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EFFECT OF *DELONIX REGIA* LEAF EXTRACT ON EGG HATCH AND LARVA MORTALITY OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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ABSTRACT

A laboratory study was carried out to evaluate the effect of different concentrations of leaf extract of *Delonix regia* at S, S/2, S/10 and S/100 on the inhibition of egg hatch and mortality of larvae of root-knot nematode, *Meloidogyne incognita*. It was observed that the mortality of larvae was significantly influenced by concentration at S, S/2, S/10, S/100. The standard concentration 'S' at 100% concentration of the leaf extract exhibited inhibited egg-hatch more than the other concentrations. The result also showed that with an increase in exposure, *M.incognita* larva mortality rate increased in all the concentrations except the control (distilled water).

Keywords: *Delonix regia*, Egg hatch Leaf extract, Larva mortality and *Meloidogyne incognita*

INTRODUCTION

Plant parasitic Nematodes are active, thread-like worms about 0.25-3mm long and too small to be seen with the naked eyes. Their juveniles hatch from eggs, move through the soil and invade roots near the root-tip. Nematodes are basically aquatic animals and require a water film around soil particles before they can move. Nematodes eggs will not hatch unless there is sufficient moisture in the soil (Kaloshian *et al.*, 1996). *Meloidogyne incognita* is an important pest of vegetable crops and causes high losses (Hughes *et al.*, 2005). The spread of some of the species is as a result of the movement of diseased or infected plants from one area to another through movement of water, farm equipments such as tractors, seedlings and ornamentals (Nickle, 1991). This pathogen brings about *different* symptoms and damages to most crops resulting in chlorosis, plant stunting and wilting, which may occur during period of peak transpiration stress on the plant. In a situation where the ends of roots are invaded, root systems may be reduced leading to slight swelling and most especially cessation of further elongation of the root (Padhi *et al.*, 2000). The main aim of nematodes control is to improve plant growth and crop yield. This may be achieved by making the nematodes ineffective or by reducing the population to below the threshold levels. For control measures to be profitable and practicable, the use of plant parts for the control of plant pathogen must be of economical value to the farmers. Taylor *et al.*, (1982) reported that the expected benefits of nematode control should exceed the expenses by ratio of at least three to one (3:1) and preferable more. The increase in the price of chemicals used as nematicidal and their side effects on the environment prompted this study on the use of botanical plants extract for the control of nematode.

Adegbite and Adesiyan (2005) reported that the root extracts of *Azadirachita indica* (neem), *Cymbopogon citratus* (lemon grass) and *Ricinus communis* (castor bean) were found to have nematicidal properties. Similarly, ethanol extract and essential oils of marigold flowers (*Tagetes eracta* and *T. Patula*) showed impressive nematicidal activity against *M. incognita* juveniles. The ED50 was 852 and 396 .umI- after 24 hours of exposure to the ethanol extract and essential oils, respectively (Debprasad *et al.*, 2000). Essential oils of

various plants have been found to be very useful in the control of nematodes. Rakesh *et al.* (2000) determined the efficacy of essential oils in *Cymbogon martinii*, *C. wintarianus*, *Mentha avensis* and *Ocimum basilicium* on the production potential of root-knot nematode, *M. incognita* and on the growth of black henbane (*Hyoscyamus niger*). They reported that populations of *M. incognita* were reduced by all the oils with maximum suppression in *C. martinii*.

Padhi *et al.* (2000) reported the effect of aqueous leaf extracts of seven plant species on hatching and mortality of second stage juvenile of *M. incognita*. They reported that there was a great reduction in hatching and an increase in nematode mortality with *Murraya koenigii* (curry leaf), *Jasminum sambac* (Jasmine), *Citrus aurantifoli* (Sour orange), *Zizyphus jujuba* (ber), *Hibiscus rosasoensis* (China rose) and *Justicia gandurosa* (*J. Gendarussa*) leaf extracts. Plant parts or by-products of *Ammi majus* L., *Artemisia annua* L., *A. Pallens* L., *Lactuca sativa* L. were reported to have nematicidal properties (Pandey and Hasseb, 1988). These extracts were tested on hatching of root-knot nematode, *M. incognita*. The results indicated that the varying dilutions of extracts of various plants showed strong nematotoxic activity by killing the nematodes and inhibiting egg-hatch.

In this study, a common woody tree from the family *Caesalpinacae*, *Delonix regia* (Bojer ex Hook.) Raf. was evaluated for the efficacy of its leaf extract to control the root-knot nematode, *M. incognita*. *Caesalpinaceae* plants are widely distributed in the tropics and temperate regions (Keay, 1989). In Bangladesh, it is said to have folkloric reputation as a medicinal agent; used as a diuretic, anthelmintic and astringent (Maniruzzaman, 1993; Lawal *et al.*, 2010). Extracts of this plant species revealed antimicrobial activity and general toxicity against some bacteria such as *Bacillus ureus*, *B.megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi*, *S.typhi*, and against fungi such as *Candida albicans*, *Aspergillus niger* and *Sacharomyces urevisiae* (Israt *et al.*,2010).

MATERIALS AND METHODS

Collection and preparation of leaf extract

Fresh leaves of *D. regia* were collected using a "Go to hell" to pluck the matured leaves from growing trees into a polythene bag and taken to the laboratory at the Department of Crop Production, Federal University of Technology, Minna, Nigeria.

Two kilograms (2 kg) of the leaf was collected and crushed into paste using mortar and pestle. Two hundred millilitres (200 ml) from the required 6 litres of distilled water was added and left on the laboratory bench for 24 h for proper disintegration of the chemical contents of the crushed leaves. After 24 h, the paste was poured into an electric blender and blended for a period of 1-2 minutes, then poured into a plastic container and the remaining 5.8 litres of distilled water was added to the paste, mixed properly using a glass rod and covered with aluminium foil paper to prevent evaporation. This was further left on the laboratory bench for another 24 h. After 24 h, there was sedimentation of the solution. The top most layer was gently decanted into a clean plastic container and filtered through Whatman No1 filter paper into another plastic container. The resultant solution was labelled standard concentration 'S'.

Serial dilution of extract

Four different concentrations level viz: undiluted solution 'S', half concentration of the standard solution 'S/2', one – tenth standard solution 'S/10', and one – hundredth of the standard solution 'S/100' were prepared. Distilled water was used as the control.

Collection of *M .incognita*

Heavily infected tomato plants (Roma V.F) were lifted carefully from the soil and taken to the laboratory in a polythene bag. The infected roots were carefully washed to remove adhering soil. The clean roots were cut into short pieces, about 2 cm for convenient handling. To remove egg masses from the gall roots, the infected roots were placed in shallow glass block and

observed under light microscope to locate egg masses. The egg masses were carefully collected using picking needle. Fresh egg masses were removed for each treatment. Care was taken to use the egg masses within 30 minutes to avoid their hatching prior to inoculation.

Experimental design

Plastic petri dishes of 5 mm diameter were used for this study. They were arranged on the laboratory working table in a completely randomized design (CRD). For each concentration of the extract, 15 ml of solution was poured into each petri-dish using a pipette. At the beginning of the experiment, two freshly removed egg masses were transferred into the solution in each of the petri dishes. Similarly, equal volume of distilled water poured into similar size Petri dishes and inoculated served as control. There were four replicates for each treatment.

The ambient temperature of the laboratory ranged from 29 – 31°C during the period of this study. The total number of larvae hatched and mortality were recorded at 3, 6, 12, 24, 48 and 96 hour intervals after inoculation. Hatched and dead larvae were counted using the stereoscopic microscope. All the data collected were analyzed using Statistics Analytical System (SAS) package (2000) and means separated with the new Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of *Delonix regia* leaf extracts on egg hatch.

The effect of *D. regia* leaf extract at different concentration and time of exposure on egg hatch are shown in Table 1. The result indicated decrease in egg hatch with increase in the concentration of *D. regia* extract. On the other hand, egg hatch increases with the time of exposure of egg masses to the extract. Throughout the time of exposure, egg hatch was highest in distilled water and lowest in standard solution of the leaf extract. The treatment with highest dilution 'S/100' shows that the leaf extract was effective even at low concentration in the inhibition of egg hatch of *M. incognita*.

Egg hatch of *M.incognita* decreased as the concentrations of the leaf extracts increased. This could be attributed to the phytotoxicity of the plant extract of the *D.regia*. Adegbite and Adesiyan (2005) reported similar inhibition of egg hatch of *M. incognita* with neem extract in Nigeria. They found that the lowest number of eggs hatched observed with the standard concentration of the *D. regia* leaf extract could be as a result of the inhibitory effect of the chemicals of the plant extract that might have some ovicidal and larvicidal properties. Ahmed *et al.* (1990) also reported that in Brinjal cv. Singnath growth characters were suppressed at the lowest concentration of the extract 'S/100' with corresponding higher galling indices, indicating drastic increase of nematode activities. The present study has supported delayed in egg hatching in all the concentration of the leaf extract.

Effect of *Delonix regia* leaf extract on larva mortality

The effect of *D. regia* leaf extract at different concentration and time of exposure on larva mortality rate are shown in Table 2. Generally, the standard concentration solution of *D. regia* leaf extract was most effective in the mortality of *M. incognita*. However, S/2 concentration of

Table 1: Effect of *Delonix regia* leaf extract on *Meloidogyne incognita* at different concentration and time of exposure

Treatment	Egg hatch (No./Petri dish) at different period					
	3 h	6 h	12 h	24 h	48 h	96 h
C	15.00^{a}	26.50^{a}	36.25^{a}	42.25^{a}	49.25^{a}	53.50^{a}
S	0.25^{d}	11.50^{d}	12.25^{d}	13.25^{e}	14.00^{e}	13.66^{e}
S/2	0.75^{d}	16.25^{c}	17.00^{c}	21.00^{d}	23.00^{d}	22.40^{d}
S/10	7.25^{c}	11.75^{d}	16.50^{c}	24.50^{c}	27.00^{c}	31.75^{c}
S/100	10.25^{b}	19.50^{b}	27.00^{b}	35.00^{b}	38.00^{b}	49.00^{b}

a,b,c: Means with same superscript on the same column are significantly different (P<0.05)

Table 2: Effect of *Delonix regia* leaf extract on mortality of *M.incognita* at different concentration and time of exposure

Treatment	Egg hatch (No./Petri dish) at different period					
	3 h	6 h	12 h	24 h	48 h	96 h
C	0.00	1.25^{c}	1.25^{c}	2.24^{d}	3.00^{c}	3.50^{d}
S	0.00	8.25^{a}	0.25^{a}	14.00^{a}	17.00^{a}	20.00^{a}
S/2	0.00	7.00^{a}	7.00^{a}	12.26^{b}	16.00^{a}	19.00^{a}
S/10	1.25	3.75^{b}	3.75^{b}	7.75^{c}	11.00^{b}	17.00^{b}
S/100	0.00	1.50^{c}	1.50^{c}	2.25^{d}	3.00^{c}	7.00^{c}

a,b,c: Means with same superscript on the same column are significantly different (P<0.05)

the extract exhibited similar effectiveness on larval mortality over time. Also, *M. incognita* larvae mortality increased with time of exposure of the egg masses to the extract concentration. At 3 hours of exposure of the egg masses to the concentrations, there was significant (P=0.5%) difference in larva mortality in spite of the low mortality value in S/10. Thereafter, both 'S' and 'S'/2 gave the highest larva mortality over time. On the other hand, both C and S/100 gave the lowest larva mortality over time.

The increase in larva mortality over time with increase in the concentration of plant extracts meant that the test plant could be more efficacious at a higher concentration than when diluted to a lower concentration level. Similar observation was reported by Adegbite and Adesiyan (2005) and Bello *et al.*, (2006). Rakesh (1990) reported that the varying dilutions of extracts of various plants showed strong nematotoxic activity at higher concentration by killing nematodes and inhibiting juvenile hatching. The highest mortality was recorded in the standard solution 'S' and one-half of standard solution 'S/2' while juvenile hatching increased with the corresponding increase in extract dilution.

CONCLUSION

For inhibition of egg hatch of *M.incognita* in *D.regia* leaf extract at standard concentration 'S' was found to be more toxic than the other concentrations. This also indicated that certain plant extracts are source of cheap and effective nematicidal of root-knot nematodes. The leaves of papaya with alkaloid have many pharmaceutical and therapeutic uses (Ghosh, 1994).The aqueous seed extract was found to be highly toxic to the root-knot nematode, *M.incognita* larvae and the reniform nematode, *Rotylenchulus reniformis*. The standard solution 'S' and one-half of standard solution, 'S/2' were found to be more promising in inhibiting and killing of the larvae. The efficacy of the leaf extract was better with longer time of exposure at standard solution 'S' and 'S/2' were effective in larva mortality. Previous phytochemical investigations of *D.regia* revealed occurrences of auroxanthin, mutatochrome and pyruvic acid (Jungalwala and Cama, 1962). Israt *et al.* (2010) reported the isolation of lupeol, epilupeol, *B*-sitosterol, stigmasterol and *p*-methoxybenzaldehyde from a methanolic extract of *D. regia*.

The present study has shown that water extracts of tested plant may be useful for nematode control, which will be an economical and environmentally safe option for control of nematodes. In view of this, the future looks bright for identifying new classes of phytonematicides from botanical plants to replace dangerous and expensive chemicals that are beyond the reach of our local farmers. However, further study on identification of active components of this extract is needed.

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