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Trapping Activity of Nematode-Trapping Fungus *Arthrobotrys dactyloides* on the Presence of Sawdust, Microorganisms, and Nematodes

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ABSTRACT

Once biological control agents of nematodes are introduced into soil, they must function in a very complex and dynamic environment. In soil, both nematodes and their antagonists are influenced by other biotic and abiotic factors. However, our knowledge of the effects of these factors in soil is limited. Hence, instead of focusing on the role of individual organisms in controlling nematode pests, this research aimed to determine the effects of sawdust, microorganisms, and nematodes on trapping activity of *Arthrobotrys dactyloides*. Experiments were conducted with "standard slide test" and "soil microcosm" using soil amended with various concentrations or without sawdust, with the presence or the absence of microorganisms, and with the presence or the absence of nematodes (*Caenorhabditis elegans* and/or *Meloidogyne javanica*). The experiments were carried out with completely randomized design and data were analyzed with analysis of variance followed by Tukey's Honestly Significant Different test. Results showed that sawdust did not have direct effect on ring formation and trapping activity of *A. dactyloides.* However, sawdust did have effect on microorganisms and *C. elegans* which then these two factors stimulated ring formation and trapping activity of *A. dactyloides.*

Keywords: *Arthrobotrys dactyloides*, *Meloidogyne javanica*, microorganisms, sawdust, Trapping activity

INTRODUCTION

The presence of organic matter in soil is known to affect the predatory behavior of fungal antagonists (Stirling, 1991). It was hypothesized that the addition of organic matter to soil stimulated nematode-trapping fungi and other inactive fungi into saprophytic competition, and because their poor competitive ability, nematode-trapping fungi avoided competition by preying on free-living nematodes (Cooke, 1977). However, there is almost no data in this area specifically for *Arthrobotrys dactyloides,* a nematode-trapping fungus that traps nematodes with constricting rings (Stirling, 1991). Results of previous study regarding this topic on the addition of organic matter into alginate granules containing *A. dactyloides* showed that sawdust, cassava, and rice husk did not have any significant effects on the growth, ring production and trapping activity of this fungus (Sudirman, 2009). Nevertheless, it was still possible that the addition of specific types of organic matter to soil could stimulate predatory behaviour in *A. dactyloides*. 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.

Most studies on organic amendments for nematode control (Lazarovits *et al*., 1999; Akhtar & 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 & Yermiyahu, 2002). Nematicidal effects, however, are relatively short-lived because ammonia concentrations remain high for a limited time (Cowling *et al*., 2001; Oka & Pivonia, 2002). Hence, instead of its nematicidal effects, the use of organic amendments is expected to control nematode through naturally occurring mechanisms.

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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 fungal-dominant soil biology and high numbers of omnivorous nematodes (Stirling *et al*., 2003).

Organic matter can also increase the activity of soil microorganisms which cause mycostatic and lytic effects on biological control agents. Stirling and Mani (1995), reported that no traps were produced by *Dactylella candida* or *Arthrobotrys dactyloides* in sand that had been amended with horse manure and compost. They suggested that the addition organic matters stimulated mycostatic and lytic effects of other soil microorganisms. Research on the predation of nematophagous fungi on the presence of various numbers of nematodes and levels of organic matter should be carried out. Result of a preliminary study (unpublished data) looking at the growth of *A. dactyloides* in four different types of organic matter showed that this fungus could grow well on organic matters with high carbon content such as; sawdust, corn cob, and rice husk, but it did not on substrate with high nitrogen content like chicken manure.

This study aimed to determine whether the addition of sawdust into soil stimulated the activity of *A. dactyloides* (ring production, trapping activity, and nematode mortality).

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 & Barker, 1973). Sterile second stage juveniles (J2) were produced by adding concentrated nematode-egg suspension to 10 ml agar (1%, 45-48^{0}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^{0}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.* *Caenorhabditis 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 (1.5% agar) in a Petri dish. The Petri dish was incubated at 27^{0}C for 5 days and then inoculated with *C. elegans*. When needed, nematodes were then washed from the plate with sterile water.

Preparation of Soil and Sawdust. 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. The nematode-free-soil was prepared by heating soil at 70^{0}C for about 60 minutes. Sterile soil was prepared by autoclaving moist soil in plastic bags for three consecutive days at 121^{0}C for 20 minutes before being used in experiments.

Sterile sawdust was prepared by the same manner with sterile soil. The carbon and nitrogen contents were 90% and 0.36%, respectively.

The Nematode-Trapping fungus, *Arthrobotrys dactyloides,* Mass Production of Mycelia and Formulation. 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 27^{0}C. 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 27^{0}C 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 27^{0}C 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.

The fungus was formulated into kaolin-alginate granules based on technique developed by Sudirman, (1997; 2009). 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 27^{0}C. 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.

Effect of Sawdust on Growth and Ring Production in Soil. This experiment aimed to determine the growth of *A. dactyloides* in soil amended with different amounts of sawdust. Six different concentrations (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0% w/w) of sawdust were separately added to sterile soil, moistened with sterile water and incubated for 8 weeks before being used. 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 mm 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 27^{0}C. 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 sawdust concentration on ring production, slide test with sterile soil amended with six different concentrations (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0% w/w) of sawdust were prepared. Three granules were put on one slide and five replicates for each treatment were prepared and processed as described previously. After 5 days incubation at 27^{0}C, the number of rings in each slide was observed.

Effect of Sawdust on Trapping activity of *A. dactyloides*. This experiment was conducted in soil microcosms using sterile soil amended and non-amended with 1% sawdust. For each treatment, 20 microcosms with granules formulated *A. dactyloides* and 20 microcosms without granules formulated *A. dactyloides* 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 and packed to a bulk density of approximately 1.3. 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 27^{0}C in an air-tight plastic container. After 5 days incubation at 27^{0}C, about 90 freshly hatched *M. javanica* (J2) were inoculated into each of five replicate microcosms of each treatment, and microcosms were re-incubated at 27^{0}C. 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 27^{0}C were counted. The inoculation of *M. javanica* was repeated at day 10, 15 and 20 and nematodes were extracted accordingly.

Effect of Sawdust and Microorganisms on Trapping Activity of *A. dactyloides*. This experiment was carried out with soil microcosms using nematode-free and sterile soil amended with or without sawdust (1% w/w). Before commencing the experiment, the number of propagules of microorganisms in each soil was determined. Forty microcosms (20 with granules and 20 without granules) for each treatment were prepared as described previously. After 5 days incubation at 27^{0}C, about 90 sterile *M. javanica* were inoculated into each of five replicate microcosms. Three days after inoculation, nematodes were extracted and counted, and the populations of microorganisms were determined by serial dilution method. The inoculation of *M. javanica* was repeated after 10, 15, and 20 days incubation.

Effect of Sawdust, Microorganisms and *Caenorhabditis elegans* on Ring Production and Trapping Activity of *A. dactyloides*. For ring production, the experiment was conducted with a standard slide test using nematode-free and sterile soil, with and without sawdust, and with and without *C. elegans*. The soil and sawdust was mixed thoroughly, about 500 *C. elegans* were added into each dish and the experiment was set up as described previously, with five replicates for each treatment. After 5 days incubation at 27^{0}C, the number of rings was counted, the population density of *C. elegans* was determined, and populations of microorganisms were determined by serial dilution method.

For trapping activity, the experiment was carried out with soil microcosms using nematode-free and sterile soil, with or without sawdust, and with or without the presence of *C. elegans*. Sawdust (1% w/w) was added and mixed thoroughly into the soil. At day 0, about 90 *C. elegans* were introduced to the appropriate microcosms. Each treatment was set with 20 microcosms with granules and 20 microcosms without granules, with microcosms being prepared as described previously. After 5 days incubation at 27^{0}C, about 90 *M. javanica* were inoculated into five replicate microcosms. Three days after inoculation with *M. javanica*, nematodes were extracted and counted. The populations of microorganisms were determined by a serial dilution method. The inoculation of *M. javanica* was repeated for each treatment after incubation for 10, 15, and 20 days.

Statistical Analysis. All experiments were conducted using a completely randomized design with five replicates. 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. Before analysis data of nematode's mortality were transformed into arc sin dx.

RESULTS AND DISCUSSION

Effect of Sawdust on Growth and Ring Production of *A. dactyloides* in Soil. Neither the presence of sawdust nor its concentration had any significant effect on the growth of *A. dactyloides* in soil. The radial growth of mycelia from granules in the extreme treatments ranged from 16.2 ± 1.64 mm to 16.4 ± 1.14 mm. In all treatments, the fungus grew at a growth rate of approximately 0.85 mm per day. Sawdust did not stimulate ring production. Regardless of the concentration of sawdust used, rings were not observed.

It has been reported that for many species of nematode-trapping fungi, trap formation and trapping activity are conditioned by the nutrient status of the medium, especially the C/N ratio (Hayes & Blackburn, 1966; Nordbring-Hertz, 1968). In this study, however, sawdust with very high C/N ratio did not induce ring formation and trapping activity. Sawdust was used to minimize harmful effects of nitrogen on nematodes (Sarathchandra *et al*., 2001; Tenuta & Ferris, 2004) in order to conserve or enhance potentially useful biological control agent of nematode, in this case nematode trapping-fungus *A. dactyloides*. *A. dactyloides* grew on most carbon sources, but did not grow on organic materials with a high N content (i.e. poultry manure) (unpublished data). This suggests that attempts to enrich soil with carbon sources or the addition of organic amendments with high C/N ration will not have a damaging effect on *A. dactyloides*. This conclusion is supported by the observation that *A. dactyloides* grew well in soil amended with various concentrations of sawdust.

Effect of Sawdust on Trapping activity of *A. dactyloides*. Sawdust, incubation time and their

interaction significantly influenced trapping activity of *A. dactyloides*. Sawdust did not have any significant effect on mortality of *M. javanica* during the first 10 days (Figure 1). After incubation for 15 days, however, the presence of sawdust significantly increased the mortality of *M. javanica* and this effect became even more apparent by day 20.

It has been demonstrated unequivocally in many reports (Stirling & Many, 1995; Sudirman, 1997; 2009), that trapping activity is strongly related to ring formation. Sawdust did not have a direct effect on ring formation or on trapping activity, as there was no significant difference in nematode mortality in amended and non-amended soils soon after *A. dactyloides* was added to sterile soil. However, sawdust seemed to serve as food source for *A. dactyloides*, because as the incubation time increased, nematode mortality in sawdust-amended soil was constantly higher than in non-amended soil (Figure 1). Barron (1992), suggested that nematode-trapping fungi are adapted to high C, low N habitats, and appear to trap nematodes to obtain N. The importance of nematodes in trap formation was demonstrated by Jaffee (2002; 2004), who reported that without the presence of nematodes, nematode trapping-fungi were never observed to form traps in microcosms containing amended sterile soil. While in non sterile condition, in which *Arthrobotrys* is supposed to use carbon sources and nematodes as a nitrogen source, is considered a relatively poor competitor (Jaffee, 2003).

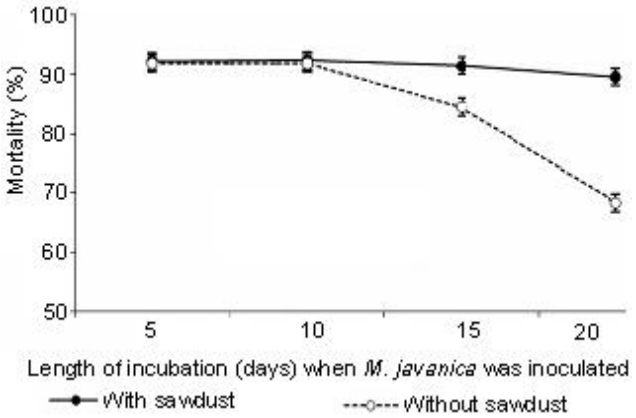

Figure 1. Mortality of *M. javanica*, introduced at various periods after introduction of granules containing *A. dactyloides* in sterile soil with and without the addition of sawdust. Bars are values of $HSD_{0.01}$

Effect of Sawdust and Microorganisms on Trapping Activity of *A. dactyloides*. Sawdust, microorganisms, incubation time, and their interaction significantly influenced trapping activity. The result showed that no significant difference in nematode mortality after introducing *M. javanica* following 5 days inoculation. (Figure 2). As incubation time increased, nematode mortality decreased significantly in microcosms containing sterile or nematode-free soil that was not amended with sawdust. In contrast,

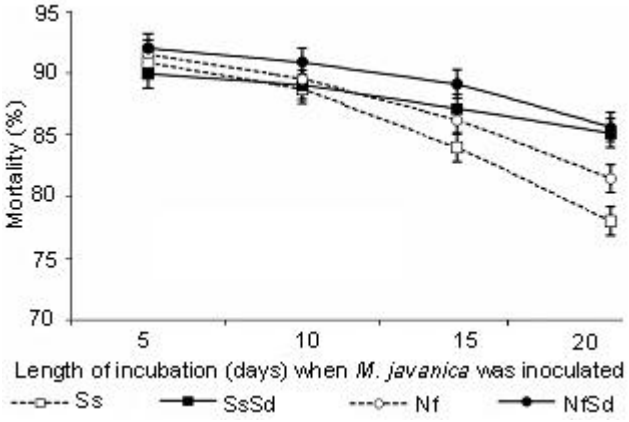

Figure 2. Mortality of *M. javanica* introduced at various periods after the introduction of granules containing *A. dactyloides* in microcosms containing nematode-free and sterile soil amended with or not amended with sawdust. Bars are values of $HSD_{0.01}$. (Ss = sterile soil, Nf = Nematode-free soil, SsSd = Sterile soil amended with sawdust, and NfSd = Nematode-free soil amended with sawdust)

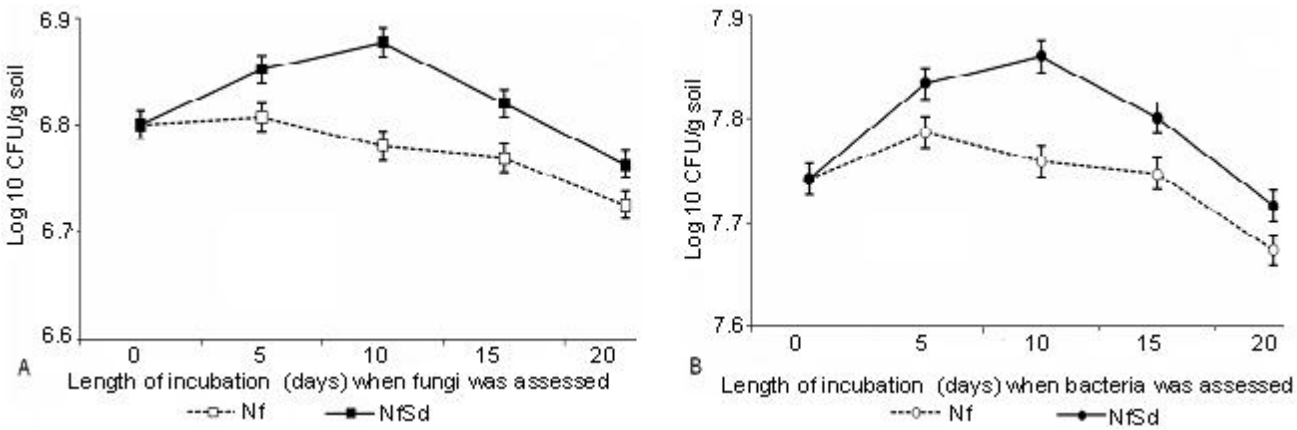

Figure 3. Populations of fungi (A) and bacteria (B) assessed after various periods of incubation in nematode-free soil with and without sawdust. Bars are values of $HSD_{0.01}$. (Nf = nematode-free soil, and NfSd = nematode-free soil amended with sawdust)

nematode mortality remained at a high level until day 15 in soil amended with sawdust, and then began to decrease. The addition of sawdust significantly increased the population of both fungi (Figure 3.A) and bacteria (Figure 3.B), especially during the first 10 days. At days 15 and 20, the population of both fungi and bacteria had decreased significantly. Fungal and bacterial populations were higher in soil amended with sawdust than in non-amended soil.

Effect of Sawdust, Microorganisms and *Caenorhabditis elegans* on Ring Production and Trapping Activity. Sawdust, microorganisms, *C. elegans*, and their interaction significantly influenced ring formation (Figure 4A). In the absence of microorganisms and *C. elegans*, no rings were formed, regardless of the presence or absence of sawdust. Microorganisms had a slight effect on ring formation, as no rings were formed in sterile soil whereas some rings were produced in nematode-free soil. *C. elegans* strongly influenced ring formation, as a large number of rings were formed when this nematode was present. Significantly more rings were formed when sawdust, microorganisms, and *C. elegans* were present together compared with when one of these factors was absent.

Sawdust did have an indirect effect on ring formation and trapping activity by influencing microorganisms and

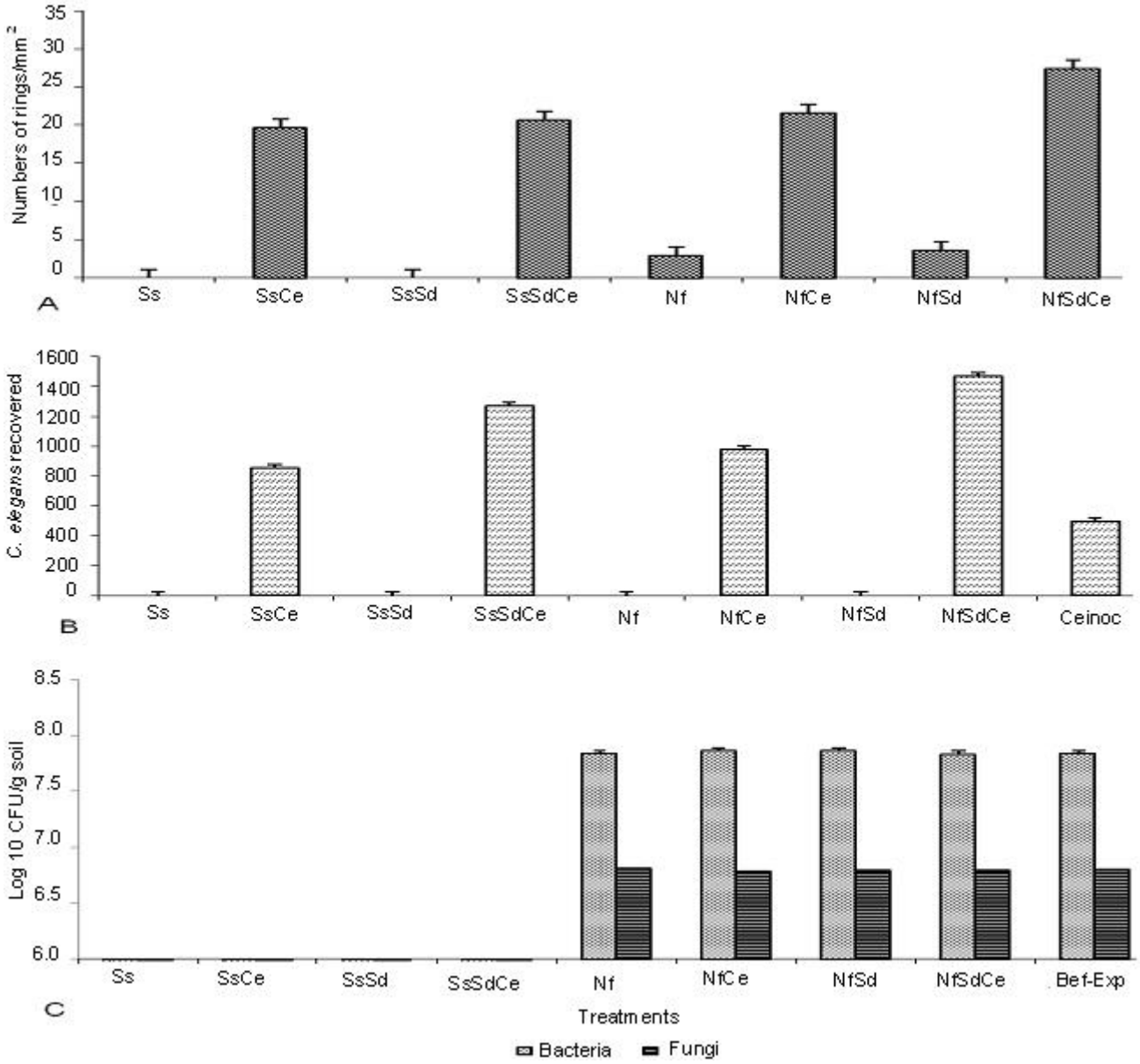

Figure 4. The effect of sawdust, microorganisms and *C. elegans* on the number of rings (A) produced by *A. dactyloides*, number of *C. elegans* recovered (B), and the population of micro-organisms (C). Bars are values of $HSD_{0.01}$. (Nf = nematode-free soil, NfSd = nematode-free soil with sawdust, NfCe = nematode-free soil with *C. elegans*, NfSdCe = nematode-free soil with sawdust and *C. elegans*, Ss = sterile soil, SsSd = sterile soil with sawdust, SsCe = Sterile soil with *C. elegans*, and SsSdCe = sterile soil with sawdust and C. elegans, Ce inoc = numbers of C. elegans inoculated, bef.exp = before experiment)

free-living nematodes or other soil biota. In non-sterile soil, the addition of sawdust caused populations of bacteria and fungi to increase. These microorganism population increases resulted in an increase in the number of rings. It is suggested that microorganisms may produce substances that stimulated ring formation. The indirect effect of sawdust on ring stimulation was apparent when microorganisms together with *C. elegans* were present in soil. Besides increasing the population of microorganisms, the addition of sawdust also increased populations of *C. elegans*. Since both of these factors can stimulate ring formation, the number of rings produced in soil amended with sawdust was much higher than in soil without sawdust. Conteh *et al.,* (1997), stated that decomposed organic amendments release organic compounds that are readily utilizable by soil microorganisms. It was not surprising, therefore, that population of bacteria and fungi increased in amended soil. Since bacteria and fungi are parts of free-living nematode's food sources, the increase of these microorganisms populations led to the increase of *C. elegans* population during decomposition process.

The presence of microorganisms significantly increased the population of *C. elegans*. This effect became more pronounced when sawdust was added to soil (Figure 4 B). The presence of *C. elegans* significantly reduced the population of bacteria (Figure 4.C).

Furthermore, microorganisms, sawdust, *C. elegans*, and their interaction significantly influenced trapping activity. There was a significant interaction between sawdust, microorganisms and *C. elegans* with regards to nematode mortality. This effect became more pronounced as incubation time increased. At day 5, there was significantly higher nematode mortality in soil without *C. elegans* and sawdust than in soil in which *C. elegans* and/or sawdust were present. As the incubation time increased, however, the mortality of *M. javanica* decreased significantly in soil without *C. elegans* and sawdust (Figure 5).

In sawdust amended sterile soil, the mortality of *M. javanica* decreased significantly at day 10. However, when sawdust was present with microorganisms, the mortality of *M. javanica* was relatively constant until day 15 and decreased significantly at day 20. When *C. elegans* and microorganisms were absent, *M. javanica* mortality decreased slightly until day 15 and then significantly at day 20. In contrast, when sawdust, microorganisms and *C. elegans* were present, mortality of *M. javanica* remained relatively high for the 20 day observation period. The mortality of *M. javanica* was always higher in sawdust-amended soil than in non-amended soil.

The addition of sawdust significantly increased populations of *C. elegans* when compared to soil without sawdust (Figure 6). In microcosms without granules, the addition of sawdust significantly increased populations of *C. elegans*. This was particularly apparent on day 15 and 20, with *C. elegans* populations being significantly higher when microorganisms were present. In microcosms with granules, the population of *C. elegans* recovered was higher when microorganisms and/or sawdust were not present.

The addition of sawdust and the presence of *C. elegans* significantly influenced the population of microorganisms (Figure 7). The population of bacteria (Figure 7 A) and fungi (Figure 7 B) increased significantly in microcosms with sawdust alone. When *C. elegans* was present, however, the population of bacteria and fungi decreased significantly.

The presence of microorganisms and *C. elegans* in sawdust amended soil, the system became more complicated, as sawdust served as a food source not only for *A. dactyloides* but also for microorganisms and *C. elegans*. In this condition, trapping activity of *A. dactyloides* appeared to decline, particularly if only the mortality of *M. javanica* was measured. There was

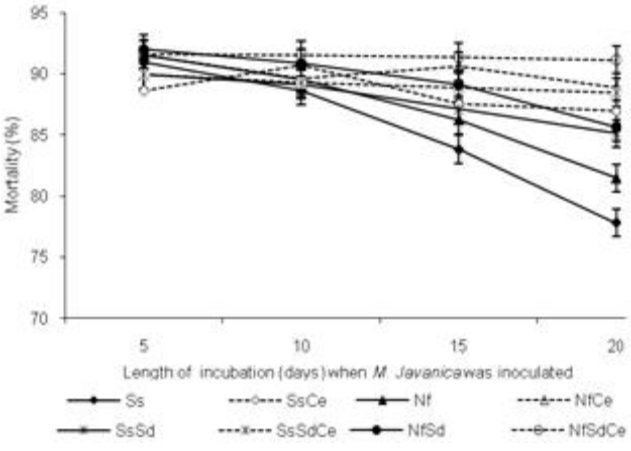

Figure 5. Mortality of *M. javanica* introduced at various periods after introduction of granules containing *A. dactyloides* in microcosms with various treatment combinations. Bars are values of $HSD_{0.01}$. Nf = nematode-free soil, NfSd = nematode-free soil with sawdust, NfCe = nematode-free soil with *C. elegans*, NfSdCe = nematode-free soil with sawdust and *C. elegans*, Ss = sterile soil, SsSd = sterile soil with sawdust, SsCe = Sterile soil with *C. elegans*, and SsSdCe = sterile soil with sawdust and *C. elegans*

higher mortality of *M. javanica* in soil without sawdust and *C. elegans* than in soil amended with sawdust and inoculated with *C. elegans*. However, if the total mortality of nematodes (*M. javanica* plus *C. elegans*) is considered, the trapping activity of *A. dactyloides* in soil amended with sawdust (in the presence of microorganisms and *C. elegans*), was much higher than in non-amended soil. This indicates that the addition of sawdust caused an increase in population of both bacteria and fungi, and an increase in population of *C. elegans* (observed from microcosms without granules). The population and the activity *of A. dactyloides* probably increased, either to escape increasing competition from other soil saprophytes or in response to the increase in the population of *C. elegans*.

The role of sawdust in influencing biological activity in soil microcosms became more apparent as the time of incubation or the length of observation increased. In soil without sawdust, the population of microorganisms and *C. elegans* decreased significantly over time, whereas in soil with sawdust, the population of both microorganisms and *C. elegans* increased at day 5 and remained high until the end of the experiment (20 days). Populations of *C. elegans* recovered from microcosms with granules were constantly low during the experiment, suggesting that the addition of sawdust did not have any significant effect on populations of *C. elegans*. However, when compared with the populations of *C. elegans* recovered from microcosms without granules, it was clear that the addition of sawdust significantly increased the populations of *C. elegans*. The differences in the number of *C. elegans* recovered from microcosms with and without granules indicate that *A. dactyloides* used *C. elegans* as one of its food sources. As a result of this continually available food source (i.e. *C. elegans*), *A. dactyloides* survived and remained active and effective in controlling plant parasitic nematodes (i.e. inoculated *M. javanica*) for a much longer period.

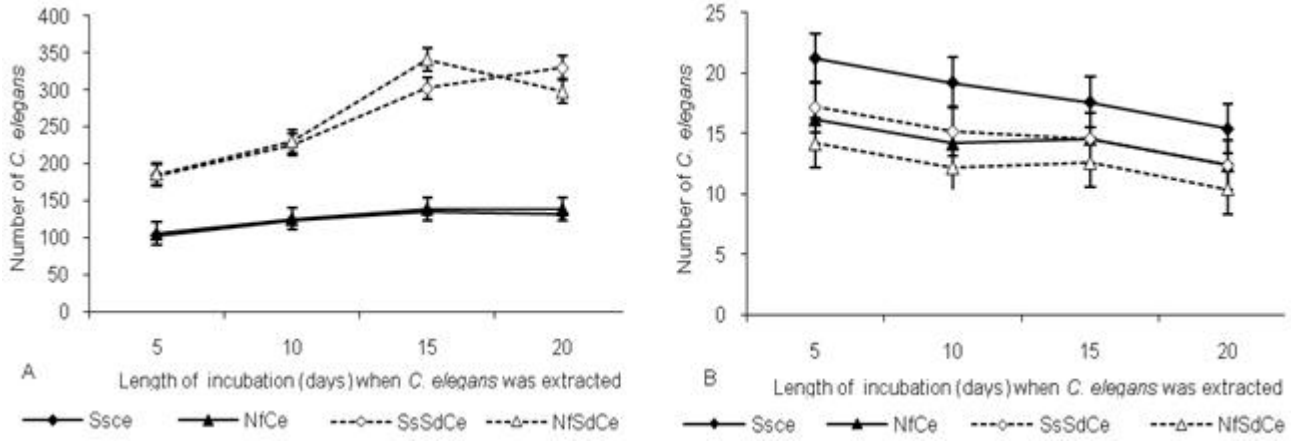

Figure 6. Number of *C. elegans* recovered after various periods of incubation in microcosms treated with various treatments. A) without granules and B) with granules. Bars are values of $HSD_{0.01}$. SsCe = sterile soil with *C. elegans*, NfCe = nematode-free soil with *C. elegans*, SsSdCe = sterile soil amended with sawdust and *C. elegans* , and NfSdCe = nematode-free soil amended with sawdust and *C. elegans*

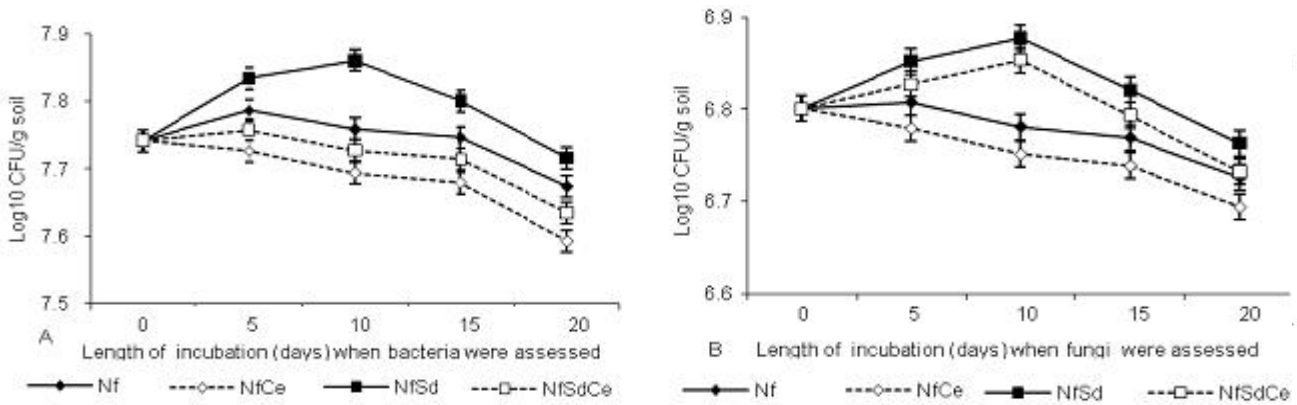

Figure 7. The population of bacteria (A) and fungi (B) after various incubation periods in microcosms with various treatments. Bars are values of $LSD_{0.01}$. Nf = nematode-free soil, NfCe = nematode-free soil with *C. elegans*, NfSd = nematode-free soil amended with sawdust, and NfSdCe = nematode-free soil amended with sawdust and *C. elegans*

CONCLUSION

Sawdust had no direct effect on ring formation by *A. dactyloides*, but it prolonged survival of the fungus in soil. Sawdust served as a food source for *A. dactyloides*, microorganisms, and *C. elegans*. Sawdust improved population of microorganisms and *C. elegans* which then significantly stimulated ring formation and trapping activity of *A. dactyloides*.

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