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Biological control of plant parasitic nematodes on black pepper with endophytic bacteria and organic materials

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ABSTRACT

Plant parasitic nematodes have been reported to cause significant demage and losses to black pepper in Bangka, Indonesia. Control of plant parasitic nematodes with pesticides is often restricted due to their high toxicity and negative impact on the environment. Endophytic microorgnisms resident within plant tissues have attracted attention due to their interesting features related to plant growth and for the bioprotection of plants against plant pests and diseases. In this study, Endophytic bacteria were collected and isolated from different varieties of healthy pepper. The isolation of endophyte was conducted using surface-sterilized method. The objective of this research was to evaluate the effect of endophytic bacteria and organic materials on yellow diseases caused by plant parasitic nematodes *Meloidogyne sp.* on black pepper. The results showed that application of endophytic bacteria and organic materials were able to suppress the number root galls caused by *Meloidogyne sp* and the juveniles of nematodes in the soil and also to increase the plant growth of pepper seedlings in the greenhouse.

Key words: endophytic bacteria, *Meloidogyne incognita*, black pepper, root gall

Introduction

Black pepper (*piper nigrum* L) is one of the important export commodities in Indonesia. However, the production of black pepper is now threatened by pests and diseases. One of the main disease on black peper is yellow diseasae caused by plant parasitic nematodes i.e *Meloidogyne* sp. and *Radopholus similis*. Several control methods have been developed to combat the nematodes, but they are still a serious problem of black pepper especially in Bangka Island. Chemical pesticides is commonlyt used for controlling the disease. The use of chemical pesticides contibuosly may caused negative impacts namely pollution to the environment, pathogens become more resistant, disruptive presence of beneficial microbes in the soil, and harmful for human.

The need for environmentally safe control strategies has increased interest in developing biological control measures. Biological control involves the reduction of inoculum potential of a disease causing pathogens or prasite in its active or dormat state by one or more organisme

accomplished naturally or by manipulation of environment, host or antagonists or by mass introduction of one or more antagonists (Baker and Cook 1974). Endophyic bacteria are bacteria that live inside plant tissues without doing symptoms on these plants. As the internal plant habitat, endophytic bacteria provides several advantages as biological control agents: 1) colonization of an ecological niche also used by plant pathogens, 2) less competition with other microorganisms, 3) sufficient supply wirth nutrients, 4) less exposure to environmental stress factors, and 5) better translocation of bacterial metabolites throughout the host plant. More recently the use of endophytic bacteria for the protection of plant against diseases has been exploited.

Several studies have shown that endophytic bacteria isolated from various plant tissues are able to suppress plant parasitic nematodes *Meloidogyne incognita* on cotton and tomato plants (Hallmann *et al.* 2001; Munif *et al.* 2001) as well as to control plant nematode *Pratylenchus* sp on patchouli (Harni *et al.* 2007). The objective of this research was to evaluate the potential of biological agents bacterial endophytes isolated from black pepper for controlling *Meloidogyne sp.*onblackpepper.

Materials and Methods

Isolation of endophytic bacteria

a total of 10 Samples of roots of healthy black pepper were taken at random Bangka (Bangka Island), Bogor and Sukabumi (West Java). The roots were transported to the laboratory for immediate processing. The roots were washed under running tap water to remove adherent soil particles and then blotted dry on tissue paper. The root material was weighed and surface sterilized with alcohol 70% for 30 seconds and in 2% sodium hypochlorite (NaOCl) containing 0.01% Tween 20 for 3 min, followed by four rinses in sterile 0.01 M potassium phosphate buffer (PB) at pH 7.0 (80 g NaCl, 2 g KCl, 11.5 g Na_2HPO_4, 2 g KH_2PO_4). To confirm complete surface sterilization (sterility check), the surface disinfected roots were imprinted on tryptic soy agar (TSA). If bacterial growth occurred within 48 hours, samples were discarded. The pepper roots were then macerated with a sterile mortar and pistil in three times PB (w/v). The macerate was decanted into sterile conical flasks and shaken for 30 second. A dilution series was made and 100 µl of each dilution was plated onto 1/10 strength TSA on petri plate. The petri plates were incubated at 24°C for 2-3 days and colony forming units (cfu) were determined. Three replicates were made per dilution. On each petri plate containing approximately 10 bacterial strains was marked and all bacterial strains from this zone were transferred and purified on full strength TSA. The bacterial strains were stored in tryptic soy broth (TSB) plus 20% glycerol at -20°C. in this research, two isolates collection of endophytic bacteria were used in this experiment.

Inoculum of nematodes and organic material

Inoculum of Nematode *Meloidogyne* sp. used in this experiment was isolated from the infected roots pepper of nematodes in Central Bangka, Province Bangka-Belitung. Subsequently the nematodes were cultured and propagated on susceptible tomato plants (cv. Ratna) for 2 months. After 2 months the plant was uprooted and the nematodes was extracted and used as a source of inoculum. Organic material that used in this experiment were commercial compost and organic manure.

Effect of endophytic bacteria on *Meloidogyne incognita*

Test the effectiveness of biological agents against *M..incognita* on pepper seedlings in the greenhouse. Two months pepper cuttings one segment were treated with isolates of endophytic bacteria. Six isolates of selected bacterial endophytes isolated from root pepper isolate MER7, AA2, HEN1, HEN3, MER9, ANIC and two isolates bacterial endophyte TT2, EH11 (isolate collection) were used in this research. First of all, the bacterial isolates were grown on TSA medium 100% for 24-48 hours at room temperature. A single colony of bacteria was transferred into 100 ml of liquid TSB medium and shake for 2 days with a speed of 150 rpm at room temperature. Furthermore, the bacterial suspension was centrifuged at 11.000 rpm for 15 minutes with a temperature of-4°C to separate the supernatant/culture filtrate with a bacterial cell culture. Suspension of bacteria was made by diluted the bacterial cell with sterile water. Three months of pepper seedlings were dipped by soaking the roots for 1 hour in bacterial suspension with a population density 10^9-10^{10} CFU (colony forming unit). The treated pepper was planted subsequently planted in pots. One week after the bacterial treatment the plants were inoculated with 1,000 larvae of the nematode per plant. Each treatment was repeated 5 times and arranged in a completely randomized design, with control (+) the plant only nematode inoculation, while control (-) plants no inoculation with nematodes. Three months after inoculation the plants were harvested and the number of galls and the population of nematodes in the roots and the soil were observed as well as the plant height and weight, root weight, number of branches and number of leaves.

Results and Discussion

Effect of endophytic bacteria against *M. incognita* on pepper

A total of 30 isolates of endophytic bacteria isolated from root pepper were tested on seedling of pepper against *M. incognita* for screening. The screening was conducted in the greenhouse. Results of the screening showed that six isolates of bacterial endophytes were able to promote the growth of pepper plants and six isolates, namely MER7, AA2, HEN1, HEN3, MER9, ANIC were able to increase the growth of the plants and to reduce the number of galls caused by *M. incognita*. The selected isolates MER7, AA2, HEN1, HEN3, MER9, ANIC and two collection

endophytic bacterial isolates, TT2 and EH11 were tested to know the biocontrol activity against M. incognita on pepper and the effect on the plant growth.

The effectiveness of the bacterial isolates showed that five isolates EH11, HEN1, HEN3, TT2, and AA2 can significantly suppress *M. incognita* populations compared to controls, while the isolates MER9, ANIC and MER7 not significantly different from controls. The highest influence in suppress galls of nematodes was showed by isolate EH11. Five Isolates of endophytic bacteria HEN1, EH11, TT2 ANIC, and AA2, are able to reduce the population of *M.incognita* in the soil respectively for 99, 97, 96, 78 and 75% compared with controls (Table 1).

Table 1. Effect of multiple isolates of biological agents against the number of galls and the population of nematodes larvae.

Treatments	Number of galls	Population of the juveniles	Population of reduction (%)
Isolate MER 7	70,4 ab	1212 b	15,36
Isolate EH11	9,0 c	23 ef	96,97
Isolate ANIC	66,0 ab	312 cde	78,21
Isolate MER 9	57,4 ab	504 c	64,80
Isolate AA2	48,8 bc	352 cd	75,41
Isolate HEN1	42,2 bc	13 f	99,0
Isolate HEN3	38,4 bc	1176 b	17,87
Isolate TT2	32,6 bc	50 ef	96,50
Control + (with nematode	101 a	1432 ab	-
Control – (without nematode)	0	0	0

Figures followed by same small letters on the same column are not significantly different at 5% Duncan test.

Biological agents can suppress the development of plant diseases through a mechanism of competition, predation and the resulting antibiotics (Kloepper *et al.* 1991, Hallmann *et al.*1997). Some research indicates that the use of biological agents endophytic bacteria through seed treatment can reduce 30-50% of the amount of gall of *M.incognita* on cotton plants (Hallmann et al. 1997). Some Bacterial endophytes isolated from the roots patchouli can suppress populations of *Pratylenchus brachyurus* 73.9% in patchouli plants in the greenhouse (Harni *et al.* 2007). Biological agents including endophytic microbial can protect plants against plant-parasitic nematodes through various ways such as by producing toxic compounds that are nematisidal activities (Sikora *et al.* 2007). In addition certain bacteria can also suppress the development of plant diseases due to the ability of bacterial endophytes in binding Fe (III), produce compounds HCN and antibiotics (Sikora et al. 2007). A treatment of culture filtrate of endophytic bacterial isolate TT2 isolated from

patchouli was able to reduce *Pratylenchus barchyurus* larvae up to 100% 24 hours after treatment (Harni et al. 2007). The results of this showed that biological agents of endophytic bacteria increased the growth of pepper plants indicated by increasing the number of branches and leaves (Table 2)

Table 2. The influence of endophytic bacteria on the growth of pepper plants inoculated with *M. incognita* 3 months after treatment.

Treatments	Plant height addition (cm)	Amounts of branches addition	Amounts of leaves addition
Isolat MER7	24,2 a	4,6 a	12,6 ab
Isolat EH11	21,6 ab	2 bcd	9,4 abc
Isolat MER9	20,4 ab	2 bcd	10 abc
Isolat TT2	20,4 ab	1,6 bcd	7,8 bc
Isolat AA2	18,8 ab	2 bcd	7,8 bc
Isolat HEN1	17,6 ab	2,8 abc	9,2 abc
Isolat HEN3	16,4 ab	2,2 bcd	9,2 abc
Isolat ANIC	14,76 b	1,2 cd	5,2 c
Control – (without nematode)	25 a	1 cd	9,8 abc
Control + (with nematode)	16 b	0,4 d	6,4 c

Figures followed by same small letters on the same column are not significantly different at 5% Duncan test.

In plants inoculated only with nematodes (K +) were significantly reduced growth (the addition of plant height, number of branches and number of leaves). The low plant height, number of branches and number of leaves of plants inoculated with nematodes, is caused by damage from the stabbing stylet and secretion of enzymes released nematodes when the nematodes feed. Sikora *et al.*(1997) reported that nematodes are taking root cells can reduce the ability of plants to absorb water and nutrients from the soil and cause symptoms such as lack of water and nutrients. Besides, the reduced concentration of plant growth regulators such as auxins, cytokinins and gibberellins that there are many root tip. Reduced plant growth regulators can occur because the nematode secretes enzymes cellulase and pektinase that can degrade the cell up to the root tip injuries and broken, this leads to auxin is not active. Inactive auxin then growth will be stunted.

Conclusion

Endophytic bacteria isolated from the roots of the pepper were able to increase the growth of pepper seedling by increasing the number of shoots, root length and number of branch roots and reduced the gall caused nematodes *Meloidogyne* spp. The highest influence on the

isolates tested in reducing the number of gall of *Meloidogyne incognita* and the population of the larvae up to 90% showed by aplication with isolate EH11.

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