EFFICACY OF ENDOPHYTIC BACTERIA IN REDUCING PLANT PARASITIC NEMATODE *Pratylenchus brachyurus*

Efikasi Bakteri Endofit dalam Mengurangi Nematoda Parasit Tanaman Pratylenchus brachyurus

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ABSTRACT

Pratylenchus brachyurus is a major parasitic nematode on patchouli that reduces plant production up to 85%. The use of endophytic bacteria is promising for controlling nematode and promoting plant growth through production of phytohormones and enhancing the availability of soil nutrients. The objective of the study was to evaluate the efficacy of endophytic bacteria to control *P. brachyurus* on patchouli plant and its influence on plant productions (plant fresh weight and patchouli oil). The study was conducted at Cimanggu Experimental Garden and Laboratory of the Indonesian Spice and Medicinal Crops Research Institute (ISMECRI), Bogor, West Java. The experiment was designed in a randomized block with seven treatments and eight replications; each replication consisted of 10 plants. The treatments evaluated were five isolates of endophytic bacteria (*Achromobacter xylosoxidans* TT2, *Alcaligenes faecalis* NJ16, *Pseudomonas putida* EH11, *Bacillus cereus* MSK and *Bacillus subtilis* NJ57), synthetic nematicide as a reference, and non-treated plant as a control. Four-week old patchouli plants of cv. Sidikalang were treated by soaking the roots in suspension of endophytic bacteria (10^9 cfu ml^{-1}) for one hour before transplanting to the field. At one month after planting, the plants were drenched with the bacterial suspension as much as 100 ml per plant. The results showed that applications of the endophytic bacteria could suppress the nematode populations (52.8-80%) and increased plant weight (23.62-57.48%) compared to the control. The isolate of endophytic bacterium *Achromobacter xylosoxidans* TT2 was the best and comparable with carbofuran.

[***Keywords*:** Patchouli, endophytic bacteria, nematode, *Pratylenchus brachyurus*]

ABSTRAK

Pratylenchus brachyurus *merupakan nematoda parasit utama pada tanaman nilam yang dapat menurunkan produksi hingga 85%. Pengunaan bakteri endofit menjanjikan untuk pengendalian nematoda dan meningkatkan pertumbuhan tanaman karena dapat meningkatkan produksi fitohormon dan ketersediaan hara tanah. Penelitian bertujuan menganalisis keefektifan beberapa isolat bakteri endofit untuk mengendalikan* P. brachyurus *pada tanaman nilam dan pengaruhnya terhadap produksi (bobot segar tanaman, dan minyak nilam). Penelitian dilaksanakan di Laboratorium dan Kebun Percobaan Cimanggu Balai Penelitian Tanaman Rempah dan Obat, Bogor, Jawa Barat. Penelitian menggunakan rancangan acak kelompok dengan tujuh perlakuan dan delapan ulangan, masing-masing perlakuan terdiri atas 10 tanaman. Perlakuan yang diuji yaitu lima isolat bakteri endofit (*Achromobacter xylosoxidans *TT2,* Alcaligenes faecalis *NJ16,* Pseudomonas putida *EH11,* Bacillus cereus *MSK, dan* Bacillus subtilis *NJ57), nematisida sintetis karbofuran (sebagai pembanding), dan kontrol (tanpa perlakuan). Bibit nilam varietas Sidikalang berumur empat minggu diperlakukan dengan suspensi bakteri endofit 109 cfu ml^{-1} (OD600=1) dengan cara merendam akar dalam suspensi bakteri selama satu jam sebelum bibit ditanam di lapangan. Satu bulan setelah tanam, tanaman diperlakukan dengan cara menyiramkan 100 ml suspensi bakteri. Hasil penelitian menunjukkan bahwa pemberian bakteri endofit dapat menekan populasi nematoda 52,8-80% dan meningkatkan berat terna nilam sebesar 23,62-57,48% dibandingkan dengan kontrol. Bakteri endofit terbaik adalah* Achromobacter xylosoxidans *TT2 yang pengaruhnya sama dengan karbofuran (nematisida sintetis).*

[***Kata kunci*:** Nilam, bakteri endofit, nematoda, *Pratylenchus brachyurus*]

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth) is an essential plant producing patchouli oil. The plant is cultivated by smallholders in several provinces in Indonesia using a simple technology, therefore its productivity is low, only 199.16 kg of oil per ha (Manggabarani 2008). The low productivity is also caused by infection of plant parasitic nematode (*Pratylenchus brachyurus*) (Harni and Mustika 2000). The damage caused by the nematode reduced plant production up to 85% (Mustika *et al*. 1995). Disease symptoms of patchouli plant attacked by *P. brachyurus* are stunted growth, red or yellowish color of the leaves, necrotic wounds of the hairy roots and root rot (Mustika *et al*. 1995). Patchouli plant infected with the nematode also becomes susceptible to other soil borne pathogens such as fungi and bacteria.

Attempts to control *P. brachyurus* on patchouli plant have been conducted by using resistant varieties (Harni *et al*. 2001), organic matters, biological agents such as trappying fungi (*Dactylaria* sp, *Dactylella*, sp. and *Arthrobotrys* sp.) and parasitic bacteria (*Pasteria penetrance*) (Mustika *et al.* 2000), as well as endophytic bacteria (Harni *et al.* 2007a). The use of endophytic bacteria was effective in *in vitro* and greenhouse experiments (Harni *et al.* 2007a; Supramana *et al.* 2007; Harni *et al.* 2010, 2011, 2012a). In addition, the use of filtrate culture of endophytic bacteria significantly reduced *P. brachyurus* populations (>80%) in *in vitro* experiment (Harni *et al*. 2010), whereas in the greenhouse experiment it reduced nematode populations by 75-81% and increased plant growth up to 50-64% (Harni *et al.* 2011). Our study has explored the efficacy of five isolates of endophytic bacteria *(Achromobacter xylosoxidans* TT2, *Alcaligenes faecalis* NJ16, *Pseudomonas putida* EH11, *Bacillus cereus* MSK and *Bacillus subtilis* NJ57) that have khitinase and protease activities (Harni *et al.* 2012a), and further developed an effective endophytic bacterium for suppressing the population of *P. brachyurus*. Endophytic bacteria also increased phenol and salicylic acid content which associated with plant resistance (Harni *et al.* 2012b).

Endophytic bacteria as a biocontrol agent for nematodes affect penetration, reproduction and population of nematodes in the roots (Sikora *et al.* 2007). Hasky-Gunther (1998) and Schafer (2007) reported that endophytic bacteria *Rhizobium etli* G12 and *Bacillus sphaericus* B43 reduced the penetration of *Meloidogyne incognita* on tomato plants by 78% and 60%, respectively, because the bacteria could induce plant systemic resistance. These bacteria also suppressed reproduction rate of *M. incognita* by reducing the number of eggs per female by 36% and 25%, respectively, compared to control. Chaves *et al.* (2009) and Harni *et al.* (2011) reported that endophytic bacteria reduced penetration rate of *P. brachyurus* and *Radhopulus similis* into the roots by 54.8% and 29%, respectively.

The study aimed to analyze the effectiveness of several isolates of endophytic bacteria to control *P. brachyurus* and its influence on production of plant fresh weight, patchouli oil and patchouli alcohol.

MATERIALS AND METHODS

Study Site and Biocontrol Agent

The experiments were conducted at Cimanggu Experimental Garden and Laboratory of the Indonesian Spices and Medicinal Crops Research Institute (ISMECRI) in Bogor, West Java. The endophytic bacteria isolates used were *Achromobacter xylosoxidans* TT2, *Alcaligenes faecalis* NJ16, *Pseudomonas putida* EH11, *Bacillus cereus* MSK and *Bacillus subtilis* NJ57 (Harni *et al.* 2011). The isolates were propagated on Tryptic Soy Agar medium (TSA) for 48 hours at room temperature. Bacterial cultures were then suspended in sterile water and their concentrations were adjusted spectrophotometrically at 600 nm wave length at the bacterial cell density of 10^9 cfu ml^{-1}.

Plant Propagation

Plant propagation was conducted in the green house of the ISMECRI. One-node cuttings of patchouli plant cv. Sidikalang were cultivated in polybags containing a mixture of soil and manure (2:1) under humid condition of a plastic cover for two weeks. The plants were watered and applied with fertilizer according to the standard protocol. After 4 weeks, the plants were used for field experiment.

Field Experiment

Field experiment was conducted at Cimanggu Experimental Garden (200-250 m above sea level) of the ISMECRI, where the soil contains high populations of *P. brachyurus* following the analyses of the nematode in the soil sample (12 *P. brachyurus* larvae per g soil). Soil preparation was done two weeks before planting by ploughing and bedding at 1 m wide, 7 m long and 30 cm high. Subsequently, planting holes were made at the distance of 75 cm x 50 cm. Organic fertilizer was added to the planting holes at a dosage of 2 kg per hole, one week before planting.

Roots of one month-old patchouli cuttings were soaked in the endophytic bacterial suspension (10^9cfu ml^{-1}) for one hour (Harni *et al.* 2007b) before planting in the field. The treatment was repeated every month for four consecutive months by drenching the bacterial suspension as much as much as 100 ml per plant. Patchouli plants were managed according to the standard protocol such as watering when soil condition was too dry, hand weeding every month, and applying inorganic fertilizers (7 g urea, 10 g SP 36 and 15 g per plant) twice every three months.

The experiment was designed in a randomized block, eight replications, and seven treatments; each treatment consisted of 10 plants. The treatments were five isolates of endophytic bacteria *(A. xylosoxidans* TT2, *A. faecalis* NJ16, *P. putida* EH11, *B. cereus* MSK and *B. subtilis* NJ57), synthetic nematicide carbofuran (for comparison), and control (without treatment).

Data Collection and Analyses

Plant height, number of branches and stem diameter were recorded every month. The patchouli plants were cut at 6 months after the treatment at 15 cm above the soil surface and then the fresh weight of the plants was calculated. Patchouli oil was obtained by steam distillation of the sun dried plants and patchouli alcohol content was analyzed with gas chromatography in the postharvest laboratory of the ISMECRI.

Data were analyzed to examine the effect of treatments on the observed variables. Analysis of variance was performed when there was a significant difference followed with the Duncan New Multiple Range Test at the 5% level.

RESULTS AND DISCUSSION

Effect of Endophytic Bacteria on Plant Growth

Patchouli plants treated with endophytic bacteria isolates significantly increased their growth parameters such as plant height, number of branches and stem diameter compared with the control (Fig. 1). The highest effect was observed on the plants treated with endophytic bacterium *A. xylosoxidans* TT2, followed with those treated with *A. faecalis* NJ16 and *P. putida* EH11. The increase in plant growth on the treated plants was associated with the decrease in *P. brachyurus* infection in the roots. Roots infected with *P. brachyurus* reduced their ability to absorb water and nutrients for the plant (Agrios 2005). *P. brachyurus* is a migratory endoparasitic nematode which causes cell death during its activities in the infected roots. In addition, isolates of endophytic bacteria used in this study produce plant growth hormones such as cytokinin and indole acetic acid that increase plant growth (Harni *et al.* 2012b). Asghar *et al.* (2002) reported that endophytic bacteria produced IAA that increases plant height (56.5%),

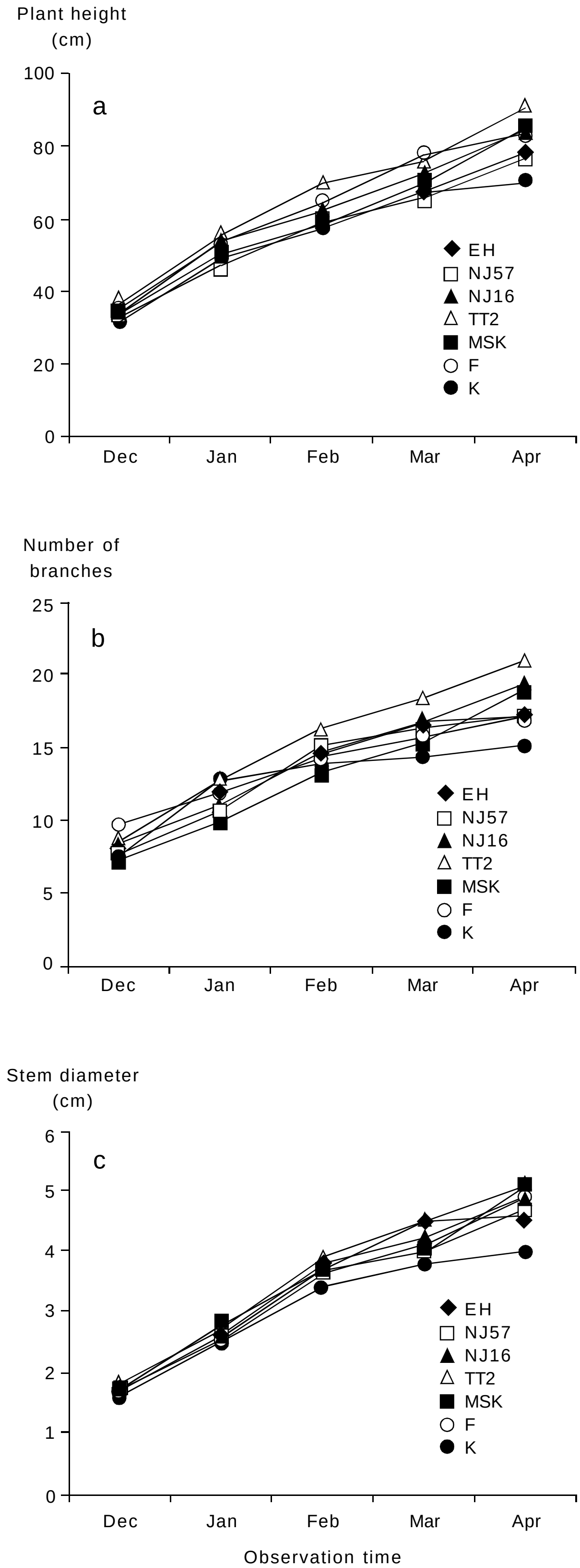

Fig. 1. Effect of five isolates of endophytic bacteria on the growth of patchouli plant; a = plant height, b = number of branches and c = stem diameter. TT = *Achromobacter xylosoxidans* TT2, MSK = *Bacillus cereus* MSK, NJ16 = *Alcaligenes faecalis* NJ16, NJ57 = *Bacillus subtilis* NJ57, EH = *Pseudomonas putida* EH11, K = control, F = carbofuran.

stem diameter (11.0%) and number of branches (35.7%) of *Brassica juncea*. In addition, Thakuria *et al.* (2004) found that rice treated with rhizobacteria increased its nutrients such as nitrogen, phosphate and other minerals, as well as produced growth hormones such as ethylene, auxin and cytokinin.

Populations of *P. brachyurus* on Roots

Application of endophytic bacteria isolates *A. xylosoxidans* TT2, *A. faecalis* NJ16 and *P. putida* significantly suppressed the penetration and reproduction rate of *P. brachyurus* in the roots of patchouli plant better than corbofuran treatment during 2-6 months following the application (Fig. 2). At two months after planting, endophytic bacteria treatment significantly reduced nematode penetration into the roots. The lowest penetration of *P. putida* was shown on the treatment of *P. putida* EH11 where the nematode population was only 2 larvae per g of roots compared with 24 larvae per g of roots in the control treatment. In addition to suppressing the penetration of the nematode, the treatment also reduced the development of the nematode in the roots. The number of the nematodes in the roots of patchouli plant treated with endophytic bacteria *A. xylosoxidans* TT2, *P. putida* EH11 and *B. subtilis* NJ57 were 7-9 larvae per g of roots at 4 months after treatments. The number was lower than that of the control (78 larvae per g of roots) and carbofuran treatment (22 larvae per g of roots). The number of nematodes on plants treated with *A. faecalis* NJ16 was similar to that treated with carbofuran, whereas that treated with *B. cereus* MSK was 49 larvae per g of roots.

Similar results were observed at 6 months after treatments. Nematode populations on patchouli plants treated with *A. faecalis* NJ16 and *A. xylosoxidans* TT2 were the lowest, i.e. 39 and 44 larvae per g of roots; much lower than those treated with carbofuran (106 larvae per g of roots) and the control (250 larvae per g of roots).

At the end of the observation, the highest fresh plant weight on *A. xylosoxidans* TT2 treatment was not significantly different from those of *A. faecalis* NJ16, *P. putida* EH11 and carbofuran treatments, but differed from those of *B. subtilis* NJ57, *B. cereus* MSK and control treatment (Table 1). This occurs because endophytic bacteria can protect the roots from nematode infection so it reduces root damage and improves water and nutrient supply to the plant so that the plants grow well and produce high fresh plant weight. Nematode population on *P. putida* EH11 treatment was high enough at the end of the observation, but did not affect the fresh plant production. This was because endophytic bacteria treatment was given only to plants for four months, consequently the well-developed nematode population was high enough. But because high attack occurs when the plant is a bit old, it does not significantly affect fresh plant production. From this data we can conclude that *P. putida* EH11 needs to be applied every month.

Effect of *B. cereus* MSK was the lowest on nematode penetration as well as nematode population in the

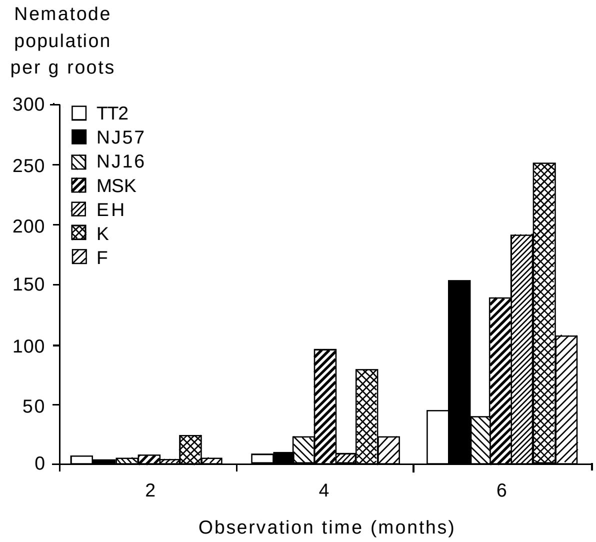

Fig. 2. Effect of endophytic bacteria on *Pratylenchus brachyurus* populations on patchouli plant. TT = *Achromobacter xylosoxidans* TT2, MSK = *Bacillus cereus* MSK, NJ16 = *Alcaligenes faecalis* NJ16, NJ57 = *Bacillus subtilis* NJ57, EH = *Pseudomonas putida* EH11, K = control, F = carbofuran.

Table 1. Effect of endophytic bacterial isolates on the fresh plant weight, patchouli oil, patchouli alcohol and total patchouli oil.

Isolates of endophytic bacteria	Fresh plant weight (kg)	Patchouli oil (%)	Patchouli alcohol (%)	Total patchouli oil (ml)
A. xylosoxidans TT	4.00 ± 1.40a	3.03a	33.43a	84a
B. cereus MSK	2.26 ± 0.84b	3.25a	34.35a	59b
A. faecalis NJ16	3.56 ± 0.36ab	3,10a	34.84a	81a
B. subtilis NJ57	2.60 ± 0.29b	2.62a	35.67a	66b
P. putida EH11	2.90 ± 0.84ab	2.55a	34.03a	73ab
Carbofuran	2.94 ± 0.37ab	3.03a	34.12a	74ab
Control	2.54 ± 0.51b	2.81a	33.15a	66b

Numbers followed by the same letters in each column are not significantly different at 0.05 level DMRT.

roots compared with other isolates either at 4-month old and 6-month old plants, as well as plant fresh weight. This occurs because *B. cereus* MSK increases IAA content in plants (Harni *et al.* 2012b) so it does not suppress nematode populations.

Effect of Endophytic Bacteria on Patchouli Oil and Patchouli Alcohol Contents

High suppression of the nematode on patchouli plants treated with *A. xylosoxidans* TT2 and *A. faecalis* NJ16 resulted in higher fresh plant weight (Table 1), implying that endophytic bacteria were effective for improving patchouli plant health and production. The endophytic bacteria treatments did not significantly affect patchouli oil and patchouli alcohol contents. In the total of patchouli oil production, however, plant treated with *A. xylosoxidans* TT produced the highest oil (84 ml per plant) than that treated with carbofuran and the control.

Plants treated with endophytic bacteria *A. xylosoxidans* TT2 produced the highest fresh plant weight and patchouli oil, namely 4 kg and 85 ml per plant, but it was not significantly different from those treated with *A. faecalis* NJ16 and *P. putida* EH11 that was 3.56 kg and 81 ml and 2.9 kg and 73 ml, respectively. These yields together with carbofuran (synthetic nematicide) treatment were 2.94 kg and 74 ml, respectively. Results of analysis on patchouli oil and patchouli alcohol content showed that endophytic bacteria treatment did not significantly affect both parameters (Table 1). Patchouli oil contents ranged from 2.55% to 3.25% and patchouli alcohol was from 33.43% to 35.67%. Although patchouli oil and patchouli alcohol levels were not significantly different, the levels were higher than those reported by Nuryani *et al.* (2005) which were 2.89% and 32.95%, respectively.

This study indicates that endophytic bacteria are potential for controlling root nematode *P. brachyurus* on patchouli plant. Further study is required to mass propagate the endophytic bacteria for large scale field experiment.

CONCLUSION

Endophytic bacteria can suppress population of root nematode (*P. brachyurus*) on patchouli plant, as well as increase plant growth and patchouli oil yield. The most effective endophytic bacteria were *A. xylosoxidans* TT2, *A. faecalis* NJ16 and *P. putida* EH11. The endophytic bacteria did not affect patchouli oil and patchouli alcohol contents. Therefore, the use of endopyytic bacteria for larger field experiment is justified.

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