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NEMATICIDAL AND INHIBITION OF EGG HATCHING ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST *Meloidogyne incognita*

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ABSTRACT

The *in-vitro* nematicidal and egg hatching activities of aqueous extracts from eleven medicinal plants were assayed against *Meloidogyne incognita*. Freshly hatched juveniles of *Meloidogyne incognita* were exposed on aqueous plant extracts for 24 hours and mortality was assayed. For inhibition of egg hatching activity eggs of *Meloidogyne incognita* were exposed on aqueous plant extracts for 72 hours. All the eleven plant extracts showed significance effect on nematode mortality and nine plant extracts have significance effect on inhibition of egg hatching. Maximum 100 percent of juvenile mortality was observed in *Ananas comosus*, *Artemisia annua*, *Costus speciosus* and *Melia azedarach*. *Albizia lebbeck, Ananas comosus*, *Artemisia annua*, *Costus speciosus, Hibiscus mutabilis, Melia azedarach, Nyctanthes arbor-tristis* and *Strychnos nux-vomica* showed lowest percentage of egg hatching with no significance difference between them. *Bacopa monnieri* and *Piper betle* have no significance effect on inhibition of egg hatching.

KEYWORDS: *Meloidogyne incognita*, nematode mortality, egg hatching, plant extracts, phytochemicals, nematicidal activity

Crop production loss due to pathogens attack is a serious threat now a days worldwide. Among all pathogens, plant parasitic nematodes are among the most devastating pathogen of the world food and fiber crop, causing an estimated loss of US $125 billion per year worldwide (Chitwood, 2003). Majority of the loss is due to infection by root-knot nematodes, *Meloidogyne* spp. Nematodes are difficult to control because of their wide host range and high rate of reproduction, with female capable of producing up to thousand eggs (Natarajan et al., 2006). There are two broad categories for nematode management practices: Chemical and Non chemical. Chemical nematicides, though effective in reducing root-knot nematode infection are not always cost effective and is economically viable only for high value crop and caused significant environmental and health problems due to their toxic residues and associated environmental damage that resulted in severe restrictions on their use (Harish et al., 2008). As general awareness of the harmful effects of chemical pesticides increases and public attitudes towards environmental pollution changes, chemical nematicides are losing their popularity among farmers. In this situation we need to develop an alternative of chemical nematicides, which would be cheap, non-phytotoxic, non pollutant and easily biodegradable. An alternative to chemical control is the use of biopesticides, obtained mainly from plants. Biopesticides have shown great promise in the sense that they are very often non-phytotoxic, non pollutant, easily biodegradable and do not leave toxic residue in the edible plant parts (Lue et al., 1984). Nematicidal phytochemicals are generally safe for the environment (Chitwood, 2002). Many plant species have been reported to have nematicidal properties but the active principles have been identified in only a few. They belong to different families and are usually herbs, shrubs and trees. The effective parts are roots, barks, leaves, fruits or seeds. A review of work on nematicidal plants including a list of 176 such plants has been provided by Sukul (1994). The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several authors (Dos et al., 2003; Pavaraj et al., 2012). But the effect of many plants have yet to be investigated for their nematicidal properties. Hence, the present study has been carried out to show the effect of some medicinal plant extracts on *Meloidogyne incognita* juvenile (J_2) mortality and inhibition of egg hatching *in vitro*.

MATERIALS AND METHODS

Preparation of Plant Extracts

Leaves of *Ananas comosus, Artemisia annua, Bacopa monnieri, Hibiscus mutabilis, Melia azedarach, Murraya koenigii, Nyctanthes arbor-tristis* and *Piper betle,* Rhizome of *Costus speciosus* and seeds of *Albizia lebbeck* and *Strychnos nux-vomica* were tested for their nematicidal properties and inhibition of egg hatching activity. *Artemisia annua* was obtained from the medicinal garden of North Bengal University, West Bengal, India. Other plant materials were collected from different part of Malda District of West Bengal. Plant extracts were prepared by

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following the methodology of Ferris and Zheng (1999). The collected materials were thoroughly washed in tap water, air dried, grind and soaked in distilled water for 24 hours. Standard extract (10% W/V) was prepared by soaking 10 g of powdered in 100 ml of distilled water. The extract was passed through a muslin cloth, filtered through Whatman No.1 filter paper. This standard stock solution was diluted 50% by using distilled water and used for *in vitro* juvenile mortality and egg hatchability test.

In Vitro Juvenile Mortality Test

Active *Meloidogyne incognita* juveniles (J_2) were allowed to hatch from egg masses obtained from a culture maintained in pots grown with tomato plants. Egg masses were hand picked using sterilized forceps from infected roots, washed in distilled water and placed in 50 mesh sieves containing a layer of tissue paper in petri-dishes with distilled water just deep enough to contact the egg masses and incubated at 28 ± 2°C to obtain second stage juvenile (J_2). To study the effect of aqueous extracts on the mortality of *Meloidogyne incognita* juvenile (J_2), two ml of each extract was poured in glass cavity blocks, each containing 100 ± 10 larvae. One cavity block containing distilled water served as the control. Experiment was replicated thrice and dead juvenile in each cavity block was counted after 24 hours. Experiment was conducted at room temperature. Data were analyzed by ANOVA, followed by Duncan's new multiple range test to compare means (Duncan, 1955).

In Vitro Egg Hatching Test

The root-knot nematode *Meloidogyne incognita* eggs were extracted from infected tomato plant roots using 0.5% Sodium hypochloride (NaoCl) solution, shaken vigorously for four minutes (Hussey and Baker, 1973). To study the effect of aqueous extract of each plant species on egg hatching of *Meloidogyne incognita* 100 ± 10 eggs were kept in a cavity block containing 2 ml of aqueous extract. One cavity block containing distilled water served as control. Each treatment was replicated thrice. The number of hatched juveniles (died or alive) were counted after 72 hours. Data were analyzed by ANOVA, followed by Duncan's new multiple range test to compare means (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of Plant Extracts on Juvenile Mortality

The effect of plant extracts on juvenile mortality of *Meloidogyne incognita* is presented in Table 1. All the eleven plant extracts have significant effect on juvenile mortality with respect to the control after 24 hours of exposure. Out of the eleven plant species tested, four, namely *Ananas comosus, Artemisia annua, Costus speciosus* and *Melia azedarach,* were found to possess greater nematicidal activity causing 100% juvenile mortality. Two plants, *Nyctanthes arbor-tristis* and *Strychnos nux-vomica* have 96% juvenile mortality. Three plants, *Albizia lebbeck, Hibiscus mutabilis* and *Murraya koenigii* have more than 91% to 94.6% juvenile mortality. The lowest percentage of mortality were observed in *Piper betle* and *Bacopa monnieri*.

Effect of plant extracts on egg hatching

The effect of plant extracts on egg hatching of *Meloidogyne incognita* is presented in Table 2. Out of eleven plant extracts tested, nine plant extracts have significantly inhibited the egg hatching as compared to the control after 72 hours of incubation. Highest inhibition of hatching over control was recorded for *Artemisia annua* with 7.0% of egg hatching followed by *Ananas comosus, Melia azedarach, Strychnos nux-vomica, Costus speciosus, Hibiscus mutabilis, Albizia lebbeck* and *Nyctanthes arbor-tristis* with no significance difeerence in egg hatching between these eight plants. *Murraya koenigii* has 20.6% of egg hatching. The difference in egg hatching percentage is not significance between *Bacopa monnieri, Piper betle* and control.

The nematicidal activity of different medicinal plant and herb extracts against *Meloidogyne* spp. has been demonstrated by different workers (Wiratno et al., 2009; Ntalli and Caboni, 2012; Nandi, 2016). Extracts of many plants with anti helminthic and antimicrobial properties have been proven effective in controlling plant parasitic nematodes (Ferris and Zheng, 1999). Many plant species produce different allelochemicals which have tremendous nematicidal potential. The compounds occurring in the plants with nematicidal activity comprise a wide variety of phytochemicals like polythienyls, acetylenes, alkaloids, fatty acids and derivatives, phenolics, terpenoids, glucosinolates, isothiocyanates, sesquiterpenes and thienyls (Chitwood, 1992). Efficacy of various plant extracts in nematode control has been established. Water extracts of Indian plants, *Fleurya interrupta, Peritrophe bicalyculata* and *Andrographis paniculata* were nematicidal and resulted in 100% mortality of root-knot larvae within 40 minutes (Mukherjee and Sukul, 1978). The results of the study indicate that all the eleven plant species have significant effect in nematode mortality. Percentage of juveniles mortality in *Nyctanthes arbor-tristis* is higher

than *Albizia lebbeck* and *Hibiscus mutabilis* but inhibition of egg hatching in this plant is lesser than the other two plants. *Costus speciosus* though showed 100% nematode mortality but in terms of inhibition of egg hatching this plant is less active than *Strychnos nux-vomica. Bacopa monnieri* and *Piper betle* though effective in juvenile mortality but are not effective significantly in reducing egg hatching. In conclusion, the present experiment showed that all the plant extracts are effective in juvenile mortality and nine plants are effective in inhibition of egg hatching activity and can be used to control plant parasitic nematodes. However for development of bionematicide on commercial scale the identification and possibly synthesis of active principle responsible for the nematicidal activity should be carried out.

Table 1: Effect of plant extracts on juvenile mortality of root-knot nematode, *Meloidogyne incognita*

Plant species	Plant Part	% of juvenile mortality
Albizia lebbeck (Linn.) Wild	Seed	91.0 ± 2.82 d
Ananas comosus Linn.	Leaf	100 ± 0 e
Artemisia annua Linn.	Leaf	100 ± 0 e
Bacopa monnieri (Linn.) Penn	Leaf	48.3 ± 3.34b
Costus speciosus (Koenig) Sm.	Rhizome	100 ± 0 e
Hibiscus mutabilis Linn.	Leaf	91.6 ± 2.48 d
Melia azedarach Linn.	Leaf	100 ± 0 e
Murraya koenigii (Linn.) Spreng	Leaf	94.6 ± 3.62 de
Nyctanthes arbor-tristis Linn.	Leaf	96.0 ± 2.12 de
Piper betle Linn.	Leaf	52.0 ± 1.87 bc
Strychnos nux-vomica Linn.	Seed	96.0 ± 2.82 de
Control	-	5.3 ± 1.77a

*Values are given in mean ± SE. Each Mean consists of three replicates. Means in each column followed by the same letters are not significantly different at p 0.05 according to Duncan's New Multiple Range Test.

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Table 2: Effect of plant extracts on egg hatching of root-knot nematode, *Meloidogyne incognita*

Plant species	Plant Part	% of eggs hatched
Albizia lebbeck (Linn.) Wild	Seed	13.3 ± 2.48 ab
Ananas comosus Linn.	Leaf	8.3 ± 2.16 a
Artemisia annua Linn.	Leaf	7.0 ± 2.54 a
Bacopa monnieri (Linn.) Penn	Leaf	85.0 ± 4.24 cde
Costus speciosus (Koenig) Sm.	Rhizome	10.3 ± 3.18 a
Hibiscus mutabilis Linn.	Leaf	12.0 ± 4.41 a
Melia azedarach Linn.	Leaf	9.0 ± 3.53 a
Murraya koenigii (Linn.) Spreng	Leaf	20.6 ± 3.34 b
Nyctanthes arbor-tristis Linn.	Leaf	16.6 ± 5.4 ab
Piper betle Linn.	Leaf	90.6 ± 4.26 de
Strychnos nux-vomica Linn.	Seed	9.3 ± 3.18 a
Control	-	93.6 ± 3.18 e

*Values are given in mean ± SE. Each Mean consists of three replicates. Means in each column followed by the same letters are not significantly different at p 0.05 according to Duncan's New Multiple Range Test

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