Research Article

Nematicidal Activity of Different Plants Extracts against Root Knot Nematodes

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Abstract | Nematicidal activities of different plants viz., *Amaranthus viridis*, *Chenopodium album*, *Solanum nigrum*, *Carica papaya* and *Euphorbia hirta* have been evaluated against root-knot nematodes. Plants were collected from different areas of Karachi. Aqueous extracts (2, 6 and 10%) of these five plant species were made for the experiment. All selected plant species inhibited egg hatching and caused larval mortality. Maximum reduction (24.3%) in egg hatching was recorded in *C. album* stem extract at 2% concentration. The minimum reduction (0.33%) was observed in the leaf extract of *A. viridis* at 10% concentration after 48 hours of exposure time. In larval mortality test, maximum larval mortality (33%) was recorded in *C. album* leaf extract at 10% concentration while minimum larval mortality (0.66%) was noted in *Solanum nigrum* at 2% concentration in leaves and stem extracts after 72 hours of exposure time as compared to control.

Received | November 16, 2020; **Accepted** | January 07, 2021; **Published** | March 20, 2021
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Citation | Afzal, J., Abid, M., Hussain, F. and Abbas, A., 2021. Nematicidal activity of different plants extracts against root knot nematodes. *Pakistan Journal of Nematology*, 39(1): 1-7.
DOI | http://dx.doi.org/10.17582/journal.pjn/2021/39.1.1.7
Keywords | Nematicidal activity, Hatching, Mortality, Plant extracts, Root-knot nematodes, Karachi

Introduction

The root- knot (*Meloidogyne* spp.) plant parasitic nematodes have significant harmful properties against almost all field crops (Perry and Maurice, 2006; Adekunle and Akinlua, 2007). It causes approximately $100 billion every year worldwide including Pakistan (Khan *et al*., 2008). Root-knot nematodes damage vast range of plants and vegetables crops and plants predominantly in subtropical and tropical countries (Osman *et al*., 2012; Youssef *et al*., 2012). Damage and crop losses in tropics are more than the temperate region because of favourable environmental condition which support more pathogen diversity. Vegetable crops usually are among the most susceptible and worst affected by the nematodes (Sharma *et al*., 2006; Anwar and Mckenry, 2007; Singh and Khurma, 2007). Vegetable yield reductions have reached as high as 30% for susceptible genotypes in the presence of plant parasitic nematodes in some production areas (Anwar *et al*., 2009). The infected plants or crops appeared stunted or chlorotic patches were observed on foliar part of plants (Archana and Saxena, 2012).

Plant protection against nematodes is difficult because nematodes cannot be eradicated completely from the field (Budai *et al*., 2005). Nematode management strategies therefore need to involve manipulation of nematode densities to non-injurious and sub-economical threshold levels (Viaene *et al*., 2006). Pesticides are generally used for the control of pests and diseases but these chemical pesticides cause human health and environmental hazards. So, there is a need to find alternative methods which are safe and suitable for environment (Abid *et al*., 2005; Khalil and Darwesh, 2017). Plants having nematicidal properties

have been used as bio-pesticides (Ibrahim *et al*., 2006; Khan *et al*., 2015; Farzana *et al*., 2016).

Plants are important sources of naturally occurring compounds having nematicidal properties, like alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls (Chitwood, 2002). The ability of plant parts/products to reduce crop damage caused by root knot nematodes, *Meloidogyne* spp., in amended soil is well documented (Begum *et al*., 2003; Jesse *et al*., 2006). Their ability, minimum toxicity to man and animal safety, safety to the environment and effectiveness in controlling the nematodes make plant parts indispensable to nematode control (Jesse and Jada, 2004).

Therefore, the aim of the present this study was to evaluate extracts of some plants commonly grown in Karachi for their nematicidal efficacy against root knot nematodes *in vitro* and check their antagonistic effects against root-knot nematode.

Materials and Methods

Site collection
Different sites including grass growing area, road side and garden area were studied and plant samples were collected from these sites in plastic bags.

Collection of plant material
Five plants viz., slender amaranth (*Amaranthus viridis*), bathwa (*Chenopodium album*), macko (*Solanum nigrum*), papaya (*Carica papaya*) and asthma-plant (*Euphorbia hirta*) were collected from different areas of Karachi.

Collection of plant samples
The collected samples were kept in polythene bags, tagged and brought to the Dr. A.G. Lab of Aerobiology and Plant Pathology, Department of Botany, FUUAST, Karachi. The collected plants were dried in screen house and ground in an electric mixture into powder separately, stored in polythene bags at room temperature until use.

Preparation of extraction
2, 6 and 10 grams of dried leaves, stem and fruits of *A. viridis*, *C. album*, *S. nigrum*, *C. papaya* and *E. hirta* were separately mixed with 100 ml distilled water and left for 24 hours at room temperature; then filtered from a Whatman No. 1 filter paper. The filtrate was considered as standard(S). Further dilutions were prepared from standard solution.

Culture of nematodes
The culture of root-knot nematode was maintained on host plants. The infected plants were uprooted; roots were thoroughly washed in running tap water then cut into 1 centimeter in length. Eggs masses were isolated using method described by (Hussey and Barker, 1973) in Petri dishes containing sterilized distilled water.

Egg hatching test
Effects of plant extracts on egg hatching were studied. One ml of root-knot egg suspension containing 50–56 eggs and 1 ml of each plant extract was transferred to each cavity block separately; treatments were replicated three times. All treatments were kept at 28±2˚C and after 72 hours the numbers of hatched eggs were observed and counted.

Mortality test
The effect of plant extracts were checked for larval mortality. Eggs of root-knot were placed in distilled water and incubated at temperature 28 ± 2 ˚C for 72 hours. A suspension of freshly hatched juveniles in distilled water containing (10-50 juveniles/ml) was prepared. 1 ml of each plant extract was transferred to each cavity block separately; treatments were replicated three times Nematodes were considered dead if they did not move when probed with a fine needle was touched (Abbasi *et al*., 2008).

Analysis of data
The observed data were analyzed by using one and two way ANOVA according to the experimental design (Gomez and Gomez, 1984). The follow up of ANOVA were included with LSD and DMRT were also applied to compare treatment means.

Results and Discussion

Egg hatching test
The five plants were selected and tested @ 2, 6 and 10% concentrations for egg hatching of root-knot nematode (Table 1). The result indicated that egg hatching was decreased with the increase in concentration. Maximum reduction (24.33%) in egg hatching was observed in *C. album* stem extract at 2% concentration as compared to control. Statistically it was found highly significant ($F=22.28$, $p<0.0003^{***}$) difference in mean. Highly significant

result was observed (F=104, p<0.0000***) in leaf, stem (F=49.244, p<0.0000***) and fruit (F= 35.11, p<0.0001***). The stem extract of S. nigrum were observed highly significant (0.33±0.33) and showed antagonistic effect (F=32.73, p<0.0001***). In distilled water extract; result showed that extract of *A. viridis* was significant in the reducing of egg hatchability. Almost all plants extract inhibited the egg hatching.

The increasing of time period and addition in concentration also decrease in egg hatchability. The *C. album* stem extract has been observed to be less toxic effecting killing capacity. When compared to other extracts but in case of *A. viridis* has been observed highest killing capacity for egg hatchability as compared to other extracts and the egg hatchability has been found to be decreasing with increasing concentrations from 2% to 10% after 48 to 72 hours exposure time. Similarly, all the leaf extracts were found increasing of egg hatchability at low concentration and decreasing at high concentration. The egg hatchability was observed in the decreasing order is *A. viridis*> *C. papaya* > *C.album*. Higher concentrations were related with higher death rates. *E. hirta* has been observed killing capacity for egg hatchability. It was highly significant in fruit (F= 252.18, p<0.0000***), stem (F= 52.52, p<0.0000***) and leaf (F= 34.69, p<0.0001***). The statistical analysis, of C. papaya were non-significant (F= 3.79, p<0.0584 ns) in leaf extract and it showed highly significantly results in fruit (F=23.97, p<0.0003***) (Table 1).

The present results of Analysis of variance for antagonistic effect of various plants on root-knot nematode show the hatching (%) of leaf, stem and fruit extract of *A. viridis* and *E. hirta* showed highly significant differences. Similarly, *S. nigrum* and *C. album* showed significant differences. However, leaf extract of *C. papaya* showed non-significant results.

Larval mortality test

The freshly hatched root-knot larvae were exposed in water extract of different plants for 72 hours in laboratory at temperature (28 ± 2 °C). The maximum larval morality (32% and 33%) was recorded in leaves and stem extract of *C. album* at highest concentration (10%) as compared to other plants (Table 2). The minimum larval mortality was observed in leaves and stems extract of *E. hirta* and *S. nigrum*, respectively. Statistically, *S. nigrum* was found highly significant (F= 56.05, p<0.0000***). Larval mortality was decreased with the increase of dilution of plants extracts. While, juvenile mortality was increased consequent to an increase of concentration. Other plants such as A. viridis caused 7% mortality in leaf extract, with non-significant (F=0.58, p<0.06436ns) results. All aqueous extracts showed potential nematicidal effects and caused mortality of juveniles (Table 2).

Table 1: *Effect of different concentrations of water extracts of plants on egg hatching of root-knot nematode after 48 hours with mean and standard error.*

Name of plants	Control*	Egg hatching % after 48 to 72 hours of exposure								
		Leaf			Stem			Fruit		
		2%	6%	10%	2%	6%	10%	2%	6%	10%
A. viridis	10±0.57	2.33±0.3	1±0	0.33±0.3	2.33±0.8	0.66±0.6	0.33±0.3	4±0.5	3.66±0.3	1.33±0.8
E. hirta	25±0.57	6±1.1	5±3.0	3.6±2.1	2±1.1	1±0.57	1±0	1.66±0.6	1±0.5	1±0
C. papaya	15±2.3	9.33±4.7	3.33±3.3	1.33±1.3	_	_	_	1.33±0.8	2.33±1.2	1±0.5
S. nigrum	12.6±1.2	2.66±0.8	1.66±1.2	0.66±0.3	2.33±0.8	0.33±0.3	0.33±0.3	_	_	_
C. album	28.3±1.4	7±4.16	5.33±2.3	2.66±0.3	24.3±10.7	6.66±2.3	1.33±0.8	_	_	_

**The control values are represented by mean number of eggs/juveniles.*

Table 2: *Effect of different concentrations of water extracts of plants on larval mortality of root-knot nematode after 72 hours with mean and standard error.*

Name of lants	Control*	Larval mortality % after 72 hours of exposure								
		Leaf			Stem			Fruit		
		2%	6%	10%	2%	6%	10%	2%	6%	10%
A. viridis	10.6±1.4	3.33±2.3	7.33±4.3	6±3.05	3±1	2.33±1.2	2.66±1.3	1.33±0.3	2±1	3.66±0.3
E. hirta	12.3±1.4	1.66±0.3	1.33±0.3	2.33±1.4	0.66±0.6	0.66±0.6	1±1	0.66±0.3	1.33±0.3	1±0.5
C. papaya	11±0.5	2.33±0.8	2.33±0.8	4.66±2.0	_	_	_	1.33±0.8	1.66±0.8	1±1
S. nigrum	11±0.8	0.66±0.3	1±0.5	1.66±0.8	0.66±0.3	1±0.5	2±0.5	_	_	_
C. album	48±1.5	15±3	18.33±3.4	32±8.5	12.3±2.3	20±2.0	33±7.9	_	_	_

*The control values are represented by mean number of eggs/juveniles.

The exposure time period has significant role in mortality in the exposure of time period. The plant extract caused significant mortality as compared to control. The results of various parts of plant shows that treatment showed highly significant results in leaf, stem and fruit of *E. hirta*, *C. papaya* and *S. nigrum* against root-knot nematode. While in mortality percentage of *A. viridis* leaf extract shows non-significant results. However, *C. album* showed significant results, respectively.

Application of plant extracts for the control of nematode populations is cheap, easy to apply and has advantages over the standard nematicides regarding environmental safety (Zurren and Khan, 1984; Adegbite, 2003). Plant extracts contain some phenolic compounds, organic acids, terpenes and terpenoids, coumarin-like compounds and other secondary metabolites (Insunza *et al*., 2001; Shaukat *et al*., 2004).

Results indicate that plants extract of weed species including *A. viridis*, *C. album*, *S. nigrum*, *C. papaya* and *E. hirta* reduced egg hatching and induced deaths of juveniles. It has been observed that nematicidal activity could be related with the release of phenolic and other secondary metabolites which are present in plants. In previous research studies, phenolic compounds such as caffeic acid, benzoic acid and p-cumaric acid induced juvenile's death in *M. javanica* (Shaukat and Siddiqui, 2001b). *A. viridis* was the significant in inhibiting the root-knot nematodes but had a negative effect on plant growth (Costa *et al*., 2003). In present study, leaf extracts of *A. viridis* were phytotoxic on root-knot nematode including all parts of plant. Plant powder extracts were toxic to larvae and cause of death in juveniles of *M. javanica*. Mortality was increased as the exposure of time increased. The plant extracts including *Eucalyptus* sp., *Prosopis juliflora*, *Samanea saman*, *Azadirachta indica* noted maximum juvenile's mortality. Soil amendment with *A. viridis* inhibited the nematode population densities in soil (Shaukat and Siddiqui, 2001c). Different species of *Eucalyptus* possess few essential oils which have toxicity against nematodes, weeds, insects, fungi and bacteria (Batish *et al*., 2008).

Liu *et al*. (2014) reported that second stage of *M. incognita* juveniles (J_2s) were inhibited by *Triadica sebifera*, *Leptopus chinensis*, *Glochidion eriocarpum*, *Croton tiglium, Phyllanthus urinaria, Ricinus communis* and *Euphorbia* spp., at the concentration of 1 mg/ml after exposure of 72 h. However, ethanol extracts with plants *R. communis*, *L. chinensis*,*E. fischeriana* and *C. tiglium* showed 100% mortality against *M. incognita* at the concentration of 1 mg/ml after 72 h exposure. In our studies, *E. hirta* aqueous extract of leaf, stem and fruit little bit toxic against juveniles. In our studies, while latex producing plant likes *E. hirta* suppressed egg hatching it was also phytotoxic against nematode. *C.album* and *S.nigrum* caused highest reduction in hatching as compared to the control and *C.album* caused highest mortality at highest concentration. Vpadhyay *et al*. (2003) reported that *E. hirta, C. album* and *Ecliptaalba* caused more than 80% reduction in hatching at 1:5 dilution. *R. communis, A. sativum*, *S. nigrum, A. indica* and *A. cepa* caused up to 90% reduction in hatching compared to the control.

In the case of screening of plants for nematicidal products against *Meloidogyne incognita*, the extract of seeds of *C. papaya* was found to be active. Methanol extracts of *C. papaya* seeds were higher nematicidal activity than petroleum ether extract but the activities of steam distilled oil from both fresh and dry seeds were by far the highest (Nagesh *et al*., 2002). In the present results indicate that the seeds of *C. papaya* possess excellent nematicidal activity towards both saprophytic and plant nematodes which are due to benzyl isothiocyanate. Isothiocyanates in plants are normally derived from the hydrolysis of glucosinolates (Daxenbichler *et al*., 1991). In our studies, *C. papaya* also showed nematicidal activity against juvenile and egg hatching. In different compounds of *Azadirachta indica*such as limonoids or triterpenes are recorded as vigorous factors for bioactivity of neem against pests and nematodes (Alam, 1993; Kraus, 1995). Guzman and Saxena (1997) reported that extracted oil of neem or neem cake have high nematicidal properties against the RKN (*M. incognita*) in green house as well as laboratory. Nimin, is considered a product of neem which can also significantly reduced the soil and RKN populations of M. inognita and promote the parameters of plant growth (Mojumder *et al*., 2004). The root exudates *E. hirta* exhibits nematicidal activity against juveniles of *Meloidogyne incognita* (Kumar *et al*., 2010).

Conclusions and Recommendations

It is concluded that all plant extracts indicates more than 30% nematicidal activity in treatment against

RKN. However, extracts of *Chenopodium album* exhibited maximum nematicidal activity as compared to remaining plant species. Contemporary finding furthers more reported that nematicidal activity of all plants species dependent on kind of extract, time exposure and dose of extract. It is plainly proved in this study that activity increased in the increment of the extracts and in the concentration and time exposure. Our present study indicated that these plant species can be utilized for biocontrol of RKN and this method of management is cheap, environmentally friendly and free from any hazards.

Novelty Statement

This study revealed that nematicidal activity of all plants species dependent on kind and dose of extract. It is also indicated that these five selected plant species can be utilized for biocontrol of RKN and this method of management is cheap environment friendly and free from hazards.

Author's Contribution

Javeria Afzal: Primary author who conducted the study.
Muhammad Abid: Supervised the research.
Faisal Hussain: Anlysed the data and wrote the manuscript.
Alia Abbas: Conducted the experiments and finalized the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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