

# EFFECT OF NEMATODE POPULATION DENSITIES ON TRAPPING ACTIVITY OF NEMATOPHAGOUS FUNGUS *Arthrobotrys dactyloides* ON *Meloidogyne javanica*

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## ABSTRACT

**Effect of nematode population densities on trapping activity of nematophagous fungus *Arthrobotrys dactyloides* on *Meloidogyne javanica*.** Trapping activities of some nematophagous fungi were suggested to be related to the population density of nematodes. This study determined the trapping activity of *Arthrobotrys dactyloides* due to the effects of (i) different populations of *Meloidogyne javanica*, (ii) the presence of *Caenorhabditis elegans*, (iii) repeated inoculation of *M. javanica*, and (iv) different populations of both *C. elegans* and *M. javanica*. Experiments were conducted using a “standard slide test” and “soil microcosm” inoculated with *A. dactyloides* formulated in kaolin-alginate granules and with low nematode populations ranged from 6 to 14 juveniles per g soil. Results showed that ring formation and trapping activity of *A. dactyloides* increased with the increase of *M. javanica* population. The presence of *C. elegans* increased *M. javanica* mortality. Repeated inoculation of *M. javanica* maintained trapping activity of *A. dactyloides*. The mortality of *M. javanica* increased with the increase of both *C. elegans* and *M. javanica* population.

Key words: Population density, trapping activity, *Arthrobotrys dactyloides*, and *Meloidogyne javanica*.

## ABSTRAK

**Effect of nematode population densities on trapping activity of nematophagous fungus *Arthrobotrys dactyloides* on *Meloidogyne javanica*.** Aktifitas pemerangkapan beberapa jamur pemangsa nematoda diduga berkaitan dengan kepadatan populasi nematoda. Penelitian ini menentukan pengaruh (i) populasi *M. javanica* yang berbeda, (ii) kehadiran *C. elegans*, (iii) inokulasi berulang *M. javanica*, dan (iv) populasi *C. elegans* dan *M. javanica* yang berbeda terhadap aktifitas pemerangkapan oleh *A. dactyloides*. Percobaan dilaksanakan dengan menggunakan “standard slide test” dan “soil microcosm” yang diinokulasi dengan *A. dactyloides* dalam formulasi butiran kaolin-alginat dan populasi nematoda yang rendah, berkisar dari 6 sampai 14 juveniles per g tanah. Hasil penelitian menunjukkan bahwa pembentukan cincin perangkap dan aktifitas pemerangkapan oleh *A. dactyloides* meningkat dengan peningkatan populasi *M. javanica*. Kehadiran *C. elegans* meningkatkan mortalitas *M. javanica*. Inokulasi berulang *M. javanica* mempertahankan aktifitas pemerangkapan *A. dactyloides*. Mortalitas *M. javanica* meningkat dengan peningkatan populasi *C. elegans* dan *M. javanica*.

Kata-kata kunci: Kepadatan populasi, aktifitas pemerangkapan, *Arthrobotrys dactyloides*, dan *Meloidogyne javanica*.

## INTRODUCTION

Some nematophagous fungi produce traps spontaneously on laboratory media, but usually trap formation occurs only in the presence of nematodes or proteinaceous material (Duddington, 1962; Pramer & Kuyama, 1963; Nordbring-Hertz, 1973). However, the importance of nematodes presence in the predatory behaviour of nematophagous fungi is still arguable.

Nordbring-Hertz (1973), and Jansson & Nordbring-Hertz (1980) observed that there was a correlation between nematode motility and trap formation. Rapidly moving nematodes such as *Panagrellus redivivus*, *Aphelenchoides fragariae* and

*Ditylenchus dipsaci* induced trap formation more rapidly in *Arthrobotrys oligospora* than slow moving species such as *D. destructor*, *Pratylenchus fallax*, and *P. penetrans*. This trap induction was not related to the mode of nematode feeding or to the degree of attraction to the fungi and was probably stimulated by nematode movement.

Nematode effects seem to be dependent on the species of antagonist. Gray (1987) reported that the activity of fungi with adhesive reticulate traps did not appear to be associated with nematode density whereas the activity of adhesive branch-forming species was closely related to the density of nematodes in soil, resulting in a predator-prey relationship. Since the

increase of activity in Gray's study was obtained from the average of several species that might behave differently, there is a need for further work on the mode of nutrition of individual species in the soil environment, especially with different species of nematodes.

Many other studies have demonstrated that the antagonist's activity is influenced by nematodes. *Meloidogyne incognita*, for instance, appears to be an important substrate for *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, since the activity of these fungi was stimulated by the presence of this nematode (Gaspard *et al.*, 1990). Jaffee *et al.* (1992) observed that an epidemic incited by *Hirsutella rhossiliensis* was characterised by temporal density dependent parasitism. Like other endoparasites, *H. rhossiliensis* appears to have little or no competitive saprophytic ability (Jaffee & Zehr, 1985) and typically sporulates upon emergence from a parasitised host (Jaffee & Zehr, 1983; Jaffee *et al.*, 1990) or on agar, regardless of the presence or absence of nematodes (Jaffee *et al.*, 1992).

Stirling (1991) and Jaffee *et al.* (1992) questioned whether the nematode-trapping fungi respond specifically to an increase in populations of plant-parasitic nematodes. It has been hypothesized that like *H. rhossiliensis*, at least some nematode-trapping fungi will exhibit density-dependent parasitism in mineral soil (Jaffee *et al.*, 1992). In testing this hypothesis, Jaffee *et al.* (1993) found that dependency on nematodes was greatest for the endoparasites (e.g. *H. rhossiliensis*), intermediate for fungi with constricting rings or adhesive knobs (e.g. *Monacrosporium elliposporum* and *A. dactyloides*) and least for fungi with adhesive nets (e.g. *A. oligospora* and *M. cionopagum*). They suggested that for some trapping fungi the failure to respond to nematode density is indirect evidence that substrates other than nematodes are important sources of food. For a nematode trapping fungus such as *A. dactyloides*, nematodes are likely to influence the production of ring traps and the predatory behavior of the fungus. However, it is still not clear yet whether ring production by *A. dactyloides* and/or parasitism of the fungus on nematodes are density-dependent.

In most studies of nematophagous fungi, plant parasitic nematodes were introduced at about 25-50 juveniles per g soil which were much higher than the commonly occur in the field prior to planting annual crops (O'Brien & Stirling, 1991). Since populations of root-knot nematode as low as 0.01-0.1 nematodes per g soil may damage susceptible crops such as tomato (Netscher & Sikora, 1990), it will be important to determine whether such low populations are also sufficient to

initiate trapping by *A. dactyloides*. A similar situation could apply to free-living nematodes. Therefore, this paper reported a study on using low population of nematodes to determine the trapping activity of *Arthrobotrys dactyloides* due to the effects of (i) different populations of *Meloidogyne javanica*, (ii) the presence of *Caenorhabditis elegans*, (iii) repeated inoculation of *M. javanica*, and (iv) different populations of *C. elegans* and *M. javanica*.

## MATERIALS AND METHODS

All experiments in this study were conducted in Laboratory of Microbiology, Faculty of Agriculture, University of Mataram, from March until September 2008.

**Preparation of Sterile Second Stage Juveniles (J2) of *Meloidogyne javanica*.** *M. javanica* cultures were maintained on susceptible tomato plants (cv. Tiny Tim) grown in sandy soil in 1.2 L pots in the glasshouse for about three months. Eggs of *M. javanica* were extracted using sodium hypochlorite method (Hussey & Barker, 1973). Sterile second stage juveniles (J2) were produced by adding concentrated nematode-egg suspension to 10 ml agar (1%, 45-48°C) and mixed well. The agar-nematode mixture was 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°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 *Caenorhabditis elegans*.** *C. elegans* was cultured on a lawn of *Escherichia coli*. The bacterial lawn was prepared by evenly spreading 1 ml of an *E. coli* suspension on the surface of one-fourth strength nutrient agar in a Petri dish. The Petri dish was incubated at 27°C for 5 days and then inoculated with *C. elegans*. When needed, nematodes were washed from the plate with sterile water.

**Preparation of Sterile Soil.** Sandy soil (22% coarse sand, 44% fine sand, 13% silt and 21% clay) taken from a field was air dried by spreading it in a shaded area for a few days. The air dried soil was then sieved with a 2

mm-aperture sieve and moistened up to approximately 15% moisture content. The moist soil was then placed in a plastic bag and autoclaved on three consecutive days at 121°C for 20 minutes before being used in experiments.

**Preparation of *A. dactyloides* and Mass Production of Mycelia.** *A. dactyloides* isolate Ampenan 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 27°C until needed. For mass production of the fungus, one square from this water culture was placed on CMA in a 9 cm diameter Petri dish and plates were incubated at 27°C for 6-7 days before use. Two 5 mm-diameter discs taken from an actively growing colony on CMA were inoculated to 250 ml Erlenmeyer flasks containing 100 ml of Glucose Peptone yeast (GPY) broth (15 g of glucose, 2 g of peptone, 5 g of yeast, 1 g of asparagine, 0.5 g of  $K_2HPO_4$ , 0.25 g of  $MgSO_4 \cdot 7H_2O$ , 0.001 g of thiamine HCl, 1 L of  $H_2O$ ; Sudirman, 1997; 2009). Flasks were incubated at 27°C on a rotary shaker at 120 rpm. After 10 days of incubation, about 0.007 g dry wt biomass/ml was produced. Mycelial suspension was prepared by homogenizing the flask culture for 15 seconds in a blender.

**Production of *A. dactyloides* Formulation.** The fungus was formulated in kaolin-alginate granules based on the technique developed by Sudirman (1997; 2009). One hundred g of kaolin (MP Biomedical Inc, Ohio, USA) and 10 g of sodium alginate were added to 1 L of water. After autoclaving, 80 ml of the blended and sterilized kaolin-alginate mixture was mixed with 20 ml of mycelial suspension. The mixture was then mixed using a magnetic stirrer in a 1 L Erlenmeyer 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 Erlenmeyer flasks containing 100 ml of GPY broth and incubated on a shaker at 27°C. After 3 days, the re-fermented granules were harvested and dried on a sterile wire mesh. The average diameter and weight of a granule were 3 mm and 3.5 g, respectively.

### **Effect of *M. javanica* Inoculum Densities on Ring Production and Trapping Activity of *A. dactyloides*.**

In the following experiments, five populations of *M. javanica* (6, 8, 10, 12, and 14 per g soil) were tested. The experiment for ring production was conducted using a standard slide test. Three granules containing *A. dactyloides* were placed at marked positions on a glass slide at the bottom of a 9-cm diameter Petri dish. The granules were covered with a piece of 100 mm-apertures nylon mesh cut to the same size as the glass slide. The Petri dish was then filled with 60 g of soil and moistened with water to approximately field capacity and inoculated with nematodes according to treatments. The Petri dish was placed in a moist-air-tight plastic container and incubated at 27°C. Five days later, the soil and nylon mesh were carefully removed by applying a little pressure with the tip of a fine forceps at the point of the nylon mesh where a granule was located. The edge of nylon mesh was lifted in such a way that granules remained in position with minimal disturbance to the growing mycelia. The mycelia were separated from granules by using a very sharp tip of a forceps to cut around the granules. The granules were removed carefully to avoid mycelia from being disarranged or dislodged. The slide was then flooded with lactoglycerol cotton blue and covered with a coverslip. Numbers of rings produced per granule were counted under a microscope from three randomly selected microscope fields. For each treatment, five replicates were provided.

For trapping activity, the experiment was carried out using soil microcosms. Microcosms were made from 38 mm internal diameter PVC pipe. Rings of 3 mm and 6 mm thick were cut from the pipe and a 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 thicker ring (approximately 7 ml) was filled with 9 g of soil and packed to a bulk density of approximately 1.3. The soil was then watered to nearly field capacity. Twenty granules (to gain rate of application 0.75% w/w) were buried in each microcosm. The microcosms were placed in 60 mm diameter Petri dishes and incubated at 27°C in an air-tight plastic container. After incubation for 5 days, 0.1 ml of a water suspension containing *M. javanica* J2 at various numbers according to treatments (6, 8, 10, 12, and 14 per g soil) was added to each microcosm and microcosms were then subjected to further incubation. Each treatment was replicated five times. Five microcosms without granules for each nematode density were provided as controls. Five days after inoculation, nematodes 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 27°C were counted. Trapping activity was expressed as percentage mortality of nematodes. The mortality was calculated as  $x = (1-a/b) 100\%$ , where  $a$  and  $b$  are the numbers of nematodes recovered from microcosms with granules and microcosms without granules, respectively.

**Effect of *C. elegans* on *M. javanica* Mortality Caused by *A. dactyloides* when *M. javanica* was Inoculated on Various Dates.** This experiment examined the effectiveness of trapping by *A. dactyloides* at various dates of *M. javanica* inoculation in the presence or the absence of *C. elegans*. Experiment was conducted using soil microcosm inoculated with 20 *A. dactyloides* granules as described previously. There were two different treatments: (i) soil infested with both granules and *C. elegans* (100 nematodes in 0.1 ml water), and (ii) soil infested with granules but without *C. elegans*. After 5, 10, 15, or 20 days of incubation at 27°C, five microcosms (replicates) from each treatment were inoculated with about 100 *M. javanica* J2 in 0.1 ml water and then re-incubated. Three days after inoculation, nematodes were extracted and *M. javanica* mortality was determined using the methods as before. Microcosms inoculated with both *C. elegans* and *M. javanica* but without granules were also provided as controls.

**Effect of Repeated Inoculation of *M. javanica* on *A. dactyloides* Trapping Activity.** This experiment aimed to determine the trapping activity of *A. dactyloides* when juveniles of *M. javanica* were added repeatedly. The test was carried out using the soil microcosm inoculated with 20 *A. dactyloides* granules. Eight treatments were applied: four treatments were one time inoculation of *M. javanica* at various dates (5, 10, 15, or 20 days after inoculation of granules), and the other four treatments were repeated inoculation of *M. javanica* at 2, 3, 4, and 5x frequencies for every five days. *M. javanica* was inoculated to each microcosm at the rate of 100 J2 in 0.1 ml water. Three days after inoculation/re-inoculation, nematodes were extracted and nematode mortality was determined using the methods as before. For each treatment level, there were five microcosms (replicates) and five microcosms without granules were provided as controls.

**Effect of *C. elegans* Inoculum Densities on *M. javanica* Mortality Caused by *A. dactyloides*.** This experiment aimed to determine the level of trapping

activity of *A. dactyloides* on *M. javanica* when different population densities of *C. elegans* were present in soil. The experiment was carried out using microcosms inoculated with *A. dactyloides* granules prepared as described previously. Six different population densities of *C. elegans* (6, 8, 10, 12, 14, and 16 nematodes/g soil) were separately inoculated into the microcosms. After 5 days of incubation, about 100 *M. javanica* J2 were inoculated into each microcosm and the microcosms were re-incubated for 3 days. Nematodes were extracted and their mortalities were determined using the methods as before. For each treatment there were five replicates. Microcosms without granules but inoculated with both *C. elegans* and *M. javanica* served as controls.

**Effect of *C. elegans* and *M. javanica* Inoculum Densities on *A. dactyloides* Trapping Activity.** This experiment examined the trapping of *M. javanica* and *C. elegans* by *A. dactyloides* when the two nematode species existed at different population densities in soil. Microcosms containing *A. dactyloides* granules were provided as described previously. After 5 days of incubation at 27°C, the microcosms were inoculated with both *C. elegans* and *M. javanica* at four different populations of each nematode (6, 8, 10, and 12 nematodes/g soil). The microcosms were re-incubated for further three days, after which the nematodes in each microcosm were extracted and nematode mortality was determined using the methods as before. There was a 4x4 factorial experiment. Each treatment was repeated five times. Microcosms without granules but inoculated with both *C. elegans* and *M. javanica* were also provided as controls.

**Statistical Analysis.** All experiments were conducted in a completely randomized design. The data were analyzed by means of analysis of variance procedure using GenStat® Discovery 2<sup>nd</sup> Edition. Before analysis nematode mortality data were transformed into arcsine "x. When the variance ratio ( $F$ ) was significant, means for each treatment were separated using Tukey's Honestly Significant Difference (HSD) test.

## RESULTS AND DISCUSSION

The number of nematodes (*M. javanica* or *C. elegans*) recovered from microcosms without granules containing *A. dactyloides* at all experiments were approximately the same as the number of nematodes when they were inoculated. In contrast, when microcosms were infested with *A. dactyloides* granules,

the number of nematodes recovered decreased significantly. Differences in the number of recovered nematodes between microcosms without granules and microcosms infested with granules were considered as nematode mortalities due to trapping activities of *A. dactyloides*.

In general, results of the experiments described in this study demonstrate that not only did nematode presence stimulate ring formation, but the increase of nematode population densities also significantly increased both the number of rings formed and trapping activities of *A. dactyloides*. The experiment on the effect of *M. javanica* inoculum densities on ring production and trapping activity of *A. dactyloides* showed that nematode population had significant effect on the number of rings produced ( $P = 0.01$ ) and nematode mortality ( $P = 0.01$ ). The number of ring and nematode mortality increased significantly as the population density of nematodes increased (Figure 1). Figure 1A showed that the lowest and the highest number of rings were observed at treatments with the lowest and the highest population densities of *M. javanica*, respectively. As there were no rings observed in control without nematodes, the results of the experiment showed that *M. javanica* stimulated ring formation. The number of rings produced by *A. dactyloides* positively correlated with *M. javanica* inoculum densities ( $R^2 = 0.97$ ), suggesting that *A. dactyloides* responded positively to the number of *M. javanica* present in soil.

In this study, trapping activity was manifested as reduced recovery of assayed nematodes. Results of the experiments on trapping activity indicated that the trapping activity by *A. dactyloides* was correlated with the number of rings formed ( $R^2 = 0.98$ ). The number of rings produced increased with the increasing populations of *M. javanica* inoculated (Figure 1A). Accordingly, the mortality of *M. javanica* significantly increased as the population of this nematode increased (Figure 1B). The lowest and the highest mortalities of *M. javanica* were observed at treatments with the lowest and the highest population of *M. javanica*, respectively.

Nordbring-Hertz (1977) demonstrated that nematodes have a dual function with regard to the nematode-trapping fungi: they act as efficient inducers of trap formation and serve as fungal prey. This dual function was illustrated by the present results with *A. dactyloides*. Nematodes had significant effects on ring formation and nematode mortality as tests with different populations of nematodes resulted in more rings being formed and more nematodes being trapped in treatments with higher populations of nematodes (Figure 1). The importance of nematodes as fungal prey become clear

with the results of the experiment on both the effect of the presence of *C. elegans* and the effect of repeating inoculation of *M. javanica* on trapping activity by *A. dactyloides*. *A. dactyloides* remained effectively trapped nematodes when *C. elegans* was present (Figure 2) or when *M. javanica* was introduced repeatedly (Figures 3). The presence of *C. elegans* had a significant effect on mortality of *M. javanica* (Figure 2). When *C. elegans* was present, mortality of *M. javanica* remained high throughout the experiment. When *C. elegans* was absent, however, mortality of *M. javanica* was more than 93% when *M. javanica* were inoculated on day 5 and day 10, then decreased significantly when *M. javanica* was inoculated on day 15 and day 20.

The roles of nematodes as ring formation inducer and prey become more obvious as repeated inoculation of *M. javanica* significantly influenced mortality of this nematode ( $P = 0.01$ , Figure 3). Mortality of *M. javanica* in microcosms that had been repeatedly inoculated with *M. javanica* was significantly higher than those receiving just one inoculation and remained constantly high during the experiment. With just one inoculation of *M. javanica*, nematode mortalities were high on days 5 and 10, then decreased significantly by days 15 and 20.

Results of this study indicated that the trapping activity of *A. dactyloides* on nematodes, manifested as mortalities of *M. javanica* and *C. elegans*, was strongly influenced by the presence and the number of *C. elegans* in soil. The importance of free-living nematodes on mortality of *M. javanica* caused by *A. dactyloides* was shown by the experiment with different numbers of *C. elegans* added into soil. The numbers of *C. elegans* significantly influenced mortality of both *M. javanica* and *C. elegans* ( $P = 0.01$ , Figure 4). The mortality of both *C. elegans* and *M. javanica* increased as the population density of *C. elegans* increased. Although fewer *M. javanica* were trapped in the presence of *C. elegans* than in its absence, the trapping activity of *A. dactyloides* slightly increased with increasing inoculum densities of *C. elegans*, suggesting density dependence of nematode population.

The indication of density dependence of *A. dactyloides* trapping activity was strengthened by the results on the experiment with various inoculation densities of both *C. elegans* and *M. javanica*. The results of this experiment showed that the number of both *C. elegans* and *M. javanica*, and their interaction significantly influenced the mortality of both *M. javanica* and *C. elegans* ( $P = 0.01$ ,  $P = 0.01$ , respectively). The increase population densities of both *C. elegans* and

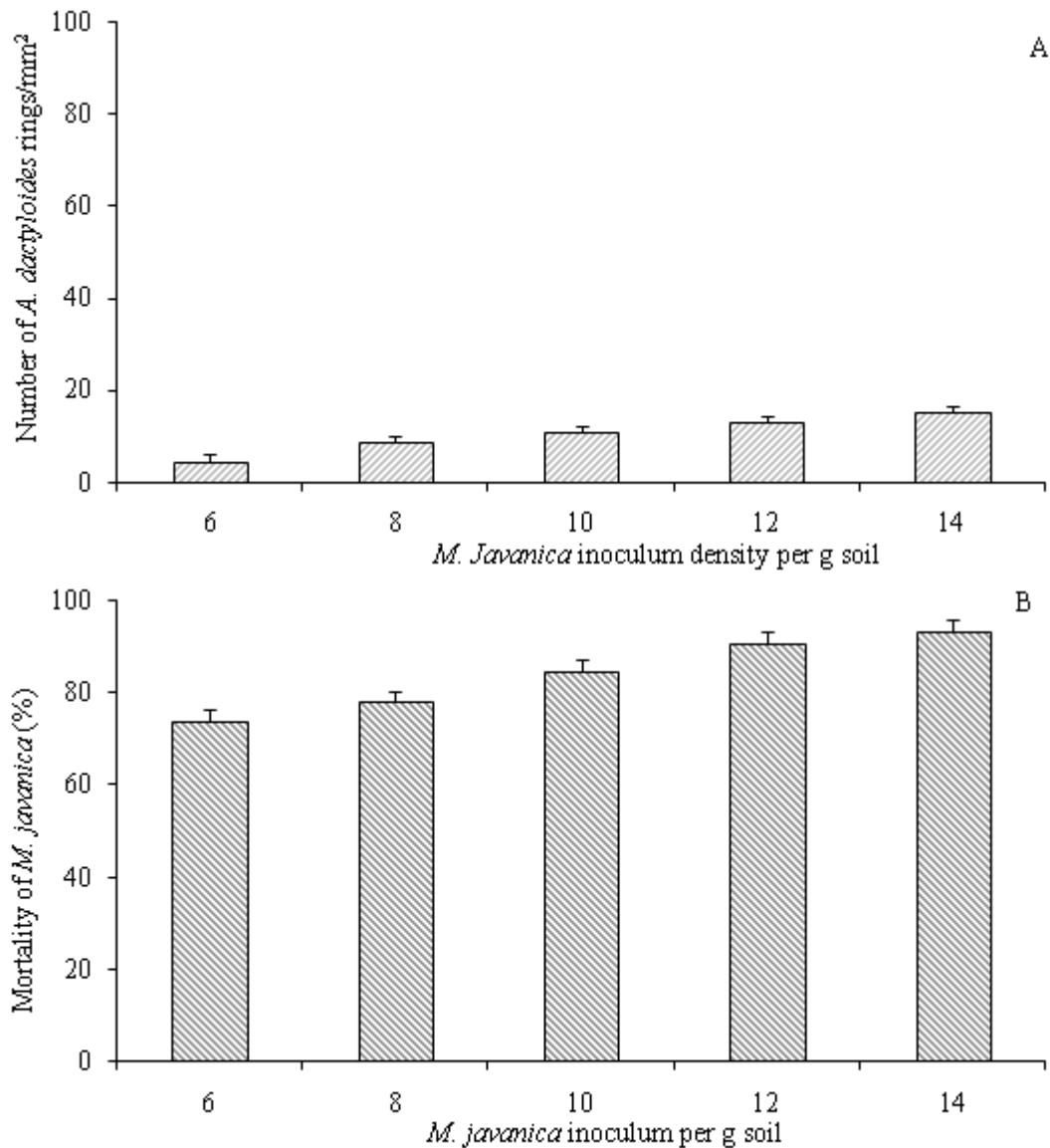


Figure 1. Number of rings (A) and percentage of *M. javanica* mortality (B) caused by *A. dactyloides* growing from kaolin-alginate granule formulation in soil with different *M. javanica* populations. Bars are the values of  $HSD_{0.01}$ .

*M. javanica* significantly increased mortality of both *M. javanica* and *C. elegans* (Table 1). This result was in accordance with the results of the other experiments, where there were more rings formed and higher nematode mortalities in treatments with higher populations of nematodes (Figure 1). The presence of both *C. elegans* and *M. javanica* and the increase of their population (Figure 2) seemed to stimulate ring formation by *A. dactyloides*, and hence to increase trapping activity of the fungus.

Results of the experiments in this study clearly demonstrated that the trapping activity remained high with the presence of higher nematode population densities of both *C. elegans* and *M. javanica*.

Interestingly, the mortalities of *C. elegans* were higher than those of *M. javanica* when these two nematodes were present together (Figure 4, Table 1). This may be due to the fact that *C. elegans* is more mobile than *M. javanica*, and hence, *C. elegans* may have had more chances to contact with and being trapped by *A. dactyloides*. It has been reported that there was a positive correlation between nematode motility and trap formation, i.e. rapidly moving nematodes induced trap formation more rapidly than slow moving species (Nordbring-Hertz, 1973; Jansson & Nordbring-Hertz, 1980). In addition, when the total number of nematodes (*M. javanica* plus *C. elegans*) were taken into account, nematode mortality was greater in the presence of *C.*



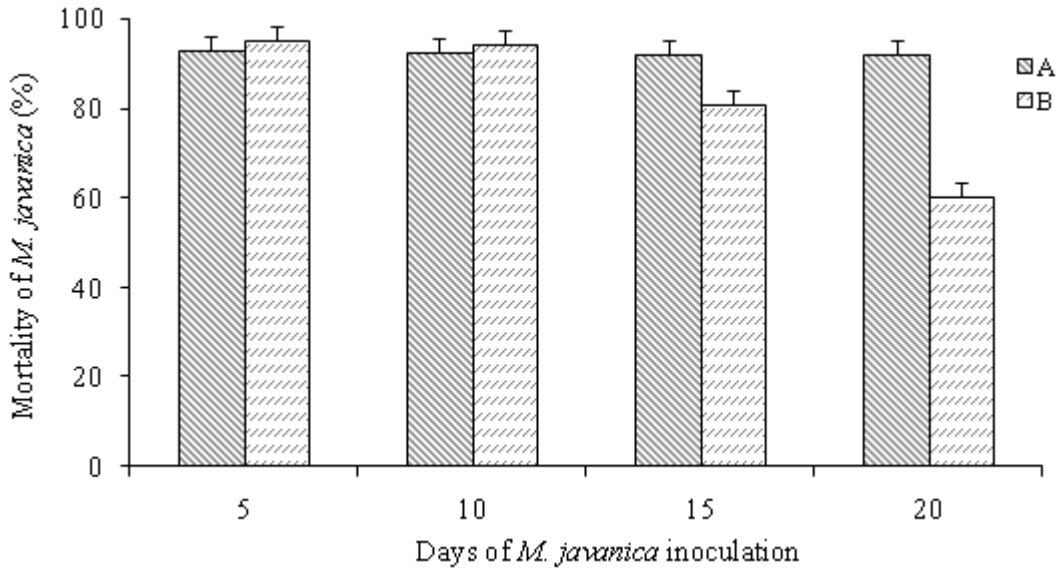


Figure 2. Mortality of *M. javanica* inoculated at various days after introduction of kaolin-alginate granules containing *A. dactyloides* in microcosms infested with (A) or without *C. elegans* (B). Bars are the values of HSD<sub>0.01</sub>.

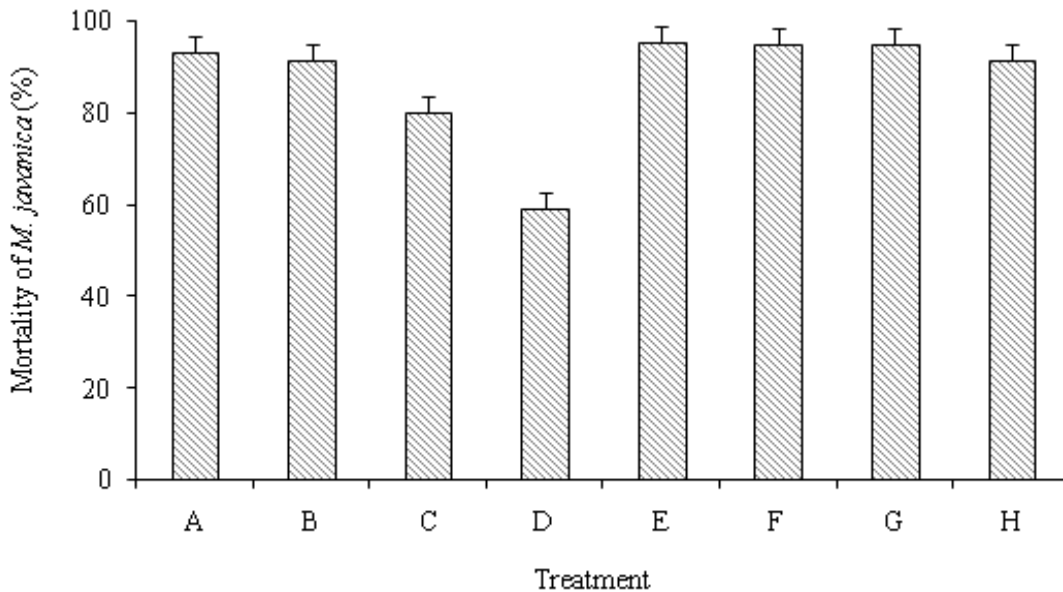


Figure 3. Mortality of *M. javanica* introduced at various dates and frequencies to soil microcosms containing *A. dactyloides* granules. A-D : 1 x inoculation of *M. javanica* at 5, 10, 15, and 20 days after introduction of *A. dactyloides* granules, respectively. E-H : 2, 3, 4, and 5 x inoculation of *M. javanica* for every 5 days, respectively. Bars are the values of HSD<sub>0.01</sub>.

*elegans*. This suggests that the trapping activities of *A. dactyloides* increase as the population of nematodes in soil increases.

The presence of *C. elegans* influenced not only the level of trapping activity but also the duration of effective trapping. In the absence of *C. elegans*, the mortality of *M. javanica* decreased as the period from inoculation increased from 15 to 20 days (Figure 2).

When *C. elegans* was present in soil, however, high mortality of *M. javanica* was maintained over time. The importance of nematodes in maintaining the trapping activity of *A. dactyloides* was confirmed in the repeated inoculation experiment of *M. javanica*, in which trapping activity remained high when nematode population was accumulated in soil (Figure 3). Furthermore, results of this study indicated that in

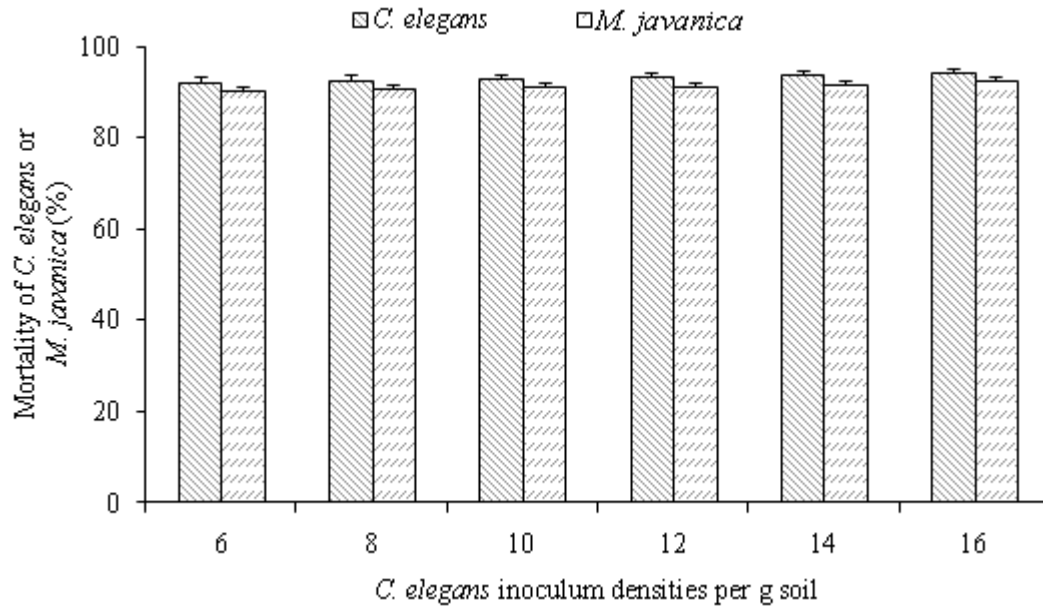


Figure 4. Mortality of *C. elegans* and *M. javanica* in microcosms with granules containing *A. dactyloides* following inoculation with different numbers of *C. elegans*. Bars are the values of  $HSD_{0.01}$ .

Table 1. Percent mortalities of *M. javanica* and *C. elegans* (in parenthesis) in microcosms with granules containing *A. dactyloides* and inoculated with different numbers of *C. elegans* and *M. javanica*

<i>C. elegans</i> inoculum density per g soil	<i>M. javanica</i> inoculum density per g soil			
	6	8	10	12
6	85.3 a*) (89.8 s)	86.5 b (90.9 st)	87.2 b (91.6 tu)	88.5 c (92.5 u)
8	86.4 b (91.5 u)	87.3 bc (92.3 uv)	88.3 c (93.1 vw)	90.3 d (94.2 wx)
10	87.9 c (92.4 uv)	90.3 d (93.2 u)	90.5 d (94.3 ux)	91.6 e (95.1 x)
12	88.2 c (93.0 v)	92.3 f (95.0 w)	92.9 f (96.0 wy)	93.3 f (96.6 y)

\*) The numbers followed by the same letters in the same rows or the same columns are not significantly different ( $HSD_{0.01} = 1.02$  and  $1.24$  for mortalities of *M. javanica* and *C. elegans*, respectively)

experiments with different densities of *M. javanica* (Figure 1), *C. elegans* (Figure 4), and both *M. javanica* and *C. elegans* (Table 1) nematode mortality increased as nematode density increased. Overall, the findings of the present study demonstrate the importance of nematodes (in this study, *M. javanica* and/or *C. elegans*) as a food source for *A. dactyloides* if it is to continue to trap nematodes effectively. Thus the ring forming fungus *A. dactyloides* appears to be dependent on population density of nematodes, a conclusion which is supported by the results of another study (Jaffee *et al.*, 1993).

## CONCLUSIONS

Ring formation and trapping activity of *A. dactyloides* increased with the increase of *M. javanica* population. The presence of *C. elegans* increased *M. javanica* mortality. Repeated inoculation of *M. javanica* maintained trapping activity of *A. dactyloides*. The mortality of *M. javanica* increased with the increase of both *C. elegans* and *M. javanica* population.



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