EFFECTIVENESS OF CHICKEN MANURE AND *ARTHROBOTRYS DACTYLOIDES* ON THE SUPPRESSION OF *MELOYDOGYNE JAVANICA*

(EFFEKTIFITAS PUPUK KOTORAN AYAM DAN ARTHROBOTRYS DACTYLOIDES DALAM MENEKAN MELOIDOGYNE JAVANICA)

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ABSTRACT

The suppression of *Meloidogyne javanica* with the application of kaolin alginate formulated *Arthrobotrys dactyloides* and chicken manure was investigated. The aims of the study were to know the effects of chicken manure on the growth, ring production, trapping activity of *A. dactyloides*, and suppression of *M. javanica*, as well as plant growth. A series of experiments consisted of slide test, soil microcosms, and pot tests were conducted with six dosages of chicken manure; 0.25%, 0.50%, 0.75%, 1.00%, 1,25%, and 1,50% (w/w) with five replicates. Treatment without chicken manure was provided as a control. All experiments were conducted with completely randomized design. The data were analyzed with analysis of variance, and means of each treatment were separated using a honestly significant difference test at 5% level. Results of the study showed that chicken manure did not have significant effect on the growth, ring production, and trapping activity of *A. dactyloides*, a fungus that effectively reduced number of nematodes entering roots of tomato seedlings. However, chicken manure significantly reduced number of *M. javanica* penetrating roots, hampered *M. javanica* development, and improved plant growth.

Key words: *A. dactyloides*, chicken manure, and *M. javanica*.

ABSTRAK

Penekanan M. javanica *dengan aplikasi* A. dactyloides *dalam formulasi kaolin alginate dan pupuk kotoran ayam telah diteliti. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pupuk kotoran ayam terhadap pertumbuhan, pembentukan cincin perangkap, aktifitas penjeratan* A. dactyloides, *dan penekanan* M. javanica, *serta pertumbuhan tanaman tomat. Serangkaian percobaan yang terdiri dari "slide test", "soil microcosms", dan percobaan pot telah dilakukan dengan enam dosis pupuk kotoran ayam; 0.25%, 0.50%, 0.75%, 1.00%, 1,25%, dan 1,50% (b/b) dengan lima ulangan. Perlakuan tanpa pupuk disiapkan sebagai control. Semua percobaan dilaksanakan dengan rancangan acak lengkap. Data dianalisa dengan analisis keragaman dan dilanjutkan dengan uji beda nyata jujur pada taraf nyata 5%. Hasil penelitian menunjukkan bahwa pupuk kotoran ayam tidak berpengaruh nyata terhadap pertumbuhan, pembentukan perangkap, dan penjeratan* M. javanica *oleh* A. dactyloides*, jamur yang secara efektif menekan jumlah* M. javanica *yang penetrasi ke dalam akar tanaman tomat.Tetapi, pupuk kotoran ayam secara nyata mengurangi jumlah* M. javanica *yang penetrasi akar, menekan perkembangan nematoda, dan meningkatkan pertumbuhan tanaman.*

Kata kunci: A. dactyloides, *pupuk kotoran ayam, dan* M. javanica.

INTRODUCTION

The addition of organic matter to soil to improve soil fertility and increase crop yield is an ancient concept (Rodriguez-Kabana, 1986). Stirling (1991) pointed out that the presence of organic matter in soil is known to affect the predatory behavior of fungal antagonists. It has been suggested that the addition of many of these materials, particularly those high in nitrogen, may be effective alternatives to nematicides for control of *Meloidogyne arenaria* (Neal) Chitwood and other plant parasitic nematodes (Mian and Rodriguez-Kabana, 1982; Rodriguez-Kabana, 1986). The addition of chicken litter to soil suppresses *Meloidogyne* spp., limits root galling caused by the nematode, and stimulates plant growth (Mian and Rodriguez-Kabana, 1982). The crop-management benefits and widespread availability of poultry litter make it of great potential use in low input, sustainable agriculture programs.

Two hypotheses have been suggested to explain the mode of action of soil amendments in terms of nematode control (Mian and Rodriguez-Kabana, 1982a; Mian *et al.*, 1982; Sayre, 1980). These are as follows: i) the amendment or its decomposition products are directly toxic; or ii) the amendment alters the soil environment so as to favor competing microbial populations, mycoflora capable of parasitizing nematode, or other soilborne antagonists that destroy or weaken these plant parasites (Stirling, 1991).

Most studies on organic amendments for nematode control (Lazarovits *et al*., 1999; Akhtar and Malik, 2000) have mainly been done with nitrogen-rich amendments such animal manure, meat and bone meal, soy meal, oil cakes and chitin. These organic materials produce nematicidal compounds such as ammonia and nitrous acid at concentrations that are sufficient to kill plant-parasitic nematodes when they are applied to soil at application rates of 5-100 t ha^{-1} (Larazovits *et al*., 1999; Oka and Yermiyahu, 2002). Nematicidal effects, however, are relatively short-lived because ammonia concentrations remain high for a limited time (Cowling *et al*., 2001; Oka and Pivonia, 2002). Hence, instead of its nematicidal effects alone, the use of organic amendments is expected to control nematode through naturally occurring mechanisms. In earlier studies with organic amendments in the sugar industry (Stirling *et al*., 2003; Pankhurst *et al*., 2005), various organic materials were added to a sugarcane-growing soil and results showed that amendments with a high C/N ratio induced suppressiveness to *Meloidogyne javanica* and *Pratylenchus zeae* 4 and 7 months after they were added to the soil. This suppression of nematodes was reported to be associated with low levels of nitrate-nitrogen in soil, a microbial-dominant soil biology and high numbers of omnivorous nematodes (Stirling *et al*., 2003).

Although previous studies have shown that ammoniac nitrogen from inorganic fertilizers and organic sources, such as chicken litter, is an effective nematode suppressant (Kaplan and Noe, 1992; Rodriguez-Kabana, 1986), the effect of this substance on nematode antagonists, particularly *A. dactyloides* – a nematode trapping fungus, in soil is still not known. The purpose of this study was to investigate the effects of chicken manure on the growth and ring production, trapping activity, and suppression of *M. javanica*, as well as plant growth.

MATERIALS AND METHODS

Preparation of Sterile Second Stage (J2) of *Meloidogyne javanica*

Meloidogyne javanica cultures were maintained on susceptible tomato plants grown in sandy soil in 1.2 L pots in the glasshouse. Eggs of *M. javanica* were extracted with sodium hypochlorite method (Hussey and Barker, 1973). Sterile second stage juveniles (J2) were produced by adding concentrated nematode-egg suspension to 10 ml agar (1%, 45-48^{o}C), mixed well, and poured into the centre of a sterile Petri dish and allowed to solidify. An antibiotic medium was prepared by adding 1.2 ml of streptomycin solution (1 g of streptomycin sulphate in 100 ml sterile distilled water) and 0.0095 g of methoxy ethyl mercuric chloride to 250 ml of water agar. The antibiotic medium was poured gently over the solidified nematode egg-agar suspension until covered to a depth of 5 mm. The plates were then incubated for 36 hours at 25^{o}C to allow J2 to hatch from eggs and migrate to the agar surface. Juveniles were washed into a sterile beaker using 10 ml of sterile distilled water.

Preparation of Chicken Manure Amended Soil

Soil used in this study was sandy soil (28% coarse sand, 55% fine sand, 7% silt and 10% clay). The soil moisture contents at field capacity and permanent wilting point were 14% and 10%, respectively. Soil taken from the field was air dried by spreading soil in a shaded area for a few days. The air dried soil was then sieved with a 2 mm-aperture sieve and stored in bins until required. Soil was then amended with six different dosages (0.25,

0.50, 0.75, 1.00, 1.25, and 1.50% (w/w)) of chicken manure. One treatment of soil without chicken manure was prepared as control. Chicken manure-amended- and non amended-soil were then separately moistened and autoclaved in plastic bags for three consecutive days at 121ºC for 20 minutes before being used in experiments. The carbon and nitrogen contents of chicken manure used were 90% and 3.36%, respectively.

The Nematode-Trapping fungus, *Arthrobotrys dactyloides* and Mass Production of Mycelia

The fungus used in this research was *Arthrobotrys dactyloides* isolate Ampenan. The fungus was grown on corn meal agar (CMA) in 9 cm diameter Petri dishes. When the whole surface of the dish was covered by mycelia, the agar was cut into squares (6 mm x 6 mm) and stored in bottles containing sterile distilled water at 27ºC. When fungus was required for experiments, one square from this water culture was placed on CMA in a 9 cm diameter Petri dish and plates were incubated at 27ºC for 6-7 days before use. The fungus was mass produced in 250 ml Ehrlenmeyer flasks containing 100 ml Glucose Peptone yeast (GPY) broth (15 g glucose, 2 g peptone, 5 g yeast, 1 g asparagine, 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$, 0.001 g thiamine HCl, 1 L H_2O) (Sudirman, 1997; 2009). Flasks were inoculated with two 5 mm-diameter discs taken from an actively growing colony on CMA as described previously and were incubated at 27ºC on a rotary shaker at 120 rpm. After 10 days incubation, about 0.007 g dry wt biomass ml^{-1} was produced. Before it was used, the mycelial suspension was homogenized for 15 seconds with blender.

Formulation of the fungus

The fungus was formulated into kaolin-alginate granules based on technique developed by Sudirman (2009b). 100 g kaolin (MP Biomedical Inc, Ohio, USA) and 10 g sodium alginate were added to 1 L water. After autoclaving, 80 ml of the blended and sterilized kaolin-alginate mixture was mixed with 20 ml of mycelial suspension prepared as described previously. The mixture was then mixed with a magnetic stirrer in a 1 L Ehrlenmeyer flask and dripped through a Pasteur pipette into a continuously shaken aqueous suspension of 0.1 M Ca-gluconate. The drops gelled upon contact with the Ca-gluconate. In order to maintain a homogenous spherical form of granules, the distance between the tip of the Pasteur pipette and the surface of the Ca-gluconate suspension was kept at about 1 cm. Granules were harvested and transferred to Ehrlenmeyer flasks containing 100 ml GPY broth and incubated in shake culture at 27ºC. After 3 days, the re-fermented granules were harvested and dried on a sterile wire mesh. The diameter and weight of a granule were 3 mm and 3.5 g, respectively.

Tests on Granules Quality

Estimation of number of propagules in granules. To determine the number of propagules in formulations, 0.1 g of dried granules were soaked overnight in vials containing 9.9 ml of sterile phosphate buffer (11.8 g KH_2PO_4 and 4.2 g Na_2HPO_4 in 1 L water at pH 6.4). The suspensions were then diluted serially in sterile water and 0.1 ml of each dilution was plated onto CMA containing 50 mg L^{-1} streptomycin sulphate. The numbers of colonies of *A. dactyloides* were counted after 5 days incubation at 27ºC.

The viability and vigor of fungus in granules were determined by placing five replicate samples of ten granules on tap water agar (TWA) and incubating the plates at 27ºC for 5 days. Viability was determined by counting the number of granules which produced mycelial growth. Each granule was also rated for vigour on a 1-4 scale (1 = sparse mycelial growth; 2 = mycelial growth covering less than 50% of the granule; 3 = mycelial growth covering 50% - 90% of granule; 4 = mycelial growth covering the whole granule with very dense mycelia).

Effect of Chicken Manure on Growth and Ring Production in Soil

This experiment aimed to determine the growth of *A. dactyloides* in soil amended with different dosages of Chicken Manure. The growth of *A. dactyloides* from granules in each soil/sawdust mixture was assessed with a standard slide test. One granule containing *Arthrobotrys dactyloides* was placed at marked positions on a glass slide at the bottom of a 9-cm diameter Petri dish. The granule was covered with a piece of nylon mesh (the same size as the glass slide) with 100 μm apertures and the Petri dish was then filled with 60 g of soil, moistened with sterile water to approximately field capacity. The Petri dish was placed in a moist air-tight plastic container (in which humidity was maintained by covering the base and top with 2 layers of moist paper) and incubated at 27ºC. Three replicates for each of 6 dosages (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50% (w/w)) of chicken manure-amended- and non-amended-soil (control) were

provided. Twenty days later, the soil and nylon mesh were carefully removed so that all granules and mycelia growing from the granules stayed in position. To achieve this, a little pressure was applied with the tip of a fine forceps at the point where a granule was located, and the edge of nylon mesh was lifted in such a way that granules remained in position with minimal disturbance to the mycelia. The mycelia were separated from granules by using a very sharp tip of a forceps to cut around the granules. The granules were then removed. This latter step was done very carefully to avoid mycelia from being disarranged or dislodged with the granules. The slide was then flooded with lactoglycerol cotton blue, a coverslip was applied and the surface of the slide was observed under microscope. Radial growth of mycelia was measured.

To determine the effect of chicken manure dosages on ring production, slide test with chicken manure-amended-soil (6 dosages) and non-amended-soil (control) were prepared. Three granules were put on one slide and three replicates for each treatment and control were prepared and processed as described previously. After 5 days incubation at 27ºC, the number of rings in each slide was observed.

Effect of Chicken Manure on Trapping activity

This experiment was conducted in soil microcosms using chicken manure-amended- (6 dosages) and non-amended-soil (control). For each treatment and control, three microcosms with granules and three microcosms without granules were prepared. Microcosms were made from 38 mm internal diameter PVC pipe. Rings 3 mm and 6 mm wide were cut from the pipe and rigid plastic mesh (2 mm diameter pore size) that had been cut to the same external diameter as the pipe was glued between the pipes. Two layers of tissue paper were then placed on the mesh and the larger ring (volume approximately 7 ml) was filled with 9 g of soil. The soil was then watered to nearly field capacity. Twenty granules were buried in soil and the microcosms were placed in 60 mm diameter Petri dishes and incubated at 27ºC in an air-tight plastic container. After 5 days incubation at 27ºC, about 90 freshly hatched *M. javanica* (J2) were inoculated into each of three replicate microcosms of each treatment, and microcosms were re-incubated at 27ºC. After 3 days, the nematodes in each microcosm were extracted by adding water to the Petri dishes to form a small Baermann tray. The nematodes that migrated through the tissue in 48 hours at 27ºC were counted.

Effect of Chicken Manure on Penetration and Growth of Tomato Seedlings

Experiment was conducted with 1.2 L pots filled with 800 g soil (amended or non amended). Ten days before seed planting, 6.4 g granules (0.8% w/w) were mixed into each pot, except pots with no chicken manure (control). One 20 day-old-seedling of tomato was planted into each pot one day before inoculation of 800 J2 *M. javanica*. J2 were inoculated by pipetting 1 ml J2 suspension at four holes made around the seedling about 2 cm from the seedling. The plants were watered and harvested at four weeks. Plant height, shoot wet and dry weight, total root length, number of galls, number of egg masses were measured and the number of nematodes inside roots were counted after dissecting roots stained with sodium-hypochlorite-acid-fuchsin (Daykin and Hussey, 1985).

Statistical Analysis

All experiments were conducted using a completely randomized design. The data were analyzed by analysis of variance using GenStat® Discovery 2nd Edition. When the variance ratio (F) was significant, means for each treatment were separated using Tukey's Honestly Significant Difference test.

RESULTS

Granules contained propagules about 24 x 10^7 CFU, with viability 100% and vigor 3,46. *A. dactyloides* grew well in soil amended with chicken manure with various dosages. Results of analysis showed that there were no significant differences on the radial growth of mecelia of *A. dactyloides* among dosages treated and control and no ring were observed in all treatments (Table 1).

Results of analysis showed that different dosages of chicken manure in amended-soil significantly decreased the numbers of *M. javanica* recovered after incubation 3 days since inoculation. The numbers of *M. javanica* recovered decreased significantly as the dosages of chicken manure increased from 0.75% (w/w) (Figure 1).Despite dosages of chicken manure, *A. dactyloides* formulated in kaolin-alginate granules significantly reduced numbers of recovered *M. javanica* (Figure 1).

Table 1. Radial growth and numbers of ring formed by *A. dactyloides* in soil amended with various dosages of chicken manure

Dosages of chicken manure (% w/w)	Radial growth of mycelia (mm)	Numbers of rings formed
0.00	18.6	0
0.25	19.4	0
0.50	19.7	0
0.75	19.2	0
1.00	18.9	0
1.25	19.0	0
1.50	19.5	0

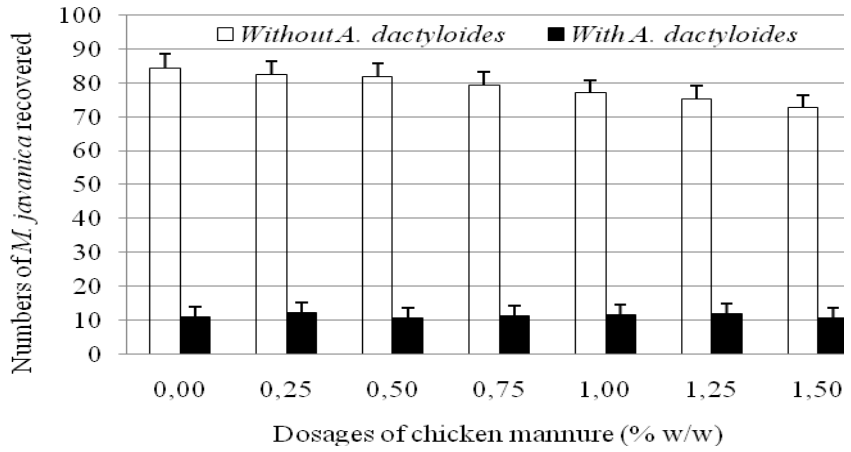

Figure 1. The numbers of recovered *M. javanica* in soil amended with various dosages of chicken manure associated with *A. dactyloides*. (Bars are the values of $HSD_{0.05}$)

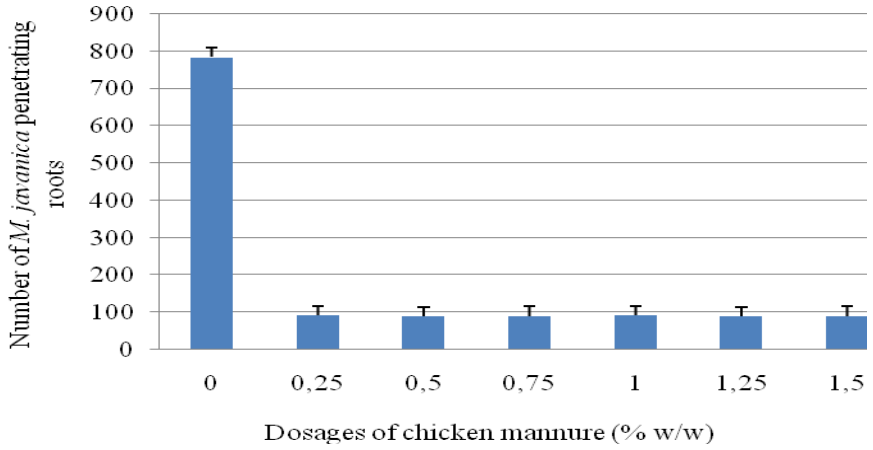

Figure 2. The numbers of recovered *M. javanica* penetrating roots of tomato seedlings grown in soil amended with various dosages of chicken manure associated with *A. dactyloides*. (Bars are the values of $HSD_{0.05}$)

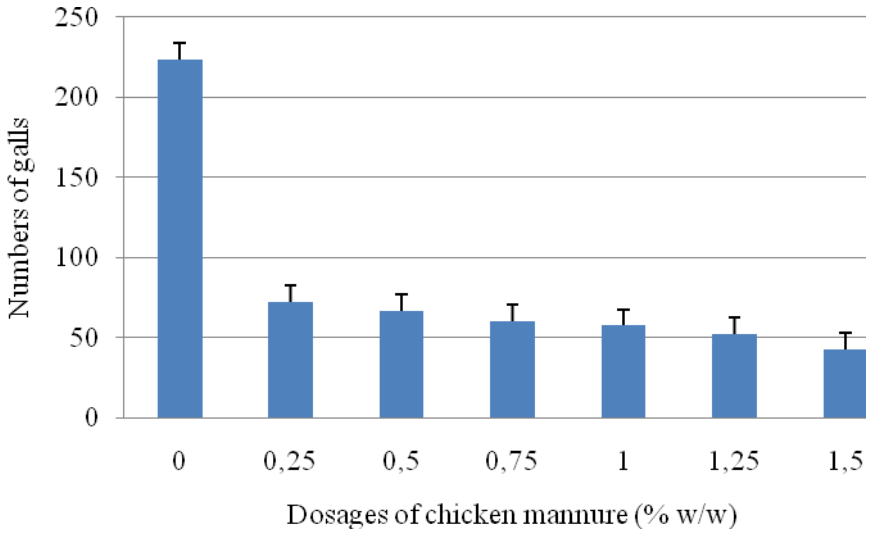

Figure 3. The numbers of galls formed on roots of tomato seedlings grown in soil amended with various dosages of chicken manure associated with *A. dactyloides*. (Bars are the values of $HSD_{0.05}$)

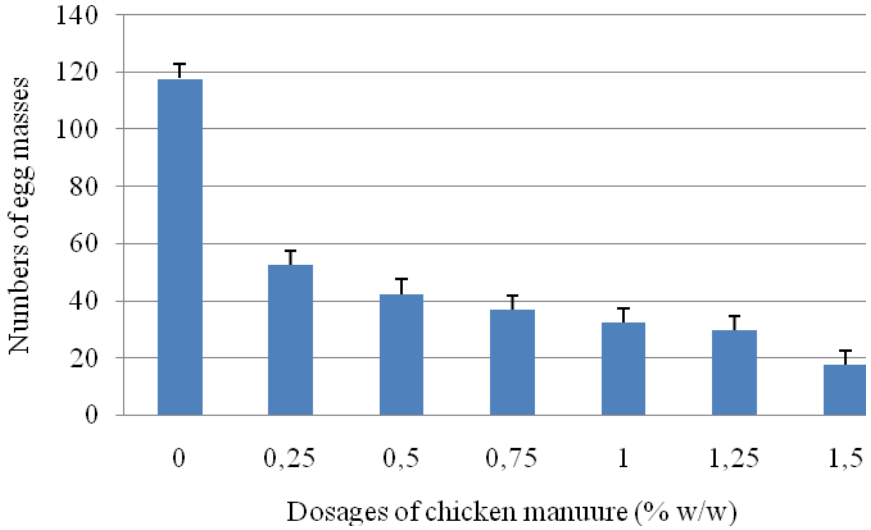

Figure 4. The numbers of egg masses formed on roots of tomato seedlings grown in soil amended with various dosages of chicken manure associated with *A. dactyloides*. (Bars are the values of $HSD_{0.05}$)

Table 2. Plant height, total length of roots, shoot fresh weight, and shoot dry weight of tomato seedlings grown on soil amended with various dosages of chicken manure

Dosages of chicken manure (% w/w)	Plant height (cm)	Total length of roots (cm)	Shoot fresh weight (g)	Shoot dry weight (g)*)
0.00	43.67	82.67	24.33	4.93a
0.25	45.33	83.33	25.67	5.24b
0.50	46.33	84.67	26.33	5.33bc
0.75	47.67	85.33	25.00	5.48bc
1.00	44.33	84.67	27.33	5.56c
1.25	45.67	87.33	26.67	5.62c
1.50	43.33	88.67	27.33	5.62c

*)The numbers followed by the same letters are significantly different ($HSD_{0.05}$ = 0.29)

Results showed that dosages of chicken manure in amended-soil significantly influenced the numbers of nematodes that entered roots, the numbers of galls, and the numbers of egg masses formed in roots. As dosages increased, the numbers of nematodes entering roots (Figure 2), the numbers of galls (Figure 3), and the numbers of egg masses (Figure 4) were reduced. The numbers of galls, egg masses, and nematodes inside roots of plants treated with only 0.25% chicken manure in amended-soil were significantly lower than the control (without granules). However, as the dosages increased, the total length of roots was increased. Although, there was no difference in plant height and shoot fresh weights, significant increases were observed in the shoot dry weights as the increase of dosages of chicken manure in amended-soil (Table 2).

DISCUSSION

Granules used in this research were in good quality as shown with their sterility, viability, and vigor. Chicken manure applied in this study did not have any detrimental effect on *A. dactyloides*. The fungus grew well on all treatments with no ring was produced (Table 1). This results suggest that nitrogen content of medium did not stimulate *A. dactyloides* to produce rings. Furthermore, results of this study strengthen the idea that organic matter does not have any significant effect on the growth and ring production of *A. dactyloides*. Results of previous studies on the incorporation organic matter into alginate granules containing the fungus showed that the three sources of carbon (sawdust, cassava, and rice husk) did not have any significant effects on the growth, ring production and trapping activity of this fungus (Sudirman, 2010). In environments with a high C/N ratio, Barron (1992) has hypothesized that nematode-trapping fungi are stimulated to increase trapping because they need to obtain nitrogen from nematodes. Since various dosages of chicken manure in this study did not stimulate ring production, it is clear, therefore, that it is not nitrogen that stimulates ring formation. The presence of nematode, however, induces ring formation to facilitate *A. dactyloides* to obtain nitrogen from nematodes (Sudirman, 2007a).

Most studies on organic amendments for nematode control (Lazarovits *et al*., 1999; Akhtar and Malik, 2000) have mainly been done with nitrogen-rich amendments such animal manure, meat and bone meal, soy meal, oil cakes and chitin. These organic materials produce nematicidal compounds such as ammonia and nitrous acid at concentrations that are sufficient to kill plant-parasitic nematodes when they are applied to soil at application rates of 5-100 t ha^{-1} (Larazovits *et al*., 1999; Oka and Yermiyahu, 2002). One of the hypotheses that have been suggested to explain the mode of action of soil amendments in terms of nematode control is that the amendment or its decomposition products are directly toxic (Mian and Rodriguez-Kabana, 1982; Mian *et al.*, 1982; Sayre, 1980). This was clearly demonstrated be the result of this study that chicken manure at dosages 1.00, 1.25, and 1.50% significantly reduced numbers of recovered nematode (Figure 1). *A. dactyloides*, in addition, significantly decreased numbers of nematodes recovered both in chicken manure-amended soil and in control. This result clearly showed that although chicken manure had a nematicidal effect, this organic matter did have any detrimental effect of the effectiveness of *A. dactyloides* to trap *M. javanica*. Cowling *et al*. (2001) and Oka and Pivonia (2002) reported that nematicidal effects of organic matter containing high nitrogen, however, are relatively short-lived because ammonia concentrations remain high for a limited time. Hence, the results of this study strengthen the idea that instead of its nematicidal effects alone, to improve its effectiveness, organic amendments may be applied in association with *A. dactyloides*. Such technique was also evaluated in earlier studies with organic amendments in the sugar industry (Stirling *et al*., 2003; Pankhurst *et al*., 2005). In these last studies, various organic materials were added to a sugarcane-growing soil and results showed that amendments with a high C/N ratio induced suppressiveness to *Meloidogyne javanica* and *Pratylenchus zeae* 4 and 7 months after they were added to the soil. This suppression of nematodes was reported to be associated with low levels of nitrate-nitrogen in soil, a fungal-dominant soil biology and high numbers of omnivorous nematodes (Stirling *et al*., 2003).

A comparison of the effects of *A. dactyloides* in chicken manure non-amended- and amended-soil showed fewer *M. javanica* (Figure 2), less galls (Figure 3) and egg masses (Figure 4) in tomato roots grown in chicken manure-amended soil, 4 weeks after planting. These numbers decreased as the dosages of chicken manure increased. Previous investigators have suggested that the amount of "protein" N in an organic soil amendment is directly related to its effectiveness in suppressing nematode population densities (Mian and Rodriguez-Kabana, 1982; Rodriguez-Kabana, 1986). Results of these earlier studies, however, support a part of results of the present study. The results of this study showed that the addition of increasing dosages of nitrogen, in the form of increasing dosages of chicken manure, was not solely responsible for suppression of *M. javanica* penetration on tomato roots, as associated *A.*

dactyloides appeared to be involved. Table 1 shows that while chicken manure reduced a small numbers of recovered *M. javanica*, *A. dactyloides* reduced a great numbers of recovered nematodes. Therefore, besides the effect of chicken manure, the numbers of nematodes penetrating roots seem to be strongly influenced by *A. dactyloides* (Figure 2) as this fungus can only trap nematodes before penetrating the roots.

The role of chicken manure as a nematicide, however, seems to be dominant once the nematode inside the roots, indicated by less numbers of galls (Figure 3) and egg masses (Figure 4) produced on roots. Of the numbers of *M. javanica* that penetrated roots, only very few of them inducing galls and producing egg masses. It has been reported previously that increasing ammonium ions hampered nematode development and egg production (Sudirman, 2007; 2009a). The soil and chicken manure used in this study had been autoclaved previously, hence any effect observed was related to either *A. dactyloides* or chicken manure. The suppression of *M. javanica* penetrating roots resulted in less galls and fewer egg masses led to the improvement of plant growth as indicated by plant dry weight (Table 2), the result that supports the finding reported by Mian and Rodriguez-Kabana (1982). The crop-management benefits and widespread availability of chicken manure make it of great potential use in low input, sustainable agriculture programs.

CONCLUSION

Results of the study showed that chicken manure did not have significant effect on the growth, ring production, and trapping activity of *A. dactyloides*, a fungus that effectively reduced number of nematodes entering roots of tomato seedlings. However, chicken manure significantly reduced number of *M. javanica* penetrating roots, hampered *M. javanica* development, and improved plant growth.

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