

Inhibitory effects *Jatropha* leaf extracts on hatching of *Meloidogyne inconita* eggs

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ABSTRACT

Plant-parasitic nematodes have been reported from all the terrains of all continents. These tiny worms have been reported from any place on earth where the annual temperature goes above 3°C. *Meloidogyne* is commonly known as root-knot nematode. A few species of this nematode is suitable for colder climatic conditions while most of its known species are having wider distribution. As per the global estimates, plant-parasitic nematodes cause a whopping loss of \$157 billion annually. In India the loss due to these unseen pests has been estimated to be \$40.3 million. The loss caused by these nematodes has been overlooked primarily as the causal organism was not seen and as such the losses were attributed to the other plant pathogen in field. With detailed analysis, now it has been possible to assign plant-parasitic nematodes as major threat to world agriculture. Initially to control these nematodes, chemical based formulations were used but due to the environmental toxicity associated with many of these chemicals, these have been withdrawn from most part of the world leaving only very little option for controlling these pests. Bio pesticides in recent times have come up as a cleaner approach for controlling many plant pest including plant-parasitic nematodes. With increasing environmental concern throughout the globe, there is increase awareness for use of eco-friendly nematicides. Recently there had been successful use of the plant extract as plant nematode control strategy. In the present paper, inhibitory potential of the *Jatropha* leaf extracts has been explored as an alternative to chemical based control strategy.

INTRODUCTION

Plants suffer endless damage in the field with variety of plant pathogens (Yadav *et al.*, 2006). Some of these pathogens are macroscopic while others are microscopic (Adityachaudhury, N. *et al.*, 1985, Banerji *et al.*, 1985). While strategies to control the macroscopic plant-pathogens are straightforward, targeting various stages of the pathogen for designing effective control strategy (Davis E.L *et al.*, 2000). For pest which are unseen to naked eyes, first it become difficult to believe that they can cause damage to such an extent, secondly identification of the stages for targeting pose another level of difficulty for their control. Among several groups of plant-parasitic nematode, endo-parasitic nematode such as root-knot nematode (*Meloidogyne spp.*) and cyst nematode (*Heterodera spp.*) are serious threat to most of the plants (Huang *et al.*, 2006). Root-knot nematodes name comes from the pathogens ability to distort the root of the affected plants in such a manner that the affected root looks like a knot. Root-knot nematode species reproduce primarily by means of apomixes making it one of the most fecund of all species. Survival on any of the crop plant makes it even more suitable to survive in any climatic condition. These two features are among the several other features that makes this nematode the nature's most serious of all plant pathogen (Chitwood D.J 2002).

Several plant varieties have been shown to possess the anti-nematode properties in the various parts of the plant (Velcher *et al.*, 1997, Egunjobi, O.A. and S.O. Afolai, 1976). Efforts to develop environmental friendly strategies to offer clean method to control the damage caused by plant-parasitic nematode has seen a long way (Parmar, B.S. and P. Dureja,

1996). Several plant species has been tested for their efficacy to control the damage caused by plant parasitic nematodes (PPN). Leaf extract and root extracts from some plants are already tested to have anti-nematode potential. Various formulations using different parts of the plant in different concentration of the active material has been tested.

Since we have very little knowledge about the biology of these nematodes (Yadav et al, 2006), we do not have many targets from the nematodes against which a specific strategy is targeted (Rich, J.R *et al.*, 1989). Endoparasitic nature of these worms makes it even more difficult to target the susceptible stages of the nematode. Earlier studies in their design of the experiment have targeted the hatching of the nematode eggs, juvenile mortality test; reduced fecundity of the females etc has been studied. Other studies have suggested the use of various parts of the plants as an organic amendment for controlling the nematode (Sasser, J.N., 1998). Some studies have targeted the chemosensory mechanism of the juvenile in an effort to disrupt the food/ host localization (Bargmann, 2006). Root-knot nematodes have been shown to undergo dauer stage in its life cycle (Elling A.A, 2013). Few studies have explored the possibility of targeting the dauer stage of root-knot nematode for designing effective control strategy. Couple of studies have targeted the reduction in reproductive potential as a strategy for controlling the nematode population (Coupland *et al.*, 2017). In an eco-friendly approach, few studies have utilized botanicals for targeting second-stage juveniles (Elbdri, 2008). Results from green house studies have confirmed the potential of the botanicals in controlling the nematode infection, reduced fecundity, less egg hatching, problems with juvenile mobility and mortality, difficulty in locating the host etc has been well documented. Effect of *Jatropha curcas* leaf extracts has been tested for its potential against *Ades* (Ojha,

Kaushiki & Pattabhiramaiah, Mahesh. 2014).). Based on the result obtained from the previous studies, idea to check the efficacy of the leaf extracts from *Jatropha curcas* has been mooted. The idea is to develop environmentally safe methods for nematode control.

MATERIALS AND METHODS

The present study was carried out at the department of botany, Govt (PG) College Fatehabad, Agra during September 2018 to July 2019. All the procedure including the maintenance of the nematode stocks, preparation of the leaf extracts, treatment using the extracts, observation of the nematode development at various intervals, monitoring of the different stages of the nematode, damage in plant roots etc. has been carried out following the standard protocol. Each of the procedure used in this study has been carried out as described below. This stock of the nematode eggs was collected from the local field from fatehabad, Agra. The collected egg was hatched in luke warm water and the resulted juveniles were used for infecting the tomato roots.

Maintenance of the nematode stock:

Meloidogyne incognita egg masses were collected from the local tomato field. The selected tomato plant was heavily infested showing the typical root-knot nematode distorted roots. 27 Single egg mass were collected and their corresponding females were carefully dissected out. Females were further subjected to perennial pattern cutting and identification. Out of the 27 females collected and kept in saline buffer, 13 were burst open during the procedure leaving only 14 for perennial pattern cutting. Corresponding egg masses from the burst open females were destroyed in absence of the identification. Further identification of the nematode females was carried out using 14 females. Perennial pattern was cut out was described by (R. H. Mulvey, et al., 1975), and typical *Meloidogyne inconita* pattern were observed in 6 among the 13 females. Rest of the females displayed

Meloidogyne javanica pattern. Corresponding egg massed from *M.incognita* females were taken for the further study. All the selected egg masses were kept for hatching in a petriplate with wire gauze fitted with water soaked filter paper. Bottom petriplate dish had enough water to keep the top filter paper wet all the time. This whole setup was kept at 28⁰C in biological incubator for 24 hours. Next day the water is from the petriplate was collected and the nematodes were counted in 100µl was taken for analysis of the active juvenile. This was done using the nematode counting grid. 50-70 active J2 were taken for infection of the already established tomato plants in author's laboratory. Individual tomato plants var. Pusa Ruby (susceptible for nematode infection) was grown from individual seeds procured from National seed corporation IARI, New Delhi. All the plants were grown in sterilized soil and sand mixture. This was done to insure the proper movement of the juvenile and making sure the access of all the roots for the feeding J2. 15 days old tomato plants with few hairy roots were used for infection analysis. Water containing active J2 (50-70) was poured near the roots of the plant and the exposed root was covered using the sterilized sand. The plants were occasionally watered and monitored. 45 days following the infection, individual plants were uprooted, roots were washed thoroughly under running tap water and examined for nematode infection and its reproduction, counting and collection of egg masses for further experiments.

Plant material :- Leaves from *Jatropha curcas* plants, luxuriantly grown in the college campus were used in this study. Leaf extract preparation was carried out as described using standard protocols. Brief method of preparation of the botanical extract. was done as follows:- 500 gm fresh leaves of *J.curcas* were collected in the morning on the day of the experiment. All the leaves were carefully washed and soaked dried using filter paper. Leaf extract was prepared by blending 250gm leaf (in two successive batches) in distilled water. Suspension of the leaf thus obtained was subjected to filtration

using sterile muslin cloth. Various concentration of the botanical was prepared using various quantities of the distilled water. All the preparation of the botanical extract was carried out as described in previous studies.

Screening of anti-nematode potential in plant extracts:- Plant extract thus obtained was subjected to screen the anti-nematode potential present in them. As described various concentration of leaf extract was prepared as described (Jumaah, Ahmed. (2015). 25-30 individual egg masses were setup in hatching in batches of 05 each. Each of the petriplate containing the egg masses over the wet wire gauze and were containing various concentration of the plant extracts. Hatching of the nematode eggs in presence of the plant extracts was carried out in an biological incubator. All the hatching was monitored at 24 hour, 48 hours and 72 hours.

RESULTS

As a control of the experiment, only distilled water was used in the petriplate. Leaf extract of the botanical visibly delayed the egg hatching. The rate of the egg hatching was different in different concentrations. Exposure timing of the leaf extracts had a significant bearing on the hatching of the nematode eggs. While the nematode egg started hatching almost immediately in control experiment (with distilled water only), the time of hatching in petriplate containing the plant extracts, differs a lot. Above all the percentage of hatching in various concentrations of the plant extracts varied significantly.

DISCUSSIONS

Result thus obtained indicates that the plant extracts had some chemical components that hinder the hatching of the nematode eggs. *Meloidogyne* being an apomictic plant pathogen produces enormous amount of eggs and as such population. Chemical control methods being non-ecofriendly is being discouraged throughout the globe. In this scenario the development of any non-chemical based control

strategy would be required to offer a clear approach for plant parasitic nematode (PPN) control.

REFERENCES

- Adityachaudhury, N., A. Bhattacharyya, A. Chowdhury and S. Pal, 1985. Chemical constituents of plants exhibiting insecticidal, antifeeding and insect growth regulating activities. *J. Sci. Ind. Res.*, 44: 85-101.
- Banerji, R., G. Misra and S.K. Nigam, 1985. Role of indigenous plant material in pest control. *Pesticides*, 19: 32-38.
- Bargmann CI. 2006. Chemosensation in *C. elegans*, in *WormBook: The online review of C. elegans biology*. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK19746/>.
- Belcher, J.V. and R.S. Hussey, 1977. Influence of *Tagetes patula* and *Arachis hypogea* on *Meloidogyne incognita*. *Plant Dis. Rep.*, 61: 525-528.
- Chitwood, D.J., 2002. Phytochemical-based strategies for nematode control. *Ann. Rev. Phytopathol.*, 40: 221-249
- Coupland J, Abd-Elgawad MMM, Askary TH (2017) Beneficial nematodes and the changing scope of crop protection. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) *Biocontrol agents: entomopathogenic and slug parasitic nematodes*. CAB International, Wallingford, pp 26–42
- Davis, E.L., Hussey, R.S., Baum, T.J., Bakker, J. and Schots, A. (2000) Nematode parasitism genes. *Annu. Rev. Phytopathol.* 38, 365– 396.
- Egunjobi, O.A. and S.O. Afolami, 1976. Effect of neem (*Azadirachta indica*) leaf extract on population of *Pratylenchus brachyurus* as growth and yield of maize. *Nematologica*, 22: 125-132.
- Elbadri, G., et al., *Evaluation of various plant extracts for their nematicidal efficacies against juveniles of Meloidogyne incognita*. *Journal of Asia-pacific Entomology - J ASIA-PAC ENTOMOL*, 2008. 11: p. 99-102.
- Elling, A. A. (2013). Major emerging problems with minor *Meloidogyne* species. *Phytopathology* 103, 1092–1102. doi: 10.1094/PHYTO-01-13-0019-RVW
- Huang, G., Allen, R., Davis, E.L., Baum, T.J. and Hussey, R.S. (2006) Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. *Proc. Natl Acad. Sci. U.S.A.* 103, 14302– 14306.
- Jumaah, Ahmed. (2015). Effect of Leaf Extracts of Some Medicinal Plants on Root-Knot Nematode *Meloidogyne Incognita* on Eggplant *Solanum Melongena*. *European Academic Research*. 3.
- Ojha, Kaushiki & Pattabhiramaiah, Mahesh. (2014). Evaluation of Phytochemicals, Larvicidal Activity of *Jatropha Curcas* Seed Oil against *Aedes Aegypti*. *Int J Appl Res Stud.* 2. 2278-9480.
- Parmar, B.S. and P. Dureja, 1996. Pesticide Management: Issues and Strategies. In: *Critical Issues in Insect Pest Management*, Heinrihs, E.A. and G.S. Dhaliwal (Eds.). Common Wealth Publishers, New Delhi, pp: 180-208.
- R. H. Mulvey, , P. W. Johnson, , J. L. Townshend, and , J. W. Potter, Morphology of the perineal pattern of the root-knot nematodes *Meloidogyne hapla* and *M. Incognita*. *Canadian Journal of Zoology*, 1975, 53(4): 370-373
- Sasser, J.N., 1998. A Perspective on Nematode Problems Worldwide. In:

Nematode Parasitic to Cereals and Legumes in Temperate Semi Arid Regions, Sasena, M.C., R.A. Sikora and Srivastava (Eds.). ICARDA, Syria, pp: 8-12.

Yadav B.C *et al*, Host-generated double-stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. *Mol. Biochem. Parasitol.* 2006; 148: 219-222