Esxon Publishers

International Journal of Applied Research and Technology
ISSN 2277-0585

Publication details, including instructions for authors and subscription information:
http://www.esxpublishers.com

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Available online: August 31, 2013

To cite this article:

Bello, L. Y., Chindo, P. S., Mamud, A. Y., Oyewale, R. O. and Asorose, A. I. (2013). Inhibitory Effects of the Leaf Extract of *Mitracarpus villosus* on Egg Hatch of *Meloidogyne incognita* at Different Concentrations. *International Journal of Applied Research and Technology*. 2(8): 115 – 121.

International Journal of Applied Research and Technology
Vol. 2, No. 8, August 2013, 115 – 121.

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Inhibitory Effects of the Leaf Extract of *Mitracarpus villosus* on Egg Hatch of *Meloidogyne incognita* at Different Concentrations

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(Received: 15 August 2013 / Accepted: 29 August 2013 / Published: 31 August 2013)

Abstract

This study was conducted to evaluate the nematicidal potential of Mitracarpus villosus in controlling the egg hatch and mortality of rook-knot nematode, *Meloidogyne incognita*. Four different concentration levels of crude leaf extract of *M.villosus* in distilled water were replicated four times in a completely Randomized Design. Significant differences were observed among the treatments from 6 hours to the 96hours of observation, but there was no significant difference ($P \leq 0.05$) between S_1 (100%) and S_2 (50%) concentration at 3hours, while S_3 (10%), S_4 (1%) of the crude extract and S_5 (distilled water) were significantly different. On larval mortality, the egg that hatched into larvae after 96hours, there were significant difference between S_1, S_2 and S_3, S_4 and S_5 throughout the period of observation.

Keywords: *Egg hatch, Meloidogyne incognita, Mitracarpus villosus, Mortality, Nematicidal potential.*

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Subject: 0813-0223

Introduction

Nematodes are well defined group of invertebrates ranked as phylum or a class in animal kingdom. Root knot nematodes are minute worm like animals often called ell worms, thread worms or round worms, and are widely spread wherever food and moisture are present causing disease in man, animal, and plants as well as facilitating the entry and establishment of pathogenic bacteria, fungi and viruses (Adesiyan *et. al.*, 1990). In Nigeria, an estimation of 140 plants is host to root – knot nematodes. This includes fruits, vegetables, field crops, tree crops and several weeds (Caveness, 1978b). Occurrences of three *Meloidogyne* species with relative abundance in the order of *M. incognita, M. Javanica* and *M. arenaria* abound in the tropics (Egunjobi, 1981). However, Omiyi (1976) reported that *M. incognita* is more prevalent in the North which is about 75% in distribution in agricultural soil in Nigeria (Adegbite and Adesiyan, 2005). The extent of damage caused by root knot nematode infection varies with host, timing of infection and cultural conditions (Lambert and Taylor, 1981). If root – knot nematodes become established in deep rooted perennial crops, control is difficult and the options of control are limited (Stirling *et. al.*, 1998). However, it has been reported that an average yield loss of the world's major crops due to plant – parasite nematodes is 12.3% (Sasser, 1998). Currently, management of these nematodes effectively involve the use of synthetic pesticides but they are neither economical nor environmentally safe (Bharadwaj and Sharma, 2007). The trend today is to reduce the amount of nematicides used and give more emphasis to cultural practices and biological control (Singh, 2005).

According to Singh (2005) the planting of nematodes non-acceptable crops or resistance varieties is the best method of nematode control in principle. This is because nematode resistance or non-host crops have the advantage of providing resistance to fungi or bacteria wilts in which nematodes is the primary invader. Galano *et. al.*, (2002) reported that the use of antagonistic crops like *Tagetes erecta* and *Crotalaria spectabilis* in nematode infested soil is effective against root- knot nematodes while Mai *et..al.*, (1998) postulated that these crop produce root exudates that contain nematicidal substances. Addition of chicken manure is very effective, in reducing nematode egg masses by 56% according to Galano *et. al.*,(2002). Singh (2005) reported that chopped pineapple leaves and leaves of *Pongamia glabra* and *Azadirachta indica* reduce root-knot damage. Green manure of rape seed was also reported to suppress *Meloidogyne* species (Zasada and Ferris, 2003). While a photochemical fraction of Sudan grass is said to delay maturity of eggs and thereby reduces the number of infective 2nd stage larvae (Wildner and Abawi, 2000).Sharon *et.al.*, (2001)reported that organic amendment increase population of fungal antagonist *Trichoderms harzianum* which parasite larvae and eggs of *M. javanica* and *M. incognita*. Nitai *et.al*, (2001) stated that metabolites of many fungi have antagonistic properties against nematodes. In view of these, Siddiqui *et.al*, and (2003) discovered that the application of *Paeciliomyces liliancinus* and *Pseudomonas aeruginosa* give good control of root-knot as well as root rot. *Arthrobotrys dactyloide* and *Nematoctonus leiosporus* are nematodes trapping fungi commonly found in plots given organic amendments (Jaffe *et.al.*, 1998).The nematophagous fungi *Pochonia robecens*, *P. chlamydosporia* and *Lecanicillium lecanii* parasitize nematode eggs and destroy it contents (Lopez-Llorea *et.al.*, 2002).Since root- knot nematode must oxidize lipids to be pathogenic, their damages can be reduce by protecting roots of host plants with lipid anti-oxidants which inhibit the oxidation of lipids in plant roots (Mjuge and Estey, 1978). These lipid antioxidants are said to be heterogenous group of chemicals which are considerably cheaper than nematicides, they are non-toxic and leaves no residues with some of them being a natural constituents of foods (Adesiyan *et.al.*, 1990).

Today, thousands of plants possessing nematocidal properties are known (Banerji *et.al.*, 1985, Grainge and Ahmed, 1988). A wide variety of plant species, representing 57 families have been shown to contain nematicidal compounds (Sukul, 1992). Adeghite *et.al*, (2005) reported that the root extract of neem, Siam weed, Lemon grass and castor bean were found to have nematicidal properties. While Bello *et.al*, (2006) also made similar observation in larva hatch of *M. incognita* when exposed to concentration of water soluble extract of parts of *Cassia Siamea*, *Isoberlina doka*, *Delonix regia* and *Cassia Sieberiana*. Mateeva, *et.al.*, (2002) reported that the standard concentration of the leaf extract of *Ocimum basilicum*, *Datura stramonium*, *Targetes patula*, *Allium sativa* and *A. cepa* were more effective than the root extract. Also Padhi *et. al.*, (2002) noted that there was a great reduction in hatching and an increase in nematode mortality with *Murraya Koenigi* (curry leaf), *Jasminum sambac* (Jasmine), *Citrus aurantifolis* (sour orange) *Rawvolfia serpentina*, *Zizyiphus jujube* (ber),*Hibiscus rosasiensis* (China rose) and *Justicia gandurosa* leaf extracts. *Mitracarpus villosus* (SW) DC, a member of *Rubiaceae* family was evaluated for efficacy of its plants leaves to control the root-knot nematodes in egg hatch and mortality. It is a weed of arable land, bush fallows and waste areas widely spread in West Africa. *M. Villosus* is commonly called "Irawo ile" among the Yoruba," Gogo masu" among the Hausas people of Nigeria and was proven to have anti-fungal properties and bactericidal properties (Irobi and Daramola, 1991, 1993).

Materials and Methods

The leaves of *M. villosus* were handpicked from growing plants located behind the staff quarters, Federal University of Technology Booso Campus, Minna for this study. The leaves were collected into polythene bags and taken into the laboratory. The leaves were carefully separated from the branches and one kilogram weighed on electric weighing balance. The leaves were crushed into paste using mortar and pestle, poured into a clean plastic container. To the paste in a plastic container, 200 ml of distilled water was added from the required 4 liters using a measuring cylinder; the mixture was carefully stirred with a clean glass rod and left on the laboratory bench for 24 hours. The plastic container was covered with aluminum foil paper to prevent evaporation .After 24 hours, the paste was poured into Philip electric blender and blended for a minute. The paste was poured into another clean plastic container; the remaining 3800 mls of the required 4 liters was added and mixed properly using a glass rod stirrer. The paste was left on the laboratory bench again for another

24 hours covered with aluminum foil paper. After 24 hours, the paste was filtered into a clean plastic container through Whatman No. 1 filter papers through a funnel. The resultant solution collected was labeled standard concentration 'S'.

Preparation of different concentration of the extract from the standard solution "S"

Four different treatments were prepared and denoted as S_1 (100%), S_2 (50%), S_3 (10%), S_4 (1%).

S_1 (100%): Contains 1000 ml of the undiluted standard solution.

S_2 (50%): Contains 500 ml of the standard solution marked up to 1000 ml with distilled water representing half concentration of the standard solution.

S_3 (10%): Contains 100 ml of the standard solution marked up to 1000 ml Marked up with distilled water representing one-tenth concentration of the standard solution

S_4 (1%): Contains 10 ml of the standard solution marked up to 100 ml marked up with distilled water representing one-hundredth concentration of the standard solution.

Ss (0%): contains 1000 ml of distilled water only and serves as the control.

Heavily infested tomato (Roman V.F) with massive root galls were uprooted from culture of *Meloidogyne incognita* pots into a polythene bag and taken to the laboratory. The infested tomato with massive galls were selected and washed to remove adhering soil particles.

To remove the egg masses needed for the experiment, the clean roots were cut into short pieces for convenient handling. The cut roots were placed in a shallow dish and observed under light microscope to locate the egg masses. The egg masses were collected using clean picking needle into each treatment. However, prompt usage of the egg masses was ensured within 1-2 hours to avoid hatching prior to inoculation.

For the purpose of this experiment, 20 plastic Petri-dishes of 5mm in diameter were used. The five treatments prepared were replicated four times and arranged on the laboratory working table in a complete randomize design (CRD). For each treatment, 15 ml of the solution was measured and poured into each Petri-dish using a pipette. Into each treatment, one or two drops of anti biotic was added to prevent bacterial growth. To each Petri-dish, two freshly removed egg masses were added. The room temperature ranged between 29 ^{0c} to 31^{0c}. The total number of larvae hatched and mortality were recorded at 3, 6, 12, 24, 48, and 96 hour intervals. Test organism response to treatment was noted using the light microscope. All the data collected were analyzed using System Analytical Statistics (S.A.S) package and means separation with the least significance difference (LSD).

Results and Discussion

In Table 1, It was observed that after three hours of exposure to the extract, there was no significant difference in the total number of egg hatch at 100% and 50% concentration, but there was a significance difference between S_3(10%), S_4(1%) and S_5(0%) respectively. However, with increase in time of exposure of the egg masses to the extract, from 6 hours to 12 hours, there was a mark significant difference between all the concentrations with distilled water with the highest number of larvae at each period of exposure. Table 2 shows the effect of the leaf extract on larval mortality at different period of exposure. The effect of the extract on the larval mortality followed similar trend as shown in table 1, but inversely proportional. There was a significance difference between S_1 (100%), after 3 hours of exposure, but no significant difference between S_4 (1%) and S_5 (0%), less number of larvae were observed to be dead. However at the time of exposure increases for S_1 and S_2, the number of larval mortality also increased, but there was no significant difference between the two concentration after 96 hours of exposure. For S_3 and S_4, there was a significance difference throughout the whole period of exposure, whereas in the case of S_4 and S_5 there were no significant differences except at 96 hours of exposure.

The effect of different concentration levels and exposure of egg masses and larval of *Meloidogyne incognita* varied. The decreasing trends of hatching with increase in concentration levels of *M. villosus* leaf extract could be attributed to a photochemical fraction of the plant extract that is nematidal. Adegbite and Adesiyan (2005) reported similar effect of neem plant extract in the inhibition of egg hatch of *M incognita* in Nigeria. They observed that the lowest number of egg hatch with the standard concentration could be as a result of the inhibitory effect of the chemicals of the plant extract that might have some ovicidal and larvicidal properties.

In table 1, egg hatched was highest in S_5 (0%) throughout the period of observation, however, there was a significant difference between S_4 (the lowest dilution medium) and S_5 (distilled water) indicating that the extract of the lowest dilution level can also prevent larval hatching up to about 50% of the larval within the medium. Similar trend was reported by Ahmed *et. al.*, (1990) in Bringal cv. Singnath. Irobi and Daramola (1993) noted that crude extract of *M villosus* is fungistatic at lower concentration and fungicidal at higher concentration. This was also in agreement with Rakesh (1990) that varying dilution of some plant extract showed strong nematoxic activity at higher concentration by inhibiting hatching and killing juveniles. The consistence increase in larvae mortality observed in this work agreed with Gommer's preposition that says "Mortality of nematode larval increased with increase in exposure period" (Gomer, 1973).

Today, thousands of plants processing insecticidal properties are known (Banerji *et. al.*, 1985), Grainage and Ahmed 1988). A wide variety of plant species representing 57 families have been shown to contain nematicidal compounds (Sukul, 1992). Nematicides of plants origin include isothioscyanates, thiophenics, glucosides, alkaloids, phenolics and fatty acids (Gommer, 1973).

Anuja and Satyawati (2007) reported nematicidal effect of *Azadirachta Indica, Carica papaya, Ocinum Sanctum, Ricinus Communis, Tagetes Patula* leaves and *Tagetes Patula* flowers on egg hatch of *M. incognita* after 48 hours of exposure. However this study revealed the efficacy and possibility of using *M. Villosus* leaf extract in the control of *M*

incognita at 100% and 50% concentration. Although complete mortality was not seen in the 1% diluents of the standard solution at 96 hours, it was however incidents that it suppressed nematodes population. Nematicidal photochemical are generally safe for the environment and humans (Chitwood, 2002) .

Conclusion and Recommendations

The present study has shown that water extracts of the test plant may be use as bio-pesticides to reduce the dependency on chemical pesticides. Also in the phase of campaign against global warming, poverty and disease, the use of plant extract which are cheaper and eco-friendly gives hope. Further investigation should be carried out to clarify and identify the nematicidal efficacy of *M. Villosus* on *M. incignita* especially the phytochemical constituents responsible for nematotoxic activities.

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Tables

Table 1: Inhibitory response of *M. villosus* leaf extract at different concentrations and time of exposure on egg hatch.

Treatment	Number of egg hatch at different periods (Hourly)					
	3	6	12	24	48	96
S_1 (100%)	0.25^{d}	11.50^{e}	12.25^{e}	13.25^{e}	14.00^{e}	18.65^{e}
S_2 (50%)	0.75^{d}	16.25^{d}	17.00^{d}	21.00^{d}	23.00^{d}	28.40^{d}
S_3 (10%)	7.25^{c}	17.75^{c}	19.50^{c}	24.50^{c}	27.00^{c}	31.75^{c}
S_4 (1%)	10.25^{b}	19.50^{b}	27.00^{b}	35.00^{b}	38.00^{b}	49.25^{b}
Ss (0%)	15.00^{a}	26.50^{a}	36.25^{a}	42.25^{a}	49.25^{a}	53.50^{a}

Means with the same letters are not significantly difference at 5%.

Table 2: Effect of *M. villosus* leaf extract at different concentrations and time of exposure on larval mortality

Treatment	Number of larval mortality at different periods (Hourly)					
	3	6	12	24	48	96
S_1 (100%)	3.25^{a}	8.50^{a}	12.25^{a}	14.00^{a}	17.00^{a}	20.00^{a}
S_2 (50%)	2.70^{b}	7.00^{a}	11.95^{a}	13.45^{a}	16.00^{a}	19.00^{a}
S_3 (10%)	1.95^{c}	3.75^{b}	5.75^{b}	7.75^{c}	11.00^{b}	17.00^{b}
S_4 (1%)	0.00^{d}	1.50^{c}	2.50^{c}	3.25^{d}	3.50^{c}	7.50^{c}
Ss (0%)	0.00^{d}	1.25^{c}	2.15^{c}	3.20^{d}	3.40^{c}	4.25^{d}

Means with the same letter are not significantly different at 5% level of probability.

Tables

Table 1: Inhibitory response of *M. villosus* leaf extract at different concentrations and time of exposure on egg hatch.

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S_3 (10%)	1.95^{c}	3.75^{b}	5.75^{b}	7.75^{c}	11.00^{b}	17.00^{b}
S_4 (1%)	0.00^{d}	1.50^{c}	2.50^{c}	3.25^{d}	3.50^{c}	7.50^{c}
Ss (0%)	0.00^{d}	1.25^{c}	2.15^{c}	3.20^{d}	3.40^{c}	4.25^{d}

Means with the same letter are not significantly different at 5% level of probability.