The effect of wheat bran soil inoculant of *Monacrosporium eudermatum* isolated from North Sumatera in the reduction of gall development by *Meloidogyne incognita* in *Nicotiana x sanderae*

L D S Hastuti¹ and J Faull²

¹ Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jalan Bioteknologi No. 1 Kampus USU, Medan 20155, Indonesia
² School of Biology, Birkbeck, University of London, Malet Street Bloomsbury, WC1E 7HX, London, UK.

*Email: liana.hastuti@usu.ac.id*

Abstract. A wheat-bran soil inoculant containing 10⁹/ml conidia of *Monacrosporium eudermatum* isolated from North Sumatera, Indonesia was used as inoculant. The potential of this fungi to control nematodes was compared with that achieved by using the chemical control agent carbofuran at 200µg/ml. A susceptible plant, ornamental Tobacco (*Nicotiana sanderae*) was inoculated with 1000 second juvenile of *M. incognita* per pot. Analysis of variance by Anova was used to find the significant differences between treatments. Based on mean value of plant height, stem fresh weight, stem dry weight, root depth and root fresh weight measurements, almost all treatments improved and enhanced the ornamental tobacco growth. Carbofuran and *M. eudermatum* was efficient in reducing the number of vermiform nematode and swollen roots after 7 days; mature, immature female and sausage shaped roots after 15 days; and immature and mature females, sausage shaped and galls after 30 days of infection by *M. incognita*. Treatment with *M. eudermatum* was seen to be comparable with carbofuran in reducing infection of *M. incognita*. Based on this study there was no evidence of resistance to *Meloidogyne incognita* in ornamental tobacco plants *N. sanderae*.

1. Introduction

The genus *Nicotiana* and their relatives occupy the second position in terms of economic plants infected mainly by Root-Knot Nematode *Meloidogyne* sp. [1]. In Indonesia *M. incognita* and *M. javanica* are always pests of economic importance in *Nicotiana* sp. culture. The creation of fungal inoculum by using water as a suspension medium to create a conidial suspension for either injection or spray in laboratory or field conditions is a common method used by researchers and growers [2]. It must be considered, that if a biopesticide such as Nematode-Trapping Fungi (NTF) inoculum is going to be used in large quantities for large-scale farming, mass production methods for industrial purposes. There are two current methods used for large scale biocontrol agent production, submerged fermentation (SmF) and solid-state fermentation (SSF) [3].

According to Brand *et al.*, [4], NTF as biocontrol applied with SSF have an advantage over those produced by submerged fermentation: aerial fungal spores are more tolerant to UV radiation; have a higher spore stability; a higher spore resistance to drying; and higher spore germination rates for
longer storage periods. Recently, wheat bran inoculum of fungi as one of SSF substrates has been introduced and increased in use for agricultural research with evidence of beneficial effects over other substrates [5]. It is envisaged that the effect could be because wheat bran contains organic ingredient that support available nutrition such as carbohydrate, protein and fat. However, there are still few researches being done in Indonesia by using this method.

In this research, production of conidia of Indonesian NTF on a pot trial bioassay using SSF was used to test the effectiveness of Indonesian NTF M. eudermatum using wheat bran soil inoculum on development of Meloidogyne incognita and production of galls on ornamental tobacco Nicotiana x sanderae. The technique, such as composition, sterilization and storage condition were noted as reference for future to evaluate if the same methods could be implemented in Indonesia.

2. Materials and Methods

2.1. Fungal Culturing and Inoculant

The strain of Nematode-trapping fungi (M. eudermatum) has been selected for further testing on the basis of proceeding experimental results. Wheat bran soil medium was used to make a dry inoculum. Strain of M. eudermatum was cultured on Potato Dextrose Agar at 25°C for 14 days. Five agar disks (5 mm in diameter) of M. eudermatum was added to 30 ml of wheat bran medium (1 unit wheat bran Bergin Fruit and Nut Company): to 4 units compost in 50-ml Erlenmeyer flasks, and the cultures were then incubated for 14 days in darkness at 25°C [6]. Numbers of conidia were counted by a dilution series method where the 1 gram of wheat bran/compost taken from flask contain of conidia was added in to 9 ml of distilled water and number of conidia was count from the 10-4 dilution. A supernatant was obtained by pouring the suspension through sterile gauze into a sterile 15 ml test tube. The conidial concentration was measured by the dilution method by counting the number of conidia in a 5 µL-drop 20 times under the microscope (100X) and multiplying the number counted by 100 to estimate the total number of conidia/ml. Each experimental pot containing 100g of sterile soil received 1g of wheat bran M. eudermatum inoculum. Inoculated pot soil was incubated in the growth cabinet (Bud Box Cabinet) 25°C for 3 days before receiving ornamental tobacco plants.

2.2. Pesticide Preparation

Carbofuran® was used as a chemical control of nematodes as it is widely used in agriculture. The pesticide was diluted to a working concentration by dissolving 2 mg/10ml of solid Carbofuran® in methanol to 200µg/ml or 2 mg/10ml liquid/suspension [7, 8]. Carbofuran® solution (10 ml) was poured on each pot, and controls received 10 ml of distilled water. The Carbofuran was poured into the pot (3 pots) together with fungal treatments 3 days before receiving a plug ornamental tobacco plant.

2.3. Plant Growth Media Preparation

In this experiment the media used for plant growth was a mixture consisting of 20 % of sea sand and 80% of soil based compost. The mixed soil was air dried for 4 days at room temperature and sieved with a 2 mm sieve. After drying and sieving, the growth media (100 gr per pot) was placed in a plastic autoclave bag and sterilized by autoclaving with a dry sterilization program of 121°C, for 15 minutes, repeated 3 times. Under sterile conditions, 100g of sterile growth media was transferred into a 9 cm pot, with three replicate pots per treatment. The soil mixture in pots was treated with either Carbofuran® or the inoculum of NTF at 1g per pot, and distilled water (10ml) was used as a negative control. In order to avoid contamination all treated pots were covered with Parafilm® on the top and bottom of each pot. All treated pots were kept in the 25°C room for three days before receiving the Nicotiana sanderae plug plant.
2.4. J2 Suspension of Meloidogyne incognita Preparation

Infected roots with galls were washed gently and cut into 1 cm section. Roots were vigorously shaken for 4 minutes in sodium hypochlorite solution (100 ml NaOCl 5-10%). Roots were poured and washed through a 250 µm sieve. Roots containing galls in the sieve were placed in a crystallizing dish, some petroleum jelly was spread on the borders of crystallizing dish to prevent escape of nematodes and it was covered with a glass lid. The crystallizing dish was incubated at 25º C temperature room for 4-7 days. A piece of tomato root was examined under the microscope to confirm infection, and the infected tomato root was used as a source of inoculum. Mature nematode egg masses were selected and 3 galls/egg mass (contain 400-600 per egg mass) were picked up with a pair of forceps. The egg mass was transferred into 15 ml tube containing sterile tap water. The 15 ml tube containing egg mass was left at room temperature to allow the juveniles (J2) to hatch. Most temperate species can be kept at 18-22ºC and tropical and sub-tropical species at 25-30ºC. Once hatched, the J2 was inoculated on to a previously transplanted ornamental tobacco host. Preparation J2 of root-knot nematode used the combined technique described by Davies [10, 11].

2.5. Host Preparation

Three months old plug plants of Nicotiana sanderae (Nicotiana white flower) were ordered from a nursery in London (www.hortipak.co.uk). Healthy plants of the average same height were selected, the original soil removed from the roots system by washing in tap water, and plants re-planted into the treatment pots and maintained in a Growth Cabinet at (Bud Box Cabinet) at 22-25ºC on a 12 hour light/dark cycle. Soil was treated 3 days before receive the plug, each treatment pot contained of three replicates (3 pots) was added with 10 ml of distilled water to control treatments and, 10 mls of Carbofuran and 10 mls containing 10^7 conidia of Monacrosporium eudermatum as treatment pots.

2.6. Harvesting and Root Assessment

The invasion and development of J2 stages, swollen root, sausage stage, pear-like (kidney-shape) of mature female [12] and galls was assessed at 7 and 15 days and 30 days. Plants were harvested by pulling out the whole rooted plant from the soil. The soil attached on the root plant was removed by soaking the root in tap water in a bucket. The ornamental tobacco plants were air dried and cut in line with the boundary between stem and the root. The height of each plant and root were measured with metric rule and the weight was measured with digital balance. Roots were stained with soaked in 5% pheloxin B in the 50ml tube for observation and number of swollen roots and egg masses (galls) were counted [12,13]. At 7, 15 and 30 days the plants were harvested to evaluate the effect of treatments on plant production in term; plant height and root depth, stem and root fresh weight and stem dry weight.

2.7. Data Analysis

The recorded data was subjected to Kruskal-Wallis–Analysis of Variance (ANOVA) and post-hoc test with Bonferroni at 0.05 level of significance for comparing the difference among treatment means by using statistical computer software SPSS ver. 21.00.

3. Results and Discussion

3.1. Assessment I (7 Days of Infection)

After 7 days infection, two stages of M. incognita were observed. Data analysis based on anova test showed that all treatments were statistically significant in the number of vermiform (P ≤ 0.05). However, there was no significant differences between treatments to the number of swollen roots (P ≥ 0.05) (Table 1; Figure 1).
Table 1. Analysis of Variance for the effect of NCA on the number of vermiform nematode and swollen root of ornamental tobacco after 7 days infection

| Test Statistics
ga,b | Vermiform | Swollen Root |
|-----------------|-----------|--------------|
| Chi-Square      | 9.804     | 7.803        |
| Df              | 3         | 3            |
| Asymp. Sig.     | .020      | .050         |
|                 | Significant | Not significant |

a. Kruskal Wallis Test  
b. Grouping Variable: Group

![Figure 1](image_url)  
**Figure 1.** Effect of NCA on the number of vermiform and swollen roots of infected ornamental tobacco plants after 7 days. Significantly different treatments are shown by annotation letter above the bars.

The least number of vermiform was on the *N. sanderae* treated with *M. eudermatum* as compared to the positive control, followed by treatment with Carbofuran. After 7 days infection, the plant height and root depth of *N. sanderae* were measured. Analysis of variance showed no statistical significance between treatments to the plant heights and root depths (Table 2; Figure 2).

Table 2. Analysis of Variance for the effect of NCA on the plant height and root depth of ornamental tobacco after 7 days infection

| Test Statistics
ga,b | Plant Height | Root Depth |
|-----------------|--------------|------------|
| Chi-Square      | 4.170        | 4.655      |
| Df              | 3            | 3          |
| Asymp. Sig.     | .244         | .199       |
|                 | Not significant | Not significant |

a. Kruskal Wallis Test  
b. Grouping Variable: Group
**Figure 2.** Effect of NCA on plant height and root depth of infected ornamental tobacco plants after 7 days. Significantly different treatments are shown by annotation letter above the bars.

Analysis of variance showed that all treatments were significantly different in terms of stem fresh weights and stem dry weights after 7 days infection ($P \leq 0.05$). Instead, there were no significant differences between treatments in terms of root fresh weights ($P \geq 0.05$) (Table 3; Figure 3). The maximum value of stem fresh weights and stem dry weights were shown on the plants treated with *M. euderrasmum* as compared to positive control.

**Table 3.** Analysis of Variance for the effect of NCA on stem fresh weight, stem dry weight, and root fresh weight of ornamental tobacco after 7 days infection

| Test Statistics$^{a,b}$ | Stem Fresh Weight | Stem Dry Weight | Root Fresh Weight |
|-------------------------|-------------------|----------------|-------------------|
| Chi-Square              | 9.462             | 9.564          | 4.747             |
| Df                      | 3                 | 3              | 3                 |
| Asymp. Sig.             | .024              | .023           | .191              |
| Significant             | Significant       | Not significant|

*a.* Kruskal Wallis Test  
*b.* Grouping Variable: Group
Figure 3. Effect of NCA on stem fresh weight, stem dry weight and root fresh weight of infected ornamental plants after 7 days. Significantly different treatments are shown by annotation letter above the bars.

3.2. Assessment II (15 Days of Infection)
In the second assessment of 15 days infection, four stages of *M. incognita* were measured. Analysis of variance showed that the treatments were significantly different to the number of immature female, mature Female and sausage shaped (P ≤ 0.05). However, the treatment did not show any significance to the number of galls (P ≥ 0.05) (Table 4; Figure 4 & 5). The minimum number of immature female and mature female were obtained in the plant treated with *M. eudermatum* compared to positive control and followed by treatment with Carbofuran.

Table 4. Analysis of Variance for the effect of NCA on the number of immature and mature female, sausage shaped cells and galls of ornamental tobacco after 15 days infection

| Test Statistics<sup>a,b</sup> | Immature Female | Mature Female | Sausage Shaped | Galls |
|-----------------------------|-----------------|---------------|----------------|-------|
| Chi-Square                  | 9.982           | 9.756         | 9.721          | 7.461 |
| Df                          | 3               | 3             | 3              | 3     |
| Asymp. Sig.                 | .019 Significant| .021 Significant| .021          | .059  |

a. Kruskal Wallis Test
b. Grouping Variable: Group
Figure 4. Effect of NCA on the number of immature and mature female of infected ornamental plants after 15 days. Significantly different treatments are shown by annotation letter above the bars.

Figure 5. Effect of NCA on the number of sausage shaped and galls of infected ornamental plants after 15 days. Significantly different treatments are shown by annotation letter above the bars.

The least number of galls was found from the plant treated by *M. eudermatum* followed by plants treated with carbofuran as compared to positive control. However, the differences in number of galls was not supported by analysis of variance. After 15 days infection by *M. incognita*, plant height and root depth of the plant were also measured. Analysis of variance showed that there was no significant differences between treatment to the plant height and root depth parameters (P ≥ 0.05) (Table 5; Figure 6).
**Table 5.** Analysis of variance for the effect of NCA on plant height and root depth of ornamental tobacco after 15 days infection.

| Test Statistics | Plant Height | Root Depth |
|-----------------|--------------|------------|
| Chi-Square      | 5.120        | 5.809      |
| Df              | 3            | 3          |
| Asymp. Sig.     | .163         | .121       |
|                 | Not significant | Not significant |

a. Kruskal Wallis Test  
b. Grouping Variable: Group

**Figure 6.** Effect of NCA on plant height and root depth of infected ornamental plants after 15 days. Significantly different treatments are shown by annotation letter above the bars.

The stem fresh weight, stem dry weight and root fresh weight were also measured 15 days after infection. Analysis of variance showed that there was no significant differences between treatments to all parameters measured (P ≥ 0.05) (Table 6; Figure 7).

**Table 6.** Analysis of Variance for the effect of NCA on stem fresh weight, stem dry weight, and root fresh weight of ornamental tobacco after 15 days infection

| Test Statistics | Stem Fresh Weight | Stem Dry Weight | Root Fresh Weight |
|-----------------|-------------------|-----------------|-------------------|
| Chi-Square      | 1.974             | 5.615           | 1.923             |
| Df              | 3                 | 3               | 3                 |
| Asymp. Sig.     | .578              | .132            | .589              |

a. Kruskal Wallis Test  
b. Grouping Variable: Group

---

8
3.3. Assessment III (30 Days of Infection)
After 30 days infection by *M. incognita*, the number of immature female, mature female, sausage shaped and galls were measured. Analysis of variance showed that almost all treatments were significantly different except immature female parameter (P ≤ 0.05) (Table 7; Figure 8 & 9). The minimum number of immature female was found from treatment by *M. eudermatum*. The result was also the same in terms of mature female as compared to the positive control. The least number of sausage shaped and galls were found from treatment by *M. eudermatum*, followed by plants treated with carbofuran as compared to positive control.

Table 7. Analysis of Variance for the effect of NCA on the number of immature and mature female, sausage shaped cells and galls of ornamental tobacco after 30 days infection

| Test Statistics\(^{a,b}\) | Immature Female | Mature Female | Sausage Shaped | Galls |
|---------------------------|-----------------|---------------|----------------|-------|
| Chi-Square                | 7.573           | 9.565         | 9.596          | 8.816 |
| Df                        | 3               | 3             | 3              | 3     |
| Asymp. Sig.               | .056            | .023          | .022           | .032  |

\(^{a}\) Kruskal Wallis Test
\(^{b}\) Grouping Variable: Group
Figure 8. Effect of NCA on the number of immature and mature female of infected ornamental plants after 30 days. Significantly different treatments are shown by annotation letter above the bars.

Figure 9. Effect of NCA on the number of sausage shaped and galls of infected ornamental plants after 30 days. Significantly different treatments are shown by annotation letter above the bars.

After 30 days infection by *M. incognita*, the plant height and root depth were also measured. Analysis of variance showed that all treatments were significantly different in terms of root depth ($P \leq 0.05$). However, there was no significant differences between treatments to the plant heights ($P \geq 0.05$). (Table 8; Figure 10). Plants treated with Carbofuran showed the minimum root depth, followed by plants treated with *M. eudermatum*. Even though the maximum of plant height was found from treatment with carbofuran, it was not significantly different.
Table 8. Analysis of Variance for the effect of NCA on plant height and root depth of ornamental tobacco after 30 days infection

| Test Statistics<sup>a,b</sup> | Plant Height | Root Depth |
|-------------------------------|--------------|------------|
| Chi-Square                    | 6.960        | 8.420      |
| Df                            | 3            | 3          |
| Asymp. Sig.                   | .073         | .038       |

a. Kruskal Wallis Test
b. Grouping Variable: Group

Figure 10. Effect of NCA on plant height and root depth of infected ornamental plants after 30 days. Significantly different treatments are shown by annotation letter above the bars

After 30 days infection by *M. incognita*, the stem fresh and dry weight, as well as root fresh weight were also measured to obtain the effect of inoculating *M. eudermatum* plant growth promoting factors. Analysis of variance showed that all treatments were significantly different to all of measured growth parameters (*P* ≤ 0.05) (Table 9; Figure 11).

Table 9. Analysis of Variance for the effect of NCA on on stem fresh weight, stem dry weight, and root fresh weight of ornamental tobacco after 30 days infection

| Test Statistics<sup>a,b</sup> | Stem Fresh Weight | Stem Dry Weight | Root Fresh Weight |
|-------------------------------|-------------------|----------------|------------------|
| Chi-Square                    | 7.821             | 8.128          | 9.667            |
| Df                            | 3                 | 3              | 3                |
| Asymp. Sig.                   | .050              | .043           | .022             |

a. Kruskal Wallis Test
b. Grouping Variable: Group
3.4. Profile of Infected Plants after 7, 15, and 30 Days Durations
The effect of inoculant *M. eudermatum* as biocontrol agent to the plant growth showed some progress over time (Fig. 12). Based on the graphs below, the NCA treatment only had an effect to the weight of plant shoots. Furthermore, the other growth parameters such as plant height, root depth and root fresh weight increased since the first until the last assessment, although there was not significant effect to those parameters being analyzed statistically. Only the negative control plant showed the better growth as compared with other treatments.
Figure 12. Sum of profiles from infected tobacco under treatment of NCA during 7, 15, and 30 days with different parameters shown in different graphs.

Based on the results from this experiment, the inoculation of Nematode Control Agent (NCA) by using wheat bran soil inoculum of Monacrosporium eudermatum as biocontrol agent in comparison with carbofuran as a chemical agent proved effective in reducing number of infections by M. incognita after 7 days, 15 days and 30 days infection. A minimum number of galls relative to those found on the infected control were seen on the treatment plants. Less invasion and development of M. incognita was obtained from roots of ornamental tobacco Nicotiana sanderae treated with M. eudermatum and carbofuran as compared to infected control.

Similar study using Arthrobotrys dactyloides as NCA proved that the fungi reduced the severity of root knot disease of tomato in pot and field experiments [14]. Inoculating A. dactyloides at the rate of $4 \times 10^6$ colony forming unit (CFU) per kg of soil successfully reduced the number of infections of root knot nematodes by up to 66% relative to infested controls in tomato [14]. According to Kumar et al., [14], the grain agar media used in these experiments consisted of: brans of pea (Pisum sativum), wheat (Triticum aestivum), rice (Oryza sativa), gram (Cicer arietinum), lentil (Lens esculentum) and pigeon pea (Cajanas cajan) with composition; each bran powder (20 g), agar (20 g) in 1000 ml distilled
water. The grain agar media consisted of grains of maize (Zea mays), wheat, sorghum (Sorghum bicolor), barley (Hordeum vulgare) with composition; each grain (20 g), agar (20 g) and 1000 ml of distilled water were used. Those media was proved to support moderate to very good growth and sporulation of NTF. This was particularly occurred if the inoculant were modified by altering the concentrations of brans by adding sand and inert plant growth materials with combination such as: compost + bran, compost + grain, compost + sand + bran or compost + sand + grain for bio-formulation of mass NTF culture with a relatively simple substrate together with limited effects or interferences to other soil biota [5].

Bran inoculum has proved the best inoculum for industrial use and mass production, for example the sand and barley bran mixture has been used as inoculum for nematophagous fungi such as Verticillium lecanii [4]. A similar experiment using wheat bran + wheat straw to produce conidia of A. oligospora and Dactylaria brochophaga showed that inoculum containing A. oligospora had better performance compared with D. brochophaga in reducing number of Root-Knot Nematode (RKN) either in sterilized soil or unsterilized soil [15]. In these experiments reported here, treatment with NCA was not significantly different in terms of the growth of Nicotiana sanderae. However, the trendline that showed growth performances from the first assessment until the end of assessment, indicated that the NCA improved the host plant growth especially to shoots. But, there is still weak evidence from this experiment that the inclusion of wheat soil inoculum containing 1 x 10⁶ conidia mL⁻¹ might have increased the growth of host plant until the end of assessment. Inoculum of NCA using M. eudermatum did not show any potencies in promoting the upper growth of host plant. Similar research has been conducted by Sharon et al., [16] using peat bran inoculum containing 10⁸ CFU/gr of Trichoderma harzianum as a BCA in the control of Meloidogyne javanica on tomato plants in the green house condition. Results from these experiments showed that T. harzianum had the potential to reduce the number of galls and increased only top fresh weight of tomato plants.

There is a reduction of root depth, fresh and dry weight of the host plant roots after 15 days infected by RKN, which is caused by the root-knot disease affecting root growth and function. However, ornamental tobacco plant treated by carbofuran showed an increase in the plant height parameter relative to the infected control. The decrease in nematode development and reproduction parameters and the increase of plant growth parameters was reported in other study. Hastuti et al., also reported the similar result using three Sumatran Nematode-trapping fungi on Deli tobacco plant in which the number of root-knot nematodes, M. incognita was suppressed, despite no growth stimulation shown from the use of fungi [17]. Other study reported the soil treatment with fungi such as T. harzianum inoculum as nematode control agent also improved the shoot fresh and dry weight of host plant. In their research, Carbofuran significantly decreased the Meloidogyne incognita infestation together with significant promotion of the growth of host plant as compared to fungal and bacterial treatment [18].

The combination of wheat bran and organic amendment and materials such as compost as inoculum substrates may provide stability in disease control and also to promote the plant growth [5]. Use of nematode trapping fungi will not only reduce the use of chemical nematicide in agricultural practice but also promote the plant vigour by uptake of essential nutrition and mineral and also reduce the reproduction and development of galls by root-knot nematode in the plant and in the soil [2]. However, it is also possible that a fungal pathogen could also grow with the fungal inoculant used as a BCA in non-sterile soils. It could be the reason why the growth of roots of plants in some experiments were inhibited [19]. Therefore it is also important to conduct further research using the sterilized and unsterilized soil to investigate the difference in damage by RKN and other fungal pathogens on the root host plant between both growth media.

4. Conclusions
Initiation of NCA inoculant by using Monacrosporium eudermatum as a biocontrol agent was effective to reduce invasion by M. incognita in ornamental tobacco (Nicotiana sanderae). Based on this research almost all stages of M. incognita can be suppressed by NCA inoculant, after 7, 15 and 30
days infection. NCA inoculant containing *M. eudermatum* is comparable with Carbofuran as gold standard manufacturer in control pest such as *M. incognita* in economically important crops such as ornamental tobacco. Even though, there is a weak evidence to support that *M. eudermatum* inoculum can be also used as growth promoting agent, our data must be considered by other researcher in the field of agriculture to alter the use of conventional pesticide with the more safer pest control agent by using this fungi. As the preventive strategies in which most commonly are related with environmental concern, application of NTF inoculant then may diminish the problems from economical and ecological aspects. As we have known, pesticide also possessed detrimental effects in the soils in causing environmental toxicity, leading to several health disorders. Furthermore, the natural enemy of *M. eudermatum* isolated from Sumatera Utara, is also easy to applied by the farmer which are counted as: affordable, effective and safe for the environment especially towards other non-target soil organisms.

**Acknowledgements**

This study was supported, in part funded by Ristekdikti period 2010–2013, from the Ministry of Research and Technology of Indonesia. The authors would also like to thank Dr. K. G. Davies for providing *Meloidogyne incognita*, the root-knot nematode used in this study, collection from Rothamstead Research as well as to Dr. Jane Faull for the generous and endless assistance.

**References**

[1] Davis EL, Rich JR, Gwynn GR and Sisson V 1988 Nematropica 18 1988
[2] Askary TH 2015 *Biocontrol Agents of Phytonematodes* 81
[3] Nunez-Gaona O, Saucedo-Castaneda G, Alatorre-Rosas R and Loera O 2010 *Brazilian Archives of Biology and Technology* 53 771
[4] Brand D, Soccol CR, Sabu A and Roussos S 2010 *Myological Control Using Paecilomyces* 22 31
[5] Larsen J, Cornejo P and Barea JM 2009 *Soil Biology and Biochemistry* 41 286
[6] Shinya R, Watanabe A, Aiuchi D, Tani M, Kuramochi K, Kushida A and Koike M 2008 *Japanese Jornal of Nematology* 38 9
[7] Kumari NS and Sivakumar CV 2005 *Commun Agric. Appl. Biol. Sci* 70 909
[8] Slaoui M, Ouhssine M, Berny E and Elyachioui M 2006 *African Journal of Biotechnology* 6 419
[9] Barker KR 1985 *Nematode extraction and bioassays in An advanced Treatise on Meloidogyne*. (pp. 19–35) (Raleigh, North Carolina: North Carolina State University Graphics)
[10] Roland N, Perry, Moens M and Starr JM 2009 *Root-knot Nematodes* (London)
[11] Trigiano R 2007 *Plant Pathology Concepts and Laboratory Exercises, Second Edition* (2nd ed.). Boca Raton: CRC Press
[12] Holbrook CC, Knautt DA and Dickson DW 1983 *Plant Disease* 57 975
[13] Southey JF 1986 *Laboratory methods for work in plant and soil nematodes*. (M. of Agriculture, Ed.) (London: Fisheries and Food. Ministry of Agriculture. HMSO Books)
[14] Kumar D, Singh K, and Jaiswal R 2005 *Mycobiology*, 33 215
[15] Bandyopadhyay P, Kumar D, Singh VK and Singh KP 2001 *Indian Journal Nematology* 31 153
[16] Sharon E, Bar-Eyal M, Chet I, Herrera-Estralla A, Kleinfeld O and Spiegel Y 2001 *Phytopathology* 91 687
[17] Hastuti LDS and Faull J 2018 *IOP Conf. Ser.: Earth Environ. Sci.* 130 012009
[18] Elgawad MMA and Kabeil SA 2011 *Crop Prot.* 30 285
[19] De-Leij FAAM and Kerry BR 1991 *Revue Nématol.* 14 157