Research Article

Phytocontrol of Root Knot nematodes in Vegetable Crops of India

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ABSTRACT

Meloidogyne spp. is a major pest of plants and causes yield loss all over the world in cultivated as well as wild plants. In India, also the major share of destruction of crops is caused by *Meloidogyne*. In the past few years, various methods were used to control this devastation-causing parasite, so as to prevent the severe yield loss. Most of the methods used were not only harmful to the environment but also to the human population. The present study relates to an important admixture of certain plants decoctions to manage and control the root knot nematode, i.e., *Meloidogyne* spp. The decoctions thus prepared from these plant extracts are successful in rendering nematodes and/or their eggs susceptible to infection by microflora. The study renders an economical concoction that is not only fatal to nematodes but also is harmless to human population and can be used as a substitute for environmentally harmful, synthetic hazardous agrichemicals, widely used by farmers and land owners to combat these phytonemas.

Key words: Bio-pesticide, Meloidogyne, Plant Extracts, Root Knot Nematode, Nematicide.

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INTRODUCTION

Meloidogyne spp. is a major pest of plants and causes yield loss all over the world in cultivated as well as wild plants.[1] From beginning of agriculture pests have been a major problem in crop production.[2] Further, agriculture sector is adversely affected due to the pests and diseases especially phyto-parasitic nematodes.[3] Plant-parasitic nematodes are nefarious and voracious parasites and are a huge threat to world's food security; these destroy at least 12.3% of global food production annually estimated at a value of approx. 11000 billion rupees.[4] In India, also the major share of destruction of crops is caused by *Meloidogyne*.[5] Roots infected by this pest harbor distinct typical root galls and infected plants show poor growth and many may even die due to vascular dysfunction. In India estimated loss due to nematodes is approx. 21000 million rupees annually.[3] Nematodes of genus *Meloidogyne*, cause large scale destruction and damage of vegetables, fruits, cereals, plantation crops and ornamental plants. They disfigure the produce, reduce its yield as well its quality.[6] They cause severe damage to tomato, chilli, potato, papaya, brinjal, jute, groundnut etc.[7] In a recent study[8] estimated crop loss of fruit crops (25.5%), vegetables (19.6%), spices (23.03%), cereal crops (18.8%), pulse crops (23%) and oilseed crops(30%) due to invasion these plant parasitic nematodes in India.

In the past few years, various methods were used to control this devastation-causing parasite, so as to prevent the severe yield loss. Most of these methods applied were not only detrimental to the environment but also harmful to the human population. One such method of management of this parasite is chemical control which requires the use of synthetic organic chemicals specific for nematodes generally called nematicides. However, apart from its prohibitive cost in terms, of price, human health these synthetic chemicals play havoc with the environment. In view of the latter two, it has become necessary to reduce or if possible totally end the usage

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of these nematicides. Therefore it has become essential to look for other efficient, ecologically safe and sound control methods. Plant extracts have extensively been used as environmentally affable means for biological control of parasitic pests, including the root-knot nematodes, instead of chemical pesticides. The present study aims to find a cost effective and eco-friendly method to control this devastating pest of plant crops.

MATERIALS AND METHODS

The experiment was performed at the experimental greenhouse. For the experiment soil was amended with powdered leaves of 10 plants (Table 1) powders being mixed at the rate of 0.1, 1 and 5% w/w and kept in 8 cm diam., plastic pots, with each pot containing 300 gm soil. Soil moisture was adjusted and maintained at 50% MHC.[9] Pots with organic matter were left for 10 days for decomposition. Non-amended soil was kept as control. Five replicas for each treatment were made. The pots were kept randomly in a complete block design. For the experiment tomato was used as test plant. After attaining a growth of two weeks, the plants were inoculated with 1000 larvae/pot.[10] The plants were pulled out of the soil, after 10 weeks, their growth parameters measured and the number of root knots determined. Twenty-five plants were examined for root-knot infection per replication in each treatment and graded on 0 – 5 scale, from which root knot indices (RKI) were calculated.[11]

Preparation of bio-control extract

Fresh leaves of each plant listed in Table 1 equal in weight were collected and crushed using Pestle and Mortar. The plant extract thus prepared was filtered using Whatman's filter paper no. 1 and the filtered decoction was vacuum evaporated in a rotary evaporator at 40°C to obtain organic crude extracts.[12] The extract was taken into two concentrations, 100% (the original one) and 50% (obtained by the dilution with distilled water).

Table 1: Plants (with botanical names) used for bio control experiments.

Sl.	Common Name	Botanical Name
1	Neem	*Azadirachta indica*
2	Ashok	*Saraca indica*
3	Madar	*Calotropis procera*
4	Pilikateli	*Argemone maxicana*
5	Sadabahar	*Catharanthus roseus*
6	Marigold	*Calendula officinalis*
7	Eucalyptus	*Eucalyptus camaldulensis*
8	Date	*Phoenix sylvestris*
9	Lemmon Grass	*Cymbopogon citratus*
10	Mint	*Mentha piperita*

Extraction of root knot nematode eggs

Eggs were isolated from root knot nematode infected roots of the experimental plant (tomato). Root knots infested with egg masses were chopped into small pieces and put in a 500 ml capacity container containing 200 ml of 0.5% solution of sodium hypochlorite (NaOCI). This mixture was shaken vigorously for 4 min in so as to digest the gelatinous matrix encasing the eggs.[13] This suspension mixture was then trickled through two nested sieves, of 200-mesh and 500-mesh, 75µm and 25µm respectively. Eggs in the 500 mesh sieve were washed in a slow stream of cold tap water. Number of eggs present in one ml of this sieved suspension was determined with the help of counting chamber. One ml of egg suspension (30-45eggs) and 1ml of aqueous extract of leaves were separately transferred to glass cavity blocks and maintained at room temperature. Three replications of each treatment were conducted and juvenile mortality was recorded for each. The number of eggs hatching, were counted after 60, 120 and 180 min of exposure under an inverted microscope. The results were recorded and % effectiveness of the preparations was analyzed. The controls were maintained to study the effect. The findings of the experiment are summarized in the Table 2.

RESULTS

In the present experiment the root-knot nematodes were found to be highly pathogenic on tomato in untreated inoculated control pots where the root-knot index (RKI) was 4.25. Root-galling caused by *M. incognita* reduced in different treatments of powdered leaves, more being at higher dose (5%w/w). In the plants treated with mixed leaves of all plants (5%w/w), the root-knot index was only 0.58 which was significantly low as compared to untreated inoculated control. The gall indices at the similar dose in other treatments were 0.95, 1.05,2.45 1.00,2.70, 1.15, 2.54, 1.16,2.65 1.04 and 0.58 in *Azadirachta indica, Saraca indica, Calotropis procera, Argemone maxicana, Catharanthus roseus, Calendula officinalis, Eucalyptus camaldulensis, Phoenix sylvestris, Cymbopogon citrates* and *Mentha piperita* respectively and the corresponding Figures of root-knot index for same treatments when applied at 1% w/w were 2.22, 2.78, 2.85, 2.75, 2.23, 2.70, 2.38, 2.72, 2.30 and 2.66 respectively (Table 2).

Table 2: Efficacy of bio control agents on *Meloidogyne incognita* infestation in tomato plant.

Treatments	Dose %w/w of soil	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Gall index*
Azadirachta indica	0.1	25.6±2.2	418±1.2	15.65±1.3	28.50±0.2	2.26±1.4
	1	27.5±1.5	425±1.3	18.75±2.1	32.50±1.2	2.22±0.5
	5	67.35±1.2*	721±2.2*	31.24±1.4*	72.35±1.3*	0.95±2.3*
Saraca indica	0.1	20.5±0.2	318±1.4	14.10±1.2	25.55±0.2	2.85±0.4
	1	21.67±1.2	400±1.6	14.20±0.2	27.85±1.4	2.75±0.2
	5	27.78±1.3	418±1.2	14.45±1.4	41.2±1.6	2.45±0.2
Calotropis procera	0.1	21.25±2.2	412±0.2	23.35±1.8	42.87±2.1	2.85±0.6
	1	30.27±1.2	412±1.2	25.25±1.2	48.75±1.2	2.28±0.01
	5	63.36±1.6	723±2.1	32.20±1.6	71.25±1.3	1.05±0.13*
Argemone maxicana	0.1	20.45±1.3	315±2.2	16.45±1.2	30.35±2.2	2.96±0.3
	1	22.34±1.0	350±3.1	16.55±2.2	31.45±1.6	2.75±0.2
	5	25.65±1.2	422±1.8	18.12±1.3	37.3±1.4	2.70±0.5
Catharanthus roseus	0.1	24.7±2.2	345±1.4	22.12±1.3	42.25±1.3	2.25±0.2
	1	24.75±1.2	415±1.0	25.12±1.1	44.57±1.3	2.23±0.5
	5	67.46±1.6*	720±1.3*	31.15±1.1*	72.23±1.2*	1.00±0.2*
Calendula officinalis	0.1	24.5±1.3	412±1.4	12.15±1.5	25.25±2.2	2.77±0.2
	1	24.35±2.2	415±1.8	15.25±1.6	26.57±1.3	2.70±0.4
	5	25.5±1.2	480±1.9	18.15±2.0	38.5±1.6	2.54±0.3
Eucalyptus camaldulensis	0.1	24.50±1.3	413±1.2	19.18±1.2	37.86±2.2	2.55±0.4
	1	32.5±1.2	525±1.2	21.20±1.2	40.35±1.2	2.38±0.6
	5	63.64±1.6*	716±1.6*	32.26±1.6*	73.36±1.0*	1.15±0.4*
Phoenix sylvestris	0.1	23.24±0.4	318±1.4	14.15±1.7	25.57±1.7	2.75±0.5
	1	27.34±0.3	350±1.3	15.15±2.2	26.55±1.8	2.72±0.6
	5	30.25±0.4	425±1.4	18.34±1.8	37.8±1.7	2.65±0.3
Cymbopogon citratus	0.1	27.25±1.8	350±1.3	21.25±1.6	42.34±1.8	2.45±0.6
	1	34.38±2.2	540±1.2	25.25±1.7	47.35±0.2	2.30±0.4
	5	64.56±1.9*	710±0.3*	30.35±0.3*	74.45±0.2*	1.16±0.1*
Mentha piperita	0.1	35.45±1.3	425±1.2	28.5±2.2	66.4±1.6	2.85±0.1
	1	37.80±2.2	436±1.0	28.67±1.7	68.5±1.4	2.66±0.3
	5	65.34±1.4*	675±2.1*	30.5±1.4*	75.52±1.1*	1.04±0.2*
Mixture	0.1	57.7±2.2	556±1.4	28.5±1.3	72.25±1.4	2.10±0.3
	1	58.5±1.3	550±1.8	28.8±1.4	72.50±1.3	1.75±0.2
	5	85.8±0.3+	780±1.4+	35.5±1.6+	82.35±1.4+	0.58±0.1+
Untreated inoculated	-	15.5±1.2#	200±1.3#	12.5±1.6#	35.5±1.8#	4.25±0.5
Untreated uninoculated	-	56.8±1.3	528±1.1	27.8±0.2	70.2±1.7	-

Gall index 1= 0 galls, 2 = 1-9 galls, 3 = 10-30 galls,4 = 31-100 galls and 5 = more than 100 galls per root system, Values are Mean ± S.E; *P<0.05*, *significantly different from Untreated inoculated at same time and concentration, # significantly different from Untreated uninoculated+ significantly different from all treatments.

Mixed leaves treatment was found to be highly effective in bringing down the nematode populations at all concentrations. The population of root knot nematodes declined significantly when treated with all ten plant leaves; however Neem, Madar, Sadabahar, Eucalyptus, Lemon Grass and Mint leaves proved more effective reducing the population in comparison to the rest of treatments. Treatment with the mixture of leaves was observed to be highly effective in reducing the nematode populations at 5% w/w. These treatments showed a comparatively lesser decline in the population of the *M. incognita* (Table 2) when applied at their lower doses (1%w/w).

After various plants leaves treatment, there was a notable enhancement in the plant development characteristics (Shoot/root length and weight), however the increase was more pronounced at higher doses where inhibition in root galling was observed to be maximum. Among all the treatments, the best plant growth characters (plant length = 85.8 cm, shoot weight = 780 g, root length = 35.5cm and shoot weight 82.35g) was observed when pots were treated with mixed leaves of all plants (5% w/w), It was followed by Neem, Madar, Sadabahar, Eucalyptus, Lemon Grass and Mint leaves at same dosage. The enhancement in the plant growth was relatively less with a similar trend when the same treatments were applied at their lower doses (Table 2).

Data in Table 3 show the effect of plant leaves extracts on hatching of root-knot nematode at different periods and concentrations. The results show a progressive decrease in the hatching of eggs with successive increase in concentration of each extract and the increase in exposure period. The maximum egg hatching was observed in *Argemone*, whilst the minimum was observed with the *A. indica*. However, the mixture of six plants Neem, Madar, Sadabahar, Eucalyptus, Lemon Grass and Mint leaves extracts was most effective to control egg hatching in the nematode at all the time and concentration, with the mean egg hatching 1.25% at 180 min followed by mixed leaves extract of all ten plants with mean egg hatching 10.14 % and in *A. indica* with mean egg hatching 11.23% in same duration and at 100% concentration. The maximum egg hatch was spotted in the control treatment, whilst the minimum egg hatch was observed in the *A. indica* extract. The most effective plant extract inhibition of egg hatching was the mixture of six plants Neem, Madar, Sadabahar, Eucalyptus, Lemon Grass and Mint leaves at the 100% concentration, which conferred the lowest egg hatching.

DISCUSSION

Numerous studies with different approaches and strategies have been performed for the control and management of nematodes, and most of them are entirely or partially dedicated for the termination or reduction of the total populace upto the damage threshold, thereby bringing down the total damage to a negligible economic value.[14] Many of these studies are based on observation and recording the effects of nematicides which may be, lab synthesized or plant or animal extracts. Numerous examples of wild plants and cultivated medicinal plants are in existence which are indicative of their nematicidal properties against root knot nematodes.[15] The current study deals with the efficacy of decoction of leaves of some commonly found plants on the biology of the root knot nematode, *M. incognita*. A series of both *in-vitro* and pot experiments was carried out to test and assess the efficacy of leaf extracts of ten common and easily available plants against *M. incognita*. However, leaves of six plants *Azadirachta indica, Calotropis procera, Catharanthus roseus, Eucalyptus camaldulensis, Cymbopogon citratus,* and *Mentha piperita* were found to be the most effective in inhibiting gall formation. This observation was further confirmed when the extract of the leaves of same plants showed significant effect on suppression of nematode egg hatching and their mixture was even more effective in inhibiting egg hatching. In a similar study the leaf extracts of plants were reported to inhibit egg hatching in *M. incognita*, *M. javanica*, and *M. arenaria*.[16] In another study comparable observations were made by[17] who described nematicidal properties of various plant extracts. The recorded observations showed that egg hatching inhibition in the nematode directly corresponds to the concentration of the extract and the maximum mortality was recorded at the maximum concentration of the aliquot/ extract which was 100%. Similar findings were recorded by[18,19] on root-knot nematode where gradual increase in inhibition of egg hatching was observed with the rise in the concentration of the root extracts.

The inhibitory effect of plant extracts on egg hatching of nematodes may be because of the presence of ovicidal phytochemicals or ovicidal phytochemical compounds which may suppress or inhibit the egg hatching in these organisms.[19] Also reported in their study that biochemicals possessing nematicidal properties may have a fatal effect on the embryonic development of nematodes. Apparently, these nematicidal properties increase with time, and therefore, the inhibition in the hatching of eggs tends to proportionately increase with increment in the exposure time of eggs in the extract. Here the vital phytochemicals may either interfere with the embryonal development or destroy and kill the eggs or may even dissolve the egg masses which is also communicated in previous studies.[20,21] In another investigation[22] confirmed the efficacy, *Pseudomonas fluorescens* and *Trichoderma viride,* as biocontrol agent for root knot nematode, *Meloidogyne incognita* on mulberry.

Results of the present study indicate that a mixture of extracts of six plants ie., *Azadirachta indica, Calotropis procera, Catharanthus roseus, Eucalyptus camaldulensis, Cymbopogon citratus,* and *Mentha piperita* recorded the best results regarding inhibition of egg hatching as compared to individual plant extracts or a combination of all the plant species taken for the study. Thus, this finding is important in the identification and

Table 3: Effect of selected plant extracts on hatching of *Meloidogyne* spp. egg at different periods.

Treatments	% Conc V/V	Mean number of egg hatching (%)		
		60 min	120 min	180 min
Azadirachta indica	25	26.05±1.3	30.13±1.4	32.04±0.2
	50	18.20±1.2	40.32±1.6	40.12±1.4
	100	12.30±0.2	14.21±1.5	11.23±1.1*
Saraca indica	25	62.23±1.3	80.14±0.5	84.35±1.3
	50	54.10±1.4	85.12±1.6	80.16±1.2
	100	52.15±1.2	70.25±2.2	76.7±0.5
Calotropis procera	25	24.13±1.2	32.24±0.2	34.24±1.8
	50	25.03±1.4	23.25±1.3	33.25±2.2
	100	17.12±1.0	14.15±1.4	16.14±1.2*
Argemone maxicana	25	41.25±1.6	85.60±2.2	86.13±1.8
	50	40.22±1.3	80.32±1.5	81.37±1.1
	100	45.13±1.2	83.2±1.4	81.25±1.3
Catharanthus roseus	25	38.29±1.3	52.35±1.3	65.35±1.6
	50	40.65±0.2	51.45±1.2	52.20±0.2
	100	42.34±1.4	22.56±1.1	10.35±1.3*
Calendula officinalis	25	42.05±1.25	45.25±1.05	76.06±1.3
	50	43.20±1.3	51.27±2.2	52.18±1.4
	100	56.23±0.2	62.20±1.6	71.35±0.4
Eucalyptus camaldulensis	25	19.32±0.23	32.45±1.6	30.48±1.3
	50	20.26±1.3	18.38±0.3	19.65±1.6
	100	20.18±0.2	28.19±1.6	18.17±1.8*
Phoenix sylvestris	25	47.08±0.2	48.19±1.4	78.29±1.9
	50	47.29±0.6	45.36±1.8	67.18±0.2
	100	54.76±1.8	48.28±0.2	72.57±0.7
Cymbopogon citratus	25	16.56±0.7	23.87±0.3	14.25±0.2
	50	15.78±0.5	21.56±0.8	20.35±0.2
	100	16.34±1.8	14.45±0.7	12.15±1.9*
Mentha piperita	25	23.24±1.4	32.56±1.8	35.16±0.9
	50	32.15±1.7	30.34±1.2	30.16±0.2
	100	18.25±0.2	17.45±0.7	13.17±0.2*
Mixture of 6 plants	25	35.04±1.3	25.14±0.7	18.11±0.3
	50	20.16±1.2	12.11±0.6	11.14±0.4
	100	10.13±0.6#	8.07±0.3#	1.25±0.2*#
Mixture of all	25	11.11±0.2	26.15±1.3	35.11±1.1
	50	20.02±1.3	24.03±0.4	30.10±1.3
	100	21.7±1.4*	15.11±1.1*	10.14±1.3*
Untreated control	-	54.7±1.2	82.4±1.3	100.0 ±0.0

Values are Mean ± S.E; *P<0.05*, *significantly different from Untreated control, #significantly different from all treatments at same concentration and time.

development of alternative strategies in the treatment and control of the root-knot nematodes. However, further work is needed to identify some of these main compounds after purification.

The selected plants are being used to test their nematicidal properties against the plant parasitic nematodes and further studies have been carried out for specifying their efficacy towards the mortality of the phyto-nematodes.

Along with that they also provide additional benefits like growth enhancement and act as soil treatments as well. The study provides an eco-friendly cost effective easy to use nematicide formulations for management of *Meloidogyne* spp.

CONCLUSION

The plants used for the study are effective in controlling the populations of the plant parasitic, root knot nematodes by virtue of their nematicidal properties. Their application to the soil for the said purpose also causes growth stimulation of the plant providing much needed micronutrients and moisture holding capacity to the soil. The study provides an economical, environment friendly, effective, easily available and convenient method for the management of the *Meloidogyne* sps. and provides for increased crop production.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RKI: root knot indices; **Sps.:** species.

SUMMARY

Meloidogyne species is a nefarious parasite of all kinds of flora. All plants cultivated or otherwise fall prey to this root knot nematode. Since it's discovery, about a century ago, efforts have been made to manage and control this phytonematode. But most of the earlier control methods were chemical based which though somewhat managed the parasite, caused irreparable harm to the environment, polluting land and water bodies alike, and causing bioaccumulation and bio magnification of organic compounds. keeping in view this environmental degradation methods have been worked out by various scientists in many countries. This study deals with an environmentally sustainable method for the management of meloidogyne species using aliquots of selected plants, in various concentrations alone and in comination. Different combinations have different effects on the mortality of the parasite. Synergistic effect of combination of aliquotes or single plant extract show various degrees of mortality of the plant parasite.

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