strains were taken from NCBI (https://www.ncbi.nlm.nih.gov/).

Pathogenicity test

In vitro test

The pathogenicity test of *P. lilacinum* isolates in vitro was carried out on *Meloidogyne* spp. eggs. The experiment was arranged in a randomized block design, consisting of six treatments and five blocks. The treatments were one control and five fungi isolates, including BioP that was isolated from NirAma, a commercial product obtained from PT. Great Giant Plantation (GGP) Plantation Group (PG) 1 Central Lampung in 2016, B4120X and B3010 that were isolated from PT. GGP PG4 East Lampung in 2016, B412G isolated from PT. GGP PG4 East Lampung in 2018 and B01TG that was isolated from Tanggamus in 2018. A drop (± 1 mL) of fungal spore suspension with a density of 10^8 spores/mL of potato water solution was added to a sterile petri dish with diameter of 9 cm. Spore suspensions were spread evenly on sterile glass petri dishes, then five masses of RKN eggs were placed in a circular position. RKN egg mass was obtained from PT. GGP PG4 East Lampung. An egg mass was taken every 12 hours to be observed under a Leica compound microscope at 400 X magnification. All eggs were chopped, including infected and healthy eggs (Figure 3). The number of eggs infected with fungus is presented in percent.

Greenhouse test

Tomato plants cv. Victoria was used as test plant. Tomato seeds were sown on a plastic tray measuring 31 cm x 24 cm x 4 cm containing sterile soil. After 14 days, they were then transplanted to a polybag filled with 3 kg sterile soil. One day before the tomato planted, the planting hole was inoculated with 1 mL of *P. lilacinum* spore suspension at a density of 10^8 spores/mL with the same type of treatment as in the in vitro pathogenicity test. One week after planting, all tomato plants were inoculated with 2000 root-knot nematode (*Meloidogyne* spp.) eggs/plant. Nematode eggs were obtained from the roots of the Crystal guava plants from PT GGP PG4, East Lampung showing symptoms of infection. Plant maintenance included watering, controlling weeds manually, and fertilizing using a mixture of N, P, K, and S with the composition of N (15%), P (15%) K (15%), and S (10%). Fertilizing was done three times, at 2, 5, and 8 weeks after replanting as much as 30g per polybag. At 98 days after planting, the soil and roots were taken for observation in the laboratory. Observations were made on the root damage, indicated by the formation of large galls or knots. The severity of the damage was determined using a scoring system based on the Zeck scale (0-10) (Zeck 1971). The intensity of root

damage was calculated using the formula of Ds = (∑vi x ni)/ (N x V) x 100%, where Ds = intensity of disease severity, vi = Zeck scale of root damage (0-10), ni = ith plant, N = number of plants measured, and V = the highest scale (10). Juvenile nematodes (J-2) were extracted using the modified Baerman method from 5 g roots randomly taken, while nematodes from the soil were extracted by stratified filtering methods and centrifuged with sugar solution from 300 mL of soil (Hooper et al. 2005). The J-2 nematode population was counted under a stereomicroscope (Leica EZ4HD, Singapore) at a magnification of 60 X (Gafur and Swibawa 2004). Data were analyzed using analysis of variance, and the separation of mean values was performed using the LSD test at a significance level of 5%.

RESULTS AND DISCUSSION

Isolates resulted from the exploration

The exploration of fungal parasites of RKN in 2016 in the Crystal guava plantations of PT GGP PG 4 East Lampung obtained two isolates. Exploration carried out in 2018 also found two isolates, each originating from the Crystal guava plantation of PT. GGP PG 4 East Lampung and the Crystal guava plantation in Tanggamus. Another isolate was obtained from PT. GGP PG 1, Terbanggi Besar, Central Lampung. The obtained isolates can be seen in Table 1.

Identification of the isolates of fungal parasites of nematode eggs

Identification based in morphological characteristics

Based on the identification, the mycelia and spores of the isolates obtained in this study were species of the genus *Paecilomyces*. The fungi grown on PSA media showed the characteristics of a colony-forming air mycelium (cotton) with a floccose-shaped edge. The color of the colony was initially white, then turned grayish violet after forming spores (Figure 4.A). At 1000 x magnification, the fungal hyphae seeped, and the conidiophores branched, forming phialides with round or oval conidia (Figure 4.B).

Molecular identification

Tracing of sequencing results using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) shows that the four isolates found had 99% similarity to *Purpureocillium lilacinum*. Based on the phylogenetic tree, the four isolates found were in the same group with *P. lilacinum* CBS 204.57 (Acc No. MH857697.1), Kw 3411 (Acc No. FR822391.1), IFM 63226 (Acc No. LC317744.1), BFM-L1048 (Acc No. AB369489.1), FMR 8251 (Acc No. FR734087.1), UWFP853 (Acc No. AY213668.1), MY2861 (Acc No. GU980027.1), CBS126685 (Acc No. MH864210), BCC2012 (Acc No. EU828665.1), MY2861 (Acc No. GU980027.1), CBS126685 (Acc No. MH864210), BCC2012 (Acc No. EU828665.1), FMR8422 (Acc No. FR832488.1), FMR8249 (Acc No. FR832489.1), CBS346.51 (Acc No. MH856891.1), FMR8253 (Acc No. FR734088.1) and CBS128764 (Acc No. MH865073.1) (Figure 5). This ensures that the four nematode egg parasite isolates found were *P. lillacinum*.

Pathogenicity of fungal nematode egg parasites discovered in this study

Five isolates of *P. lilacinum* tested proved to have high pathogenicity. At 60 hours after infestation (HAI), the five fungi were able to infect RKN (*Meloidogyne* spp.) eggs in the range of 86.4% to 100%. The 100% infection was produced by B3010 and B412G isolates (Table 2).

The pathogenicity test in the greenhouse also showed relatively similar results to in vitro test. The isolates of *P. lilacinum* were seen to be effective in controlling nematode populations. The population of J-2 nematodes in the roots of tomato plants in control treatments was higher than the population of nematodes in the roots of plants treated with the fungi (Table 3). Likewise, the J-2 nematode population in the soil was seen to be higher in control treatments than plants treated with *P. lilacinum* fungi. J-2 nematode populations in roots and soil appear to be influenced by the type of fungal isolate applied (Table 3). In plants treated with B01TG isolates, nematode populations reached 848.2 individuals/5 g roots and 424.8 individuals/300 mL of soil, which were lower than nematode populations in plants treated with other isolates whose populations were in the range of 1216 (B3010)-2571 (control) individuals/5 g roots and 615.2 (B412G)-1261.6 (control) individuals/300 mL of soil.

The application of *P. lilacinum* isolates was seen to be able to suppress the formation of root galls or knots. Root galls formed in control treatment reached a score of 7 from a maximum score of 10, while in the plants treated with *P. lilacinum,* the root galls formed were in the score of 2-4 of the maximum score of 10. The root damage intensity was also high in the control plants, reaching 66%, while in plants applied with the fungi, it was only around 18-40%. B01TG isolates were able to suppress the formation of root galls (score 2) and decrease the damage intensity (18%) (Table 4).

Discussion

Purpureocillium lilacinum was originally described as *Paecilomyces lilacinus*. Based on the 18sRNA sequence analysis results, Internal Transcribed Spacers (ITS) and partial Translation Elongation Factor 1-α (TEF), Luangsa-ard et al. (2011) moved *Paecilomyces lilacunus* into the genus of *Purpureocillium* and named *Purpureocilium lilacinum*. *P. lilacinum* is widely known as the fungus of the egg mass of the root-knot nematode (RKN) *Meloidogyne* spp. In the Crystal guava plantations in Lampung, the fungal egg parasites of RKN is easily found so that it has the potential to be developed into bionematicide for controlling RKN.

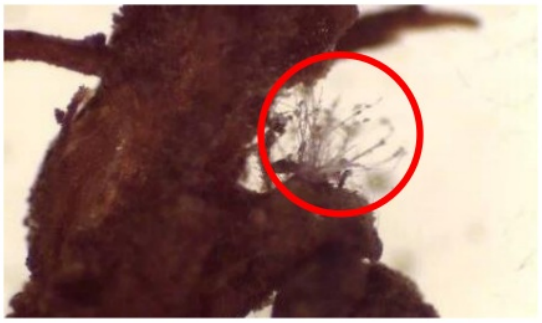

Figure 2. The root gall containing egg mass of Root Knot Nematode (RKN) which was infected by the fungi. The red circle showed the fungi which was emerged

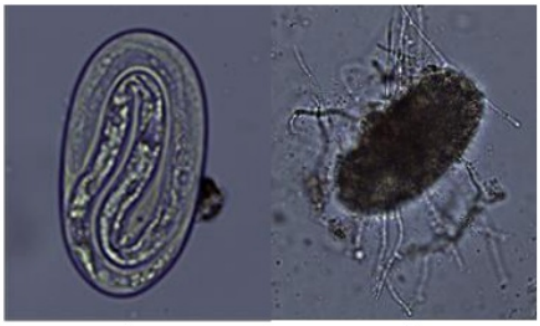

Figure 3. Healthy (A) and infected (B) eggs of Root-knot nematode (RKN) *Meloidogyne* sp.

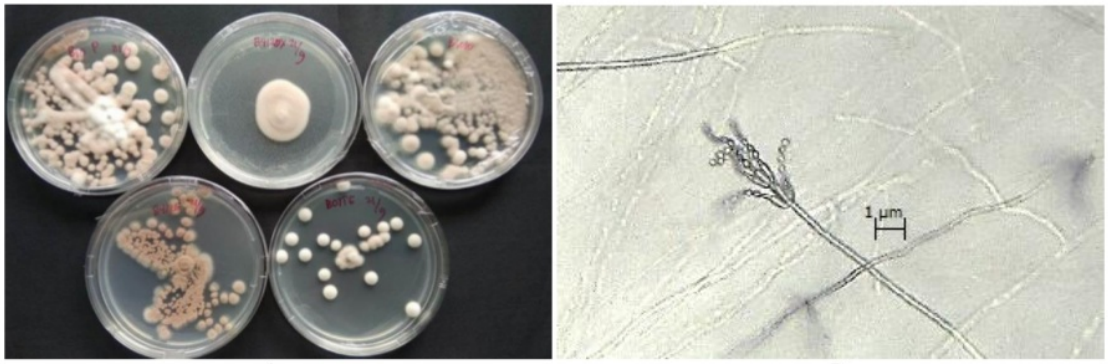

Figure 4. Fungal egg parasites of RKN discovered in the Crystal guava plantations of PT NTF; Colony form (A); the shape of hyphae, conidiophore and conidia (B).

Table 1. The origin of isolates of fungal nematode egg parasites

Isolate name	Isolate origin	Location	Year of isolation
B4120X	Eggs of *Meloidogyne* spp.	PT. GGP PG 1, Central Lampung	2016
B3010	Rhizosphere of Crystal guava	PT. GGP PG4, East Lampung	
BioP	Commercial products (NirAma)	Unknown	
B412G	Rhizosphere of Crystal guava	PT. GGP PG4, East Lampung	2018
B01TG	Eggs of *Meloidogyne* spp.	Tanggamus	

Table 2. Percentage of the root-knot nematode eggs infected by each isolate of *P. lilacinum*.

Treatment of *P. lilacinum* isolates	% Root-knot nematode eggs infection by fungi Hours after infestation (HAI)				
	12	24	36	48	60
Control	0^{b}	0^{b}	0^{c}	0^{c}	0^{c}
BioP	11.3^{b}	11.3^{b}	56.8^{ab}	82.1^{ab}	93.6^{ab}
B4120X	1.1^{b}	1.1^{b}	70.9^{ab}	72.6^{b}	86.4^{b}
B3010	4.9^{b}	12.1^{b}	48.8^{ab}	82.7^{ab}	100.0^{a}
B412G	0.0^{b}	20.9^{b}	34.4^{bc}	89.8^{a}	100.0^{a}
B01TG	81.6^{a}	85.1^{a}	88.6^{a}	93.7^{a}	99.5^{a}
P>F	0.0001^{**}	0.0001^{**}	0.0107^{**}	0.0001^{**}	0.0001^{**}

Note: ** = significantly different based on F test; values followed by the same letters in the same column are not significantly different based on LSD test at 5%

Table 3. Population of root-knot nematodes in the soil and in the roots of tomato plants previously inoculated with root-knot nematodes and each of *P. lilacinum* isolates

Treatment of *P. lilacinum* isolates	Nematode population	
	Indiv./5 g roots	Indiv. /300 mL soil
Control	2 571.0 a	1 261.6 a
BioP	1 951.2 b	899.2 b
B4120X	1 552.6 bc	813.6 b
B3010	1 216.0 cd	629.2 c
B412G	1 396.0 c	615.2 c
B01TG	848.2 d	424.8 d
P>F	0.0001	0.0001

Note: Values followed by the same letters in the same column are not significantly different based on LSD test at 5%.

Table 4. The root damage of tomato plants inoculated with root-knot nematodes and each of *P. lilacinum* isolates

Treatment of *P. lilacinum* isolates	Root damage	
	Zeck scale of root damage (0-10)	Damage intensity (%)
Control	7 a	66
BioP	4 b	40
B412X	4 b	36
B3010	3 c	30
B412G	3 c	30
B01TG	2 d	18

Note: Values followed by the same letters in the same column are not significantly different based on LSD test at 5%.

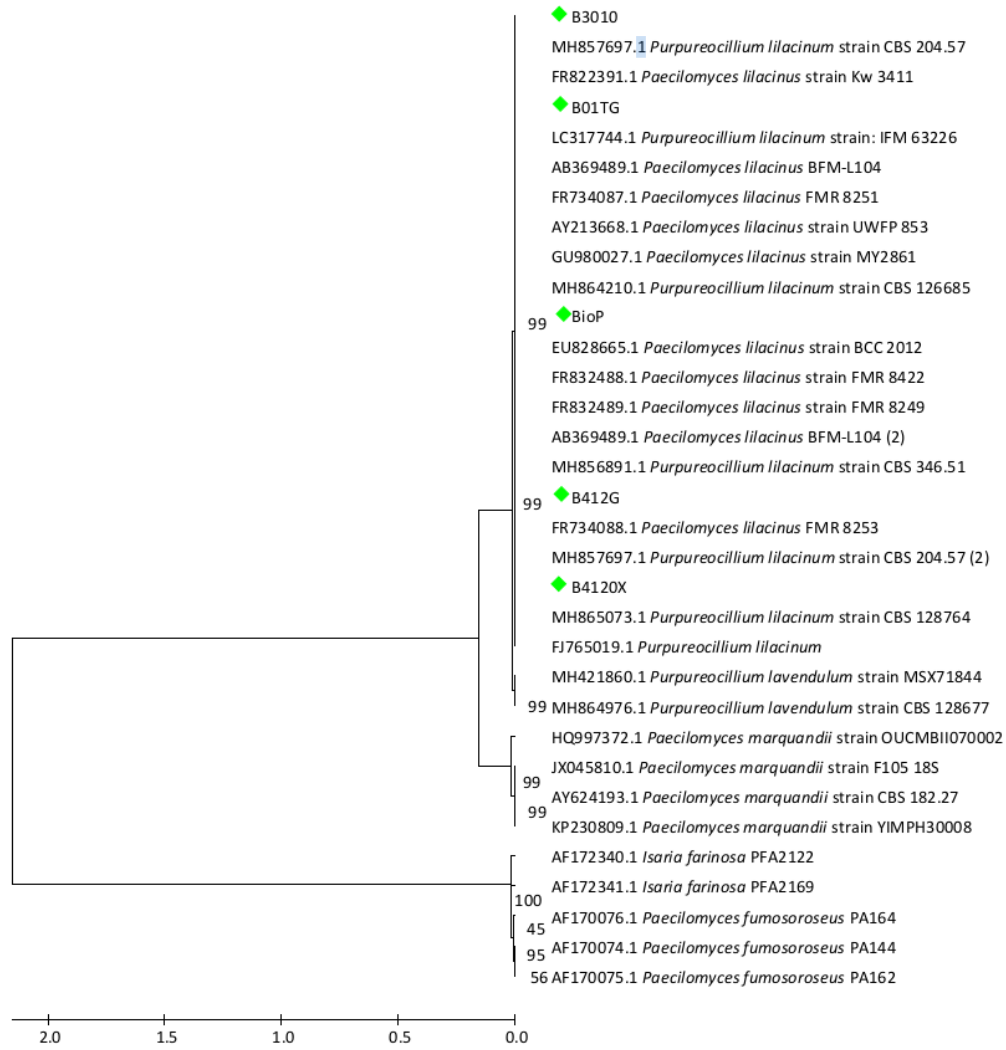

Figure 5. Phylogenetic tree based on the sequencing of ITS 1-ITS 4 using UPGMA method performed with Mega 7 program (Kumar et al. 2016). ♦ : The isolates of fungal nematode egg parasites discovered.

Based on the morphological characteristics and the analysis results of the ITS1 and ITS4 sequences, the isolates of fungal of nematode egg parasites found were *P. lilacinum*. Fungal colonies were seen forming air mycelia (cotton) with floccose-shaped edges. This characteristic is shared by the fungus in the *Paecilomyces* group (Barnett 1969). Mounfort and Rhodes (1991) stated that *P. lilacinum* has oval conidia of 2.5 μm long and 1.5 μm wide. Meanwhile, Esser and El-Gholl (1993) mentioned that *P. lilacinum* has a colony of light purple to dark purple, producing smooth to coarse conidia from a group of phialides growing from conidiophores. Khan et al. (2012) explained that *P. lilacinum* forms a white air mycelium, branched conidiophores arising from hyphae, and elliptic fusiform conidia, which are rarely round, single, or grouped.

Purpureocillium lilacinum isolated from the Crystal guava plantation in Lampung infected RKN eggs since 12 HAI, and after that time, the number of eggs infected by the fungi increased. The pathogenicity of the fungi isolates tested varied. For instance, the pathogenicity of B01TG isolates from Tanggamus was higher than that of other isolates. Winarto et al. (2017) reported that there was a variation in the pathogenicity of *P. lilacinum* isolates against *Meloidogyne* spp. The pathogenicity variation is thought to be influenced by the biophysical conditions of the environmental origin of the isolate. Isolates with high pathogenicity have the potential to be developed as bio nematicides. Fungal parasites of nematode egg are reported producing protease and chitinase enzymes to reduce the hatching of RKN eggs (Khan et al. 2004; Bonants et al. 1995). In vitro pathogenicity test conducted by Paz et al. (2015) showed that a mixture of *P. lilacinus* and *Pochonia clamidosporia* infected 80% of eggs and killed 90% of J-2 larvae of *Meloidogyne incognita*. In line with the research of Paz et al. (2005), the results of this study showed that the five *P. lillacinum* isolates had high infection ability. In 60 HAI, five fungal isolates were able to parasitize RKN eggs by more than 80% (86.4-100%).

The research in the greenhouse also showed that the five *P. lilacinum* fungi had the ability to be pathogen of root-knot nematode. The application of fungal suspension in tomato plants infected with RKN can reduce J-2 nematode populations in soil and roots and decrease the root damage of tomato plants (unpublished data). Dahlin et al. (2019) reported that the application of *P. lilacinum* combined with chemical nematicides could reduce the population of root-knot nematodes and root damage caused by root-knot nematodes.

Based on the result, it can be concluded that the five fungal isolates used in this study were *P. lillacunum*. All the fungal isolates showed good capability to be used as biological control agent RKN, which were resulted in more than 80% of egg mass infection.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Research and Higher Education for funding this study through Institutional National Strategy Grant Research Scheme based on Decree Number 062/SP2H/LT/DRPM/2018 and Contract Pledge No. 393/UN26.21/PN/2018. Gratitude is also expressed to the management of PT Great Giant Pineapple Lampung for their help and support in this research.

REFERENCES

Barnett HL. 1969. Illustrated Genera of Imperfect Fungsi. Second Edition. Burgers Publishing Company, USA.

Barnett HL, Hunter BB. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. Shop APS, USA.

Bonants PJM, Fitter PLR, Thijs H, den Belder E, Waalwjck C, Henfling JWD. 1995. A basic serine protease from *Paecilomyces lilacinus* with biological activity against *Meloidogyne hapla* eggs. Microbiology 141: 775-784.

Brand D, Soccol CR, Sabu A, Roussos S. 2009. Production of fungal biological control agents through solid state fermentation: A case study on *Paecilomyces lilacinus* against root-knot nematodes. Micol Appl Intl 22 (1): 31-48.

Dahlin P, Eder R, Consoli E, Krauss j, Kiewnick S. 2019. Integrated control of *Meloidogyne incognita* in tomatoes using fluopyram and *Purpureocillium lilacinum* strain 251. Crop Prot 124: 104874.

Dawabah AAM, Al-Yahya FA, Lafi HA. 2019. Integrated management of plant-parasitic nematodes on guava and fig trees under tropical field conditions. Egypt J Biol Pest Contr. DOI: 10.1186/s41938-019-0133-9

Esser RP, El-Gholl NE. 1993. *Paecilomyces lilacinus* a fungus parasitizes nematode eggs. Nematology Circular, Fla. Dept Agric and Consumer Serv. Division of Plant Industry, Miami, FL.

Ecoman Biotech Co., Ltd. 2014. Ecoman® Bio-Nematicide (*Paecilomyces lilacinus*). https: // www.en.ecomanbiotech.com/bio-nematicide. [March 2019]

Gafur A, Swibawa IG. 2004. Methods in Nematodes and Soil Microbe Research for Belowground Biodiversity Assessment. In: Susilo FX, Gafur A, Utomo M, Evizal R, Murwani S, Swibawa IG. Conservation and Sustainable Management of Below-Ground Biodiversity in Indonesia. Universitas Lampung, Lampung. [Indonesian]

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95-98.

Hernadez-Leal T, Lopez-Lima D, Carrion G. 2016. Effect of the application of nematophagous fungus *Purpureocillium lilacinum* over nutrients availability on agricultural soil and yield of *Avena sativa*. Revista de la Facultad de Ciencias Agrarias 48 (2): 1-12.

Hooper DJ, Hallmann J, Subbotin SA. 2005. Methods for Extraction, Processing and Detection of Plant and Soil Nematodes. In: Luc M, Sikora RA, Bridge J (eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd ed. CAB International Publisher, Wallingford, UK.

Hore J, Roy K, Maiti AK. 2018. Evaluation of Bio-Nematon (*Pupureocillium lilacinum* 1.15 WP) against root-knot nematode (*Meloidogyne incognita*) in tomato. J Entomol Zool Stud 6 (4): 1700-1704.

Jatala P. 1986. Biological control of plant parasitic nematodes. Ann Rev Phytopathol 24: 453-489.

Kepenekci I, Toktay H, Oksal E, Bozbuga R, Imren M. 2018. Effect of *Purpureocillium lilacinum* on root lesion nematode, *Pratylenchus thornei*. Tarim Bilimleri Dergisi J Agric Sci 24: 323-328

Khan A, Williams KL, Nevalainen HKM. 2004. Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol Control 31: 346-352.

Khan A, Williams KL, Nevalainen HKM. 2006. Control of plant parasitic nematodes by *Paecilomyces lilacinus* on *Monacrosporium lusipagum* in pot trials. BioControl 51: 643-658.

Khan Z, Ahmad S, Al-Gimlas F, Al-Mutairi S, Josep L, Chandy R, Sutton DA, Guarro J. 2012. *Purpureocillium lilacinum* as a Cause of Cavitary Pulmonary Disease: a New Clinical Presentation and

Observations on Atypical Morphologic Characteristics of the Isolate. J Clin Microbiol 50 (5):1800-1804.

Kumar S, Stecher G, Tamura K. 2016. MEGA:7 Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874

Kumar S, Rawat S. 2018. First report on the root-knot nematode *Meloidogyne enterolobii* (Yang and Eisenback, 1988) infecting guava (*Psidium guajava*) in Udham Singh Nagar of Uttarakhand, India. Int J Curr Microbiol App Sci 7 (4): 1720-1724.

Luangsa-ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA. 2011. *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiol Lett 321:141-149.

Mounfort DO, Rhodes LL. 1991. Anaerobic growth and fermentation characteristic *Paecilomyces lilacinus* isolate from mulet gut. Appl Environ Microbiol 57 (7): 1963-1968.

Paz BAO, Piedrahita OAG, Caycedo JL. 2015. In vitro effect of *Purpureocillium lilacinum* (Thom) Luangsa-Ard *et al*. and *Pochonia chlamydosporia* var. *catenulata* (Kamyschko ex Barron & Onions) Zare & Gams on the root-knot nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood and *Meloidogy nemayaguensis* Rammh & Hirschmann]. Bol Cient Mus Hist Nat 19 (2): 154-172.

Prabu S, Kumar S, Subramanian S. 2009. Mass production and commercial formulation of *Paecilomyces lilacinus*. Madras Agric J 95 (7-12): 415-417.

Razak AR, Lim TK. 1987. Occurrence of the root-knot nematode Meloidogyne incognita on guava in Malaysia. Pertanika 10 (3): 265-270.

Singh S, Panday RK, Goswami BK. 2013. Bio-control activity of *Purpureocillium lilacinum* strains in managing a root-knot disease of tomato caused by *Meloidogyne incognita*. Biocontrol Sci Technol 23 (12): 1469-1489.

Stanes T and Company Limited. 2017. Bio-Nematon. http://www.tstanes.com/products-bio-nematon.html. [June 2017].

Stirling GR. 2014. Biological Control of Plant-parasitic Nematodes (2nd Edn). CAB International Publisher, Wallingford, UK.

Sundararaju P, Cannayane I. 2002. Production of nematode egg parasitic fungus, *Paecilomyces lilacinus,* on banana wastes and certain plant leaves. Indian J Nematol 32 (2): 183-233.

Swibawa IG, Saputri ER, Yuliana E, Fitriana Y, Solikhin. 2017. Nematoda puru akar dan jamur parasitnya pada pertanaman jambu biji di Lampung. Seminar Nasional dan Kongres XIV Perhimpunan Fitopatologi Indonesia, 3-5 Oktober 2017, Kendari. [Indonesian]

Winarto, Darnetty, Liswarni Y. 2017. Potensi jamur *Paecilomyces* isolat lokal Sumatera Barat untuk pengendalian nematoda bengkak akar (*Meloidogyne* spp.) pada tanaman sayuran. Laporan Akhir Penelitian Unggulan Perguruan Tinggi. Universitas Andalas, Padang. [Indonesian]

Wulandari C, Budiono P, Ekayani M. 2019. Impacts of the new decentralization Law 23/2014 to the implementation of community based forest management in Lampung Province, Indonesia. IOP Conf Ser: Earth Environ Sci 285: 012006. DOI: 10.1088/1755-1315/285/1/012006

Zeck WM. 1971. A rating scheme for field evaluation of root-knot infestations. Pflanzenschutz Nachrichten Bayer AG 24: 141-144.

Morpho-molecular identification and pathogenicity test on fungal parasites of guava root-knot nematode eggs in Lampung, Indonesia

ORIGINALITY REPORT

6%

SIMILARITY INDEX

PRIMARY SOURCES

1	repository.lppm.unila.ac.id Internet	74 words — 2%
2	mafiadoc.com Internet	38 words — 1%
3	atrium.lib.uoguelph.ca Internet	21 words — < 1%
4	fr.scribd.com Internet	18 words — < 1%
5	www.mdpi.com Internet	11 words — < 1%
6	Z. B. Xie, J. G. Zhu, H. Y. Chu, Y. L. Zhang, Q. Zeng, H. L. Ma, Z. H. Cao. "EFFECT OF LANTHANUM ON RICE PRODUCTION, NUTRIENT UPTAKE, AND DISTRIBUTION", Journal of Plant Nutrition, 2006 Crossref	11 words — < 1%
7	Jennifer Luangsa-ard, Jos Houbraken, Tineke van Doorn, Seung-Beom Hong et al. "Purpureocillium, a new genus for the medically important Paecilomyces lilacinus", FEMS Microbiology Letters, 2011 Crossref	11 words — < 1%
8	wwwnc.cdc.gov Internet	11 words — < 1%

9	"Innovations as Key to the Green Revolution in Africa", Springer Science and Business Media LLC, 2011 Crossref	11 words — < 1%
10	digilib.unila.ac.id Internet	11 words — < 1%
11	link.springer.com Internet	10 words — < 1%
12	Fayez Raiesi, Mahmoud Ghollarata. "Interactions between phosphorus availability and an AM fungus (Glomus intraradices) and their effects on soil microbial respiration, biomass and enzyme activities in a calcareous soil", Pedobiologia, 2006 Crossref	10 words — < 1%
13	"Management of Phytonematodes: Recent Advances and Future Challenges", Springer Science and Business Media LLC, 2020 Crossref	10 words — < 1%
14	Liu, X.. "Photosynthetic acclimation to high temperatures associated with heat tolerance in creeping bentgrass", Journal of Plant Physiology, 200812 Crossref	10 words — < 1%
15	hdl.handle.net Internet	10 words — < 1%
16	Bioformulations for Sustainable Agriculture, 2016. Crossref	10 words — < 1%
17	P. J. M. Bonants. "A basic serine protease from Paecilomyces lilacinus with biological activity against Meloidogyne hapla eggs", Microbiology, 04/01/1995 Crossref	10 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		