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EFFICACY OF ESSENTIAL OILS FROM BARK AND LEAF OF *CINNAMOMUM ZEYLANICUM* ON ROOT KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* IN RICE SEEDLINGS AND YOUNG RICE PLANTS

L.D. AMARASINGHE[1*], W.K.A.G.A. WIJESINGHE[1] and B. K. JAYAWARDHANE[2]

[1]Department of Zoology, University of Kelaniya, Kelaniya, 11600, Sri Lanka
[2]Department of Chemistry, University of Kelaniya, Kelaniya, 11600, Sri Lanka

ABSTRACT

The present study was conducted to evaluate the efficacy of essential oils from leaf and bark of cinnamon, *Cinnamomum zeylanicum* in controlling *Meloidogyne graminicola* and their effects on rice seedlings and young rice plants. This study was done; to determine the LC_{50} of essential oils of the extracts of cinnamon leaf and bark to kill 50% of second stage juveniles (J_2) of *Meloidogyne graminicola* in rice root galls; to determine the efficacy of essential oils of cinnamon; in controlling J_2 in rice seedlings and young rice plants; and on the growth of rice plants.

Cinnamon bark oil sample used in the current study contained about 72% cinnamaldehyde and the leaf sample contained about 86% eugenol. LC_{50} for cinnamon leaf oil and cinnamon bark oil for killing 50% of juveniles of *M. graminicola* in rice root galls after three days of the treatment was 0.326 ppm and 0.454 ppm respectively. Number of galls in the root system of nematode infested rice seedlings was significantly reduced when they were treated with 0.9 ppm of cinnamon leaf oil and bark oil compared to untreated controls. However, the nematicidal activity between the cinnamon leaf oil and bark oil was not significantly different. The root gall index of infested young rice plants was significantly lowered when they were treated with either cinnamon leaf oil or bark oil compared to untreated controls. It was revealed that the mean plant height and the mean number of roots of essential oil treated plants were positively correlated with the number of root galls whereas, the number of dead plants and the mean percentage chlorosis were negatively correlated. Nevertheless, the mean root length did not show any significant correlation with number of root galls. This study concludes that essential oils of cinnamon

[*] Corresponding author: Email: deepika@kln.ac.lk

leaf which contained 86% eugenol and bark which contained 72% cinnamaldehyde are similarly effective in suppression of *M. graminicola* in rice seedlings and young rice plants. According to the rates of application of essential oils of cinnamon leaf and bark, none of them have significant negative effect on the plant growth. Hence, they can be used as an alternative nematicide against the rice root knot nematode, *M. graminicola* in early stage of rice plants.

Keywords: *Cinnamomum zeylanicum*; essential oils; *Meloidogyne graminicola;Oryza sativa*

INTRODUCTION

Meloidogyne graminicola (L. Golden and Birchfield) is recorded as a pest of rice,*Oryza sativa* in Sri Lanka **(**Ekanayake & Toida, 1997, Anon, 2001; Nugaliyadde *et al*., 2001; Amarasinghe *et al*., 2007). Nugaliadde *et al*., 2001 reported that there is a yield loss due to this nematode species when more than 75% of the root system of the plant is infested.

The characteristic damage symptoms of *M. graminicola* are the enlargement of roots and formation of hooked like galls in the root tips of infested plants. They cause arrestment of root growth. Usually, there are no distinct symptoms on foliage of affected plants unless the nematode infestation becomes severe. In very severe cases, newly emerged leaves appear distorted and dried up along the margins and chlorosis can be seen. Infested plants show stunting growth resulting of a considerable yield loss (Ou, 1985). Integrated nematode management is the widely recognized method of controlling the *Meloidogyne* species (Bridge & Aploeright, 2005; Whitehead, 1998; Amarasinghe *et al*., 2007) that aim to prevent further distribution of the nematode and to decrease the yield loss. In Sri Lanka, suppression of *M. graminicola* has been achieved with varying success by integrating a number of physical, chemical, and cultural measures that introduced by the Department of Agriculture, Sri Lanka (Anon., 2001).

In later years, several authors have demonstrated to use essential oils and their components from variety of plant species as alternative bio control agents against plant parasitic nematodes (Abbas *et al*., 2009; Choi *et al.*, 2007; Kong *et al.*, 2007; Albuquerque *et al*., 2007). The present study was carried out to determine the efficacy of essential oils of leaf and bark of *Cinnamomum zeylanicum* on the second stage juveniles of *M. graminicola* and their effects on rice seedlings and young rice plants.

MATERIALS AND METHODS

Source of *Meloidogyne graminicola* inoculum

Identification of *Meloidogyne graminicola* was done to the species level using the characteristic cuticular pattern that surrounds the vulva and anus of the adult female nematodes (Taylor & Netscher, 1974). Infested rice plants were maintained in a 36cm diameter and 18cm deep plastic basin half filled with moisten de-faunated soil to raise the nematode population. The root galls positive for nematodes and active juveniles collected from them were used for experiments.

Extraction of essential oils from bark and leaf of cinnamon, *Cinnamomum zeylanicum*

A 40g sample of plant material, cinnamon bark and cinnamon leaf, was treated with a 1 % solution of cellulase and 1 % pectinase enzyme and incubated with 50ml of pH 7.4, 0.05 M phosphate buffer for 14 days separately. After the incubation period, the plant material was transferred to two flasks and essential oils were extracted separately for 3h using steam distillation. A 1µl of each sample was analyzed using Hewlett Packard gas chromatograph (Model 5890) equipped with a FID detector (Flame Ionization Detector). Gas chromatography profile was compared with reported values. Cinnamon bark oil sample used in the current study contained about 72% cinnamaldehyde and the leaf sample contained about 86% eugenol. 0.5µl of Tween-20 was used to dissolve 1µl of cinnamon oils. Concentrations of essential oils were prepared using serial dilution with distilled water.

Experiment I: Screening the effect of different concentrations of cinnamon leaf oil and bark oil on J_2 of *Meloidogyne graminicola* in the laboratory.

5ml each from 0.01, 0.03, 0.1, 0.3 and 0.9 ppm of cinnamon leaf oil and 0.1, 0.3, and 0.9 ppm of cinnamon bark oil were added separately into cleaned petri dishes each measuring 5cm diameter. Each concentration was replicated 10 times. 5ml each of 0.5µl Tween-20 and distilled water were added separately into 10 petri dishes each and they were used as two sets of controls. Three root galls each expected to contain about 70 juveniles of *M. graminicola* were introduced into each petri dish. Closed Petri dishes were arranged in a complete randomized design at 26 ± 2 ° C room temperature. Three days after inoculation, root galls were teased individually and all J_2 stage nematodes were counted on a sedgewick rafter cell held under the microscope with 10 x 10 magnification.

Nematodes were confirmed as dead if they were motionless to a mechanical pricking and their bodies held straight after transferring them to distilled water.

Experiment II: Screening the effect of cinnamon leaf oil and bark oil on *Meloidogyne graminicola* in seedling rice.

Approximately 250 rice seeds were washed well with 0.5 mgl^{-1} potassium permanganate and then rinsed thoroughly with distilled water. They were equally spread on fifteen wetted filter papers each of 9 cm diameter. They were laid on the bottom of fifteen plastic containers each with 9 cm diameter and 6 cm height. Seeds were maintained until they are germinated in the laboratory. After one week, well grown 10 seedlings were allowed to remain in each pot and others were removed. Ten ml each of the following treatments were given separately into five containers and the treatments were replicated three times. T_1 - Distilled water with 100 juveniles; T_2 - 0.9 ppm cinnamon leaf oil with 100 juveniles; T_3 - 0.9 ppm cinnamon bark oil with 100 juveniles; T_4 - Distilled water only; T_5 - 0.5 ppm Tween-20 only**.** After one week of the treatment, a soil solution was added into each container to maintain the growth of the seedlings. Seedlings were allowed to remain for one more week in same containers to deploy the root system. The plants were then removed from containers and the number of root galls due to nematodes in each seedling was counted separately.

Experiment III: Screening the effect of cinnamon leaf oil and bark oil on *Meloidogyne graminicola* in potted rice plants

Twenty eight plastic containers (9 cm diameter and 6 cm height) were filled with moisten de-faunated soil up to 1/3 each of it. Rice plants at the rate of ten plants per container were maintained for two weeks from germination. Calculated amounts of NPK fertilizer was added into each pot as recommended by the authority of the Department of Agriculture, Sri Lanka. Four pots were given 10 ml of one of the following treatments. T_1 - Distilled water with 100 juveniles; T_2 - 0.9 ppm leaf oil with 100 juveniles; T_3 - 0.9 ppm bark oil with 100 juveniles; T_4 - Distilled water; T_5 - 0.9 ppm cinnamon leaf oil; T_6 - 0.9 ppm cinnamon bark oil; T_7 - 0.5 ppm Tween-20. Pots were maintained in a completely randomized design outside the laboratory. The height of the plant, the number of dead leaves, the number of roots, the length of roots, and the number of root galls in each plant and the number of dead plants in each pot, were recorded after three weeks from inoculation.

Data Analysis

Data were corrected for control mortality using Abbott's formula (Abbott, 1925). The LC_{50} values were calculated by profit values of adjusted mean percentage mortality (Minitab version 14). Data were subjected to analysis of variance including Least Significant Difference (LSD) and Duncan's multiple Range Test (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Experiment I: Screening the effect of different concentrations of cinnamon leaf and bark oil on J_2 of *Meloidogyne graminicola* in the laboratory

Figure 1 shows the adjusted percentage mortality of J_2 stage of *M. graminicola* against different concentrations of cinnamon leaf oil and bark oil. It indicates that the highest mortality of the nematode was in 0.9 ppm of both cinnamon leaf oil and bark oil. There was no significant difference in the nematode mortality between two concentration levels of leaf oil namely 0.3 ppm and 0.9 ppm. However, the nematode mortality was significantly different between 0.1 ppm and 0.9 ppm leaf oil ($p = 0.001$, $t = 3.89$, $df = 19$). Figure 2 envisages that the LC_{50} for cinnamon leaf oil is 0.326 ppm and bark oil for 50% mortality of J_2 *M. graminicola* is 0.454 ppm (Figure 3). It is envisaged from LC_{50} values that the concentration of cinnamon leaf oil required to kill 50% of J_2 *M. graminicola* is lower than that of cinnamon bark oil. However, there is no significant difference between the percentage mortality of J_2 in respective concentrations of cinnamon leaf oil and cinnamon bark oil ($p = 0.819$, $df = 1$).

Experiment II: Screening the effect of cinnamon leaf oil and bark oil on *Meloidogyne graminicola* in seedling rice

Table 1 shows the mean number of root galls in cinnamon leaf oil treated and cinnamon bark oil treated seedlings at the end of the experiment. The number of root galls were significantly lowered in the cinnamon leaf oil treated rice plants ($p = 0.033$, $t = -2.38$, $df = 13$) and cinnamon bark oil treated rice plants ($p = 0.01$, $t = -3.24$, $df = 9$) compared to untreated controls. However, the differences of gall formation are not significant between two essential oils ($p = 0.24$, $t = -1.24$, $df = 12$).

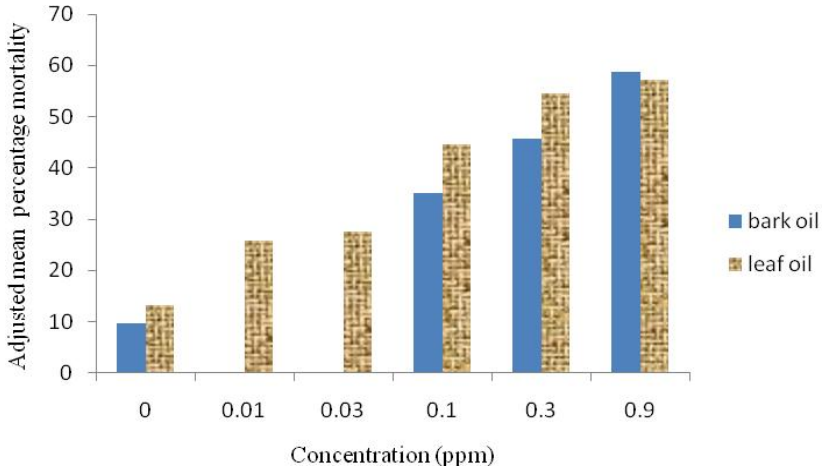

Figure 1: The adjusted percentage mortality (±SE) of second stage juveniles of *M. graminicola* against the different concentrations of cinnamon leaf oil after three days of treatment.

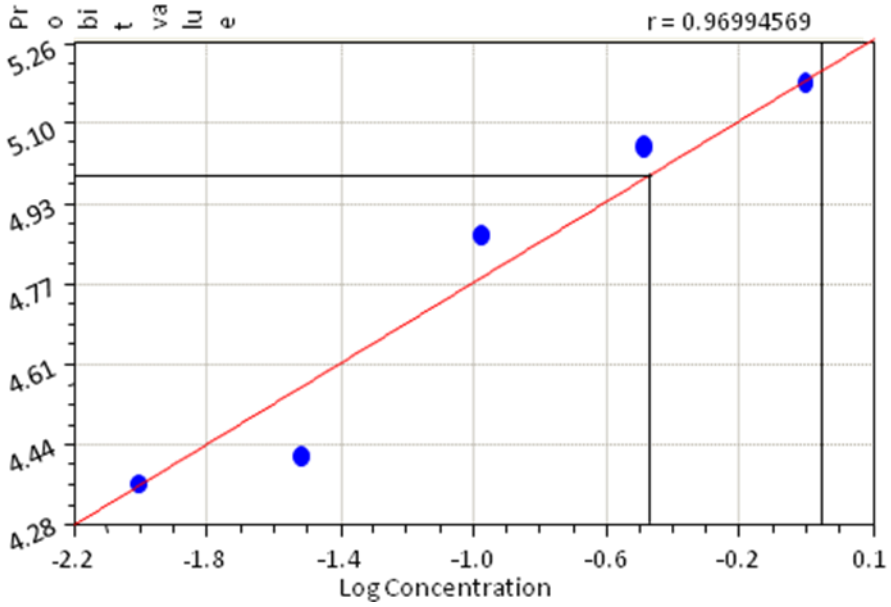

Figure 2: Probit values of adjusted percentage mortality of J_2 against log concentration of cinnamon leaf oil

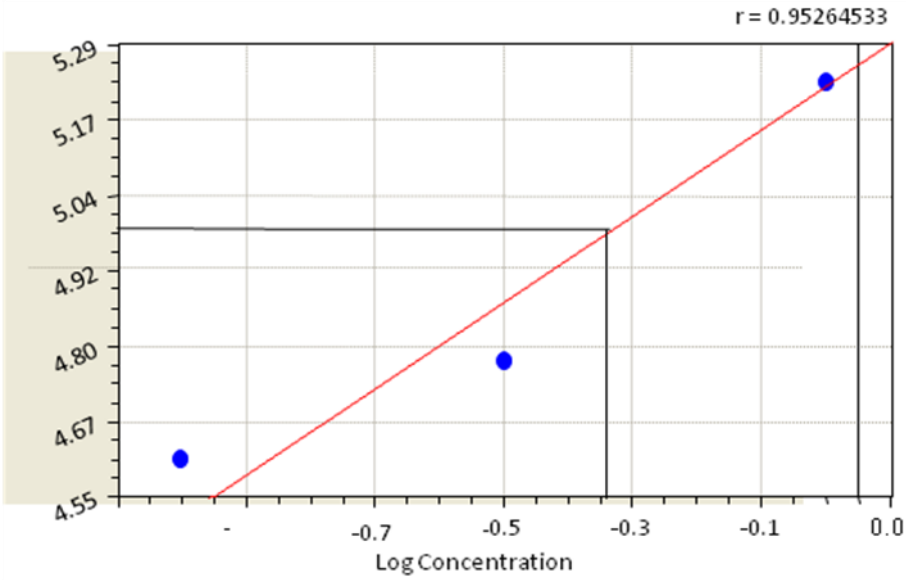

Figure 3: Probit values of adjusted percentage mortality of J_2 against Log concentration of cinnamon bark oil

Table 1: Mean total root galls in cinnamon leaf oil and cinnamon bark oil treated seedlings

Treatment	Mean total root galls (± SE)
Distilled water + juveniles (untreated)	16.0 ± 0.4^{a}
0.9ppm leaf oil + juveniles	4.0 ± 0.2^{b}
0.9 ppm bark oil + juveniles	1.0 ± 0.1^{b}
Distilled water	0
0.5 ppm Tween-20	1.0 ± 0.1^{b}

Experiment III: Screening the effect of cinnamon leaf oil and bark oil on *Meloidogyne graminicola* in potted rice plants

It was observed that the percentage chlorosis and mean number of dead plants have been increased in pots treated only with second stage juveniles (Table 2) ($p = 0.003$, $F = 6.58$). However, the mean plant height did not show and significant difference between treatments. This is indicative that there is no significant phyto-toxicity due to cinnamon leaf oil or Tween-20 on rice plants. The root gall formation was significantly reduced in

cinnamon leaf oil treated rice plants (n = 8.5 ± 0.13; p = 0.002, t = 3.34, df = 45) and bark oil treated plants (n= 8.3 ± 0.12; p = 0.000, t = 5.64, df = 45) compared to untreated plants (n = 16.1 ± 1.6). However, the gall indices between cinnamon leaf oil treated rice plants and cinnamon bark oil treated rice plants was not significant (p = 0.218, F = 1.89). An increased number of dead plants, the lowest plantheight and the highest chlorosis were observed in the plants treated only with nematode juveniles. However, neither the dead plants (p = 0.441, F = 0.82) nor plant height (p = 0.054, F = 0.82) was significantly different among treatments.

Table 2: Post-treatment measurements of cinnamon leaf oil treated young rice plants (±SE)

Treatment	Mean % dead plants	Mean plant height	Mean % chlorosis	Mean No. of roots	Mean root length	Mean No. of galls
Distilled water + J_2	20.0 ± 7.1^{ab}	14.4 ± 1.1^{b}	62.5 ± 6.7a	7.2 ± 0.7	4.9 ± 0.51	16.1 ± 1.6^{a}
0.9 ppm leaf oil + J_2	32.5 ± 4.7^{a}	15.4 ± 0.7^{ab}	59.1 ± 4.6^{ab}	6.9 ± 0.6	5.4 ± 0.54	8.5 ± 0.1^{b}
0.9 ppm bark oil + J_2	25.0 ± 0.5^{a}	19.9 ± 1.1^{ab}	49.5 ± 7.2^{ab}	10.1± 0.8	6.7 ± 0.58	8.3 ± 0.1^{b}
Distilled water	20.0 ± 2.1^{ab}	21.2 ± 5.4^{a}	43.2 ± 5.4^{b}	7.2 ± 0.3	5.1 ± 0.5	0
0.9 ppm leaf oil	5.5 ± 3.7^{c}	15.4 ± 0.9^{ab}	42.5 ± 2.8^{b}	7.2 ± 0.3	4.7 ± 0.16	0
0.9 ppm bark oil	15.0 ± 1.4^{b}	16.4 ± 1.5^{ab}	44.6 ± 2.9^{b}	9.2 ± 1.3	5.5 ± 0.34	0
0.5 ppm Tween-20	17.5 ± 2.7^{b}	17.3 ± 0.4^{ab}	28.1 ± 2.9^{c}	8.6 ± 0.4	5.4 ± 0.39	0

Data indicated by the same letters are not significantly different at p=0.05 according to Duncan's multiple range test.

These results show that the essential oils of cinnamon have shown nematicidal activity. Studies conducted on the effectiveness of the cinnamon oil on the stored grain insects, fungal pests and nematodes have also shown the potential of these botanicals as replacement for synthetic insecticides (Paranagama *et al.*, 2003, Kong *et.al*, 2007). In a detailed study have shown a higher nematicidal activity of trans-cinnamaldehyde against the *Meloidogyne javanica* (Oka, 2001). This study further reports that EC_{50} value of trans-cinnamaldehyde for juvenile immobilization and hatching inhibition *in vitro* were as low as 15µl/l and 11.3µl/l respectively. In a micro plot experiment, soil treatment with trans-cinnamaldehyde (50ml/m^2) has caused a reduction of the galling index and has increased the shoot weight of tomato plants (Oka, 2001). Galling incidence and size of the gall are dependent upon nematode density and the nematode species. In the present study, the

number of root galls has been considered as an index of the nematode population. The root gall formation in rice seedlings and young rice plants due to *M. graminicola* was significantly reduced in the treated plants with cinnamon leaf oil and cinnamon bark oil. None of the treatments, cinnamon leaf oil, cinnamon bark oil or Tween-20 showed a significant phytotoxicity in rice plants. Cinnamon leaf oil and cinnamon bark oil can be used as an environmental friendly nematicide against the rice root knot nematode *Meloidogyne graminicola* in rice seedlings and in young rice plants.

CONCLUSIONS

LC_{50} for cinnamon leaf oil for killing 50% of juveniles of *M. graminicola* in rice root galls after three days of the treatment was 0.326 ppm and that for cinnamon bark oil was 0.454 ppm.

Nematicidal activity is not significantly different between cinnamon leaf oil and bark oil.

Concentration of 0.9 ppm of cinnamon leaf oil and bark oil is required to reduce the formation of root galls in rice plants infested with *M. graminicola*.

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