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The Potential of Endophytic Bacteria for the Biological Control of Plant Parasitic Nematodes on Black Pepper

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Abstract

Plant parasitic nematodes cause damage and losses significantly to black pepper in Indonesia. Control of plant parasitic nematodes with pesticides is often restricted due to their high toxicity and negative impact on the environment. The need for environmentally safe control strategies has increased interest in developing biological control measures.Endophytic bacteria are ubiquitous in most plant species and reside within healthy plant tissue without producing symptoms of damage. Bacterial endophytes provide several advantages as biological control agents, namely the colonization of an ecological niche also used by plant pathogens, less competition with other microorganisms, sufficient supply with nutrients, less exposure to environmental stress factors, and better translocation of bacterial metabolites throughout the host plant. The objective of this work was to evaluate the effect of endophytic bacteria isolated from black pepperand other plants to control *Meloidogyne incognita* on black pepper.Three months of pepper seedlings were used in this research. Suspension of 5 isolates of selected endophytic bacteria were applied by soil drensing at pepper seedlings and 2 weeks later 2000 juveniles of *M. incognita* were inoculated. The plants were harvested 4 months after nematodes application. The resultsshowed that some endophytic bacteria are able reduce the root gall caused by *M. incognita*and juveniles in the soil up to 70%and also to promote the growth of pepper seedlings.

Keywords: endophytic bacteria, *Meloidogyneincognita*, black pepper, root gall

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ABSTRACT

Plant parasitic nematodes cause damage and losses significantly to black pepper in Indonesia. Control of plant parasitic nematodes with pesticides is often restricted due to their high toxicity and negative impact on the environment. The need for environmentally safe control strategies has increased interest in developing biological control measures. Endophytic bacteria are ubiquitous in most plant species and reside within healthy plant tissue without producing symptoms of damage. The internal plant habitat provides several advantages for endophytic bacteria 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. The objective of this work was to evaluate the effect of endophytic bacteria isolated from black pepper to control *Meloidogyne incognita* on black pepper. The results of the research showed that some endophytic bacteria are able to reduce the root galls caused by *Meloidogyne incognita* and juveniles of nematodes in the soil up to 70% and also promote the growth of pepper seedlings.

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. The use of chemical pesticides with persistent pesticides can result in negative impacts on the environment, pathogens become more resistant, disruptive presence of beneficial microbes in the soil, and human health. Use of Pesticides also provide residual effects on pepper which is currently an one obstacle for consumers, mainly for export purposes that are very concerned about the health and environmental aspects. In connection with the need to develop a system of agricultural production, including plant disease control systems are environmentally sound one of them by optimizing the use of biological agents.

Endopyitic bacteria are bacteria that live inside plant tissues without doing symptoms on these plants. As the internal plant habitat, endophytic bacteria provides several advantages for endophytic bacteria 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. 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 200) as well as to control plant nematode *Pratylenchus* sp on patchouli (Harni 2010). In addition, some bacterial isolates endofite have been reported to enhance plant growth because it can increase the availability of nutrients to induce plant resistance. The objective of this research was to determine the potential of biological agents bacterial endophytes isolated from pepper for controlling *Meloidogyne incognita* on pepper and their effects on the plant growth.

Materials and Methods

Isolation of endophytic bacteria

A total of 10 samples of healthy pepper plants taken at random each of pepper plants cultivated in Bangka, Bogor and Sukabumi. The peppel plant 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 disk. 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 parasitic nematodes

Inoculum of Nematode *Meloidogyne incognita* 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..

Effect of endophytic bacteria on *Meloidogyne incognita*

The effectiveness of biological agents against *M. incognita* on pepper seedlings was done in the greenhouse. Two months pepper cuttings one segment were treated with isolates of endophytic bacteria. Eight isolates of selected bacterial endophytes isolated from root pepper isolate MER7, AA2, HEN1, HEN3, MER9, ANIC TT2 and EH11 were used in this study. The bacterial isolates were grown on TSA medium 100% for 24-48 hours at room temperature. A single colony of the bacterial isolate 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 in pots. One week after the bacterial treatment, the plants were inoculated with 1000 larvae of the nematode *M. incognita* 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

In this present study, eight isolates of endophytic bacteria isolated from root pepper and patchouli were tested on seedling of black pepper against *M. incognita* in the greenhouse. The isolates of bacteria were isolate MER7, AA2, HEN1, HEN3, MER9, ANIC, TT2 and EH11. All of isolates of bacterial endophyte were able to reduce gall and juveniles of M. incognita. Out of eight isolates, four isolates EH11, HEN1, HEN3 and TT2 significantly reduced gall nematodes compared to the control. Isolate EH11 showed the highest in reducing gall nematodes compared to other isolates (Table1). The isolates of bacteria were also able to suppress juveniles of nematode in the soil compared to control. Six isolates EH11, AA2, HEN1, MER9, ANIC and TT2 significantly reduced number of juveniles compared to control, while The highest influence in suppressing number of juveniles of *M.incognita* was showed by isolate HEN1 and followed by EH11. Five Isolates of endophytic bacteria HEN1, EH11, TT2, ANIC, and AA2 are able to reduce the population of *M.incognita* in the soil for 99, 97, 96, 78 and 75% respectively.

Table 1. Effect of isolates of biological agents bacterial endophyte 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	-

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

Biological agents can suppress the development of plant diseases through a mechanism of competition, predation and the resulting antibiotics (Kloepper *et* al. 1991). 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% on patchouli plants in the greenhouse (Harni *et al.* 2007).

Biological agents,including endophyticmicrobial can protect plants against plant-parasitic nematodes through various ways such as by producing toxic compounds that are nematisidal activities (Sikora *et al*. 2007; Yang et al. 2011). In addition certain bacteria can also suppress the development of plant diseases due to the ability of bacterial endophytes in binding Fe (III) and to produce compounds HCN (Keel *et al*. 1992). A treatment of culture filtrate of endophytic bacterial isolate isolated from patchouli was able to reduce *Pratylenchus barchyurus* larvae up to 100% 24 hours after treatment (Harni 2007).

Application of several bacterial endophytes can increase plant growth of black pepper. Out of 8 isolates tested, plant hight of pepper, number of branches and number of leaves was significantly increased only by isolate MER7 (Table 2). Isolate EH11, MER9, TT2 were able to increase plant height compared to control, while isolate HEN1 increased the number of brances compared to control. Vetrivelkalai et al. (2010) reported that bacterial endophytes can suppress the gall number of nematodes on bhendi, but not all of bacteria increased the plant growth.

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)	Numbers of branches increment	Numbers of leaves increment
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 + (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.

Inoculation with nematodes caused damage from the stabbing stylet and secretion of enzymes released nematodes when the nematodes feed. Hallmann 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 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 then growth of plant will be stunted. Unique endophytes could be used directly to treat seeds or transplants limiting substantially the side-effects of abiotic and biotic factors on the biological agent by almost immediately protecting them within plant tissue. In the endosphere, mutualistic endophytes are in protected environments that give them a competitive advantage over organisms of the rhizosphere and phyllosphere—consistent nutrient flow, pH, moisture, as well as protection from high numbers and densities of competitors (Backman and Sikora 2007).

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