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Nematicidal potential of Plant extracts against the Root Knot Nematode, *Meloidogyne incognita*

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ABSTRACT: Plant parasitic nematodes (PPN) are the most effective parasites in nature causing enormous crop yield loss. Due to their ability to excel in a wide variety of the host plants and our lack of knowledge about the biology of these plant parasites, there are limited ways to control them. From among various groups of PPN, endo-parasitic nematodes, *Meloidogyne* sps., commonly known as root-knot nematodes, are the most damaging one and also difficult to control. Because of the accessibility of the whole genome sequences of a few *Meloidogyne* species, biotechnological interferences are being used to learn the secrets of their unrestricted survival. The use of chemicals to control these nematodes has been widely documented. The environmental toxicity of these known compounds and the limitations on the use of nematicides in deterrence of nematodes necessitates the screening and exploration of eco-friendly methods. The current study was carried out to assess the nematotoxic potential of leaf extracts from red spiderling (*Boerhavia diffusa*), atrilal (*Peristrophe bicalyculata*), chaff-flower (*Achyranthes aspera*), castor oil plant (*Ricinus communis*) and pigweed (*Anagallis arvensis*). The usage of the aqueous extracts hindered the ability of the nematode eggs to hatch, the mobility of second stage juveniles (J2), and the feasibility of the J2. The eggs were exposed to numerous concentrations of the certain extracts for periods ranging from 24 hours to 6 days. The aqueous extracts had an impact on nematode juvenile mortality and egg hatching. Less egg hatching and increased nematode juvenile mortality indicate that leaves of the selected plant possess anti-nematode potential. The results of the study reveal the effectiveness of the natural products and could possibly result in the development of ecologically friendly management strategies for the PPNs.

Keywords: Root-knot nematode, juvenile mortality, management, green approach.

INTRODUCTION

One of the most successful multicellular creatures that parasitize practically all plants are plant parasitic nematodes (PPN). PPNs evolved gradually to devour a range of plants between 120 and 135 million years ago (Blaxter and Koutsovoulos 2015). Nematodes have an edge over other creatures in terms of their ability to live in a variety of environmental situations due to their diversification (Kern *et al*., 2020; Lorenzen, 1994). Plant-parasitic nematodes endured many parasitic episodes during evolution, according to phylogenetic analyses of worm genomes (Perry and Moens, 2011). These findings unequivocally prove that advantageous genes were picked up during nematode evolution (Crook, 2014). Nematodes have taken over every conceivable space in an ecosystem. Root-knot nematodes have a definite sexual dimorphism and a consistent life cycle. In *Meloidogyne*, the female nematode has a tear-drop shape and dwells inside the plant root while the male worm is always vermiform and only feeds on the plant's exterior surface (Kyndt *et al.*, 2013). After fertilisation, the implanted female lays her eggs in a gelatinous matrix, leaving egg masses on the roots' surface. *Meloidogyne* egg masses range in size from 400 to 700 eggs (Ferris, 2010). In the optimum setting, the root-knot nematode takes 28 days to complete its life cycle. PPNs are a most devastating biotic stressor for plants that have a negative impact on agricultural crop production (Postnikova *et al*., 2015). These parasitic worms have developed unique feeding habits as a result of their parasitic adaptations (Mesa-Valle *et al*, 2020). According to earlier studies on the types of harm caused by plant parasite nematodes and other secondary illnesses, plants sustain considerable field damage that results in substantial economic loss (Nicol *et al.*, 2011). By damaging crop plants, these nematodes impound huge financial losses. Nematodes that parasitize plants severely hinder plant growth, which eventually affects yield (Blaxter *et al.*, 1998). Usually, the secondary infection causes the plant to start wilting before worm attack occurs (Desmedt *et al.*, 2020). It has also been reported that nematode damage to the plant parts cause substantial crop yield losses by diverting nutrients, disrupting water transport and may also act as vector for viruses, which in turn mitigates the harm (Bird and Kaloshian 2003). A number of coordinated projects over several years have thoroughly

quantified the damage caused by various nematodes. According to Indian estimates, crop losses would be 21.3% higher than expected, or $1.58 billion yearly. PPNs are thought to result in $173 billion in annual losses globally (Elling, 2013). Also, the anticipated loss from India is a note worthy concern. It justifies making major efforts to manage nematodes that parasitize plants. Several agricultural plant species have been subjected to extensive research into the negative impacts of nematodes (Kumar *et al.*, 2020). It has been discovered that the most severe economic harm to rice plants is caused by *Meloidogyne* spp (Rusinque *et al.*, 2021).

Currently, a variety of techniques are employed to lessen the harm that plant-parasitic nematodes cause. Techniques used to restraint PPN damage are used to maintain nematode numbers in fields below a predetermined threshold. Farmers frequently employ chemical control techniques in the field (Sasanelli *et al*., 2021). Chemical-based management techniques offer quick fixes, but because of their toxicity, they also harm the environment and undesirable species (Dieterich *et al.*, 2009). Besides chemical control, other methods are also being utilized. The idea that an enemy's enemy is a friend underpins the concept of biological control of PPNs. As biological weapons against PPNs, numerous bacterial and fungi strains are used (Hamidi *et al*., 2020). The possibility of finding additional biological agents that could lessen the harm caused by the plant-parasitic nematodes has also been investigated (Meyer, 2003). In an effort to minimise the effect caused by PPNs, many methods of physical control, regulatory control, and cultural control have been explored. Researchers have come up with an idea for using multiple strategies rather than just one to control the plant-parasitic worm. Integrated pest management (IPM) approach is employed for overall improvement of crop yield (Masonbrink *et al.*, 2021).

Scientists are growing more concern about unrestrained use of chemical-based nematicides which might lead to worm resistance. Endo-parasitic nematodes are the most harmful group of worms. The control strategies are directed mostly at endo-parasitic nematodes such cyst nematodes and root-knot nematodes. It is critical to consider alternatives to the chemical control strategy for treating these nematodes. Therefore, it is need of the hour to establish eco-friendly management methods. Many plant components have been found to possess anti-nematode potential (Mwamula *et al*., 2022). Plant extracts have been employed in certain studies to reduce PPN damage both *in vivo* and *in vitro*. The discovery of secondary metabolites, which act as natural defence molecules in plants and, when scaled up, can prove to be a better source for nematode management, sparked the idea of controlling plant-parasitic nematodes using chemicals derived from plants. The development of plant-based nematode control methods is further aided by this concept. Previous research has shown that a variety of plants have anti-nematode properties. These bio-based control techniques are simple to develop and secure to apply in field conditions. There have been numerous attempts to use plant-based remedies to manage different forms of root-knot nematodes (Shakya, 2022). Different plants have distinct effects on root-knot nematode isolates. It will therefore be necessary to search for additional plants that display anti-nematode characteristics. The aim of this study was to evaluate the anti-nematode potential of leaves from five specific plants against the root-knot nematode *M. incognita*.

MATERIALS AND METHODS

The standard protocol described below was followed to collect root-knot nematodes, identify them, maintain nematode stocks on the susceptible tomato plant *Lycopersicon esculentum* var. Pusa Ruby, prepare leaf extracts, hatch nematode eggs, treat nematode eggs (hatching) in occurrence of active compound, monitor hatching, and inhibit hatching (if any) over a designated period of time.

Nematodes. The root-knot nematodes used in this study came from tomato plants that were produced nearby in Agra. Under running water, the roots of infected tomato plants were carefully washed to remove adhered soil particles. The root was properly cleaned under running water to remove any last bits of dirt after dipping them in a bucket of water for two hours. With the aid of a laboratory forceps and a light microscope, individual egg masses were meticulously removed. For continued pattern cutting and identification, corresponding females were cut out, preserved in water, and removed under a microscope right away. For hatching, egg masses that matched female *Meloidogyne incognita* were procured. 35 mm glass petriplate were filled with water and added with individual egg masses to it. In a biological incubator, the petriplate was incubated at 28°C for 24 hours. Thirty egg masses were used for hatching separately. The following day,100µl of water was removed from each petriplate using a glass pipette. The number and viability of the young active juveniles in each petriplate were examined under a microscope (J2). Active J2 was injected into the roots of newly planted tomato plants using 15 ml of water.

Maintenance of *Meloidogyne incognita* Infection on Tomato Plants. Root-knot nematode infections are particularly prone to affect tomato plants. The tomato seeds (*Lycopersicon esculentum* var. Pusa Ruby) were procured from national seed company Beej Bhawan, Pusa Complex, IARI, New Delhi. For the purpose of starting nurseries, a 3:1 mixture of soil and sand was sterilised in a laboratory autoclave in a number of small batches. In a large container, fifty tomato seeds were planted, and they were given three weeks of tap water irrigation. Each plantlet was moved into a single container with sterile soil and sand mixtures after three weeks and allowed to grow for a further two days. 15 ml of water containing live juveniles were then poured over and close to the plant roots after the surrounding soil had been dug up. The soil was then placed on top of the roots. After the infestation, every plant was moved into the shadow. After the first three days without irrigation, the plants received all the water they required. The plants were allowed to become infected for 45 days. Once the stipulated time had passed, the

infected plant was dug up, and the established method outlined above was used to cut the perennial pattern and the corresponding female species was identified. The pure culture of *Meloidogyne incognita* was thereafter maintained in the tomato plants.

Collection of Eggs from Infected Plants. Egg masses were retrieved from the *Meloidogyne incognita* population that was being maintained on the Pusa Ruby tomato variety using surgical needles and lab forceps. Galled roots were preserved by being divided into tiny pieces and placed in a 0.1 percent sodium hypochlorite solution. Then this liquid was swiftly blended for two minutes in an electric blender. A series of nested sieves with varying pore diameters were used to filter the resulting egg suspension. Above sieves with mesh sizes of 400 and 500, a sieve with a mesh size of 100 was placed. After assembling, the sieves were used to filter the suspension from the blender. For one minute, the assembly was submerged in gently running tap water. In a glass beaker, the contents of the 400- and 500-mesh sieves were combined (Borosil). Further samples of each sieve's contents were taken for microscopic examination. After being separated, the eggs were put in water-filled petri dishes. All of the egg masses utilised for hatching were put on filter paper with wire mesh on top of these petriplates filled with water. This setup was done to prevent drying up of the filter paper. This setup was kept at 28°C for 24 hours in an incubator. Plant extracts were tested using the egg masses collected using a 400- and 500-mesh filter, and the offspring were used to maintain nematode stock levels.

Leaf Extracts preparation. The Government PG College campus in Fatehabad, Agra, Uttar Pradesh, India was the location for the collection of the leaves from five different plants: the pimpernel (*Anagallis arvensis*), the castor oil plant (*Ricinus communis*), the atrilal (*Peristrophe bicalyculata*), the chaff-flower (*Achyranthes aspera*), and the red spiderling (*Boerhavia diffusa*) (Latitude 27.022492 Longitude 78.307349). After being carefully selected, the leaves were thoroughly cleaned under running water, then placed on filter paper to dry in the shade. 10 grams of the leaves from each plant were added to 100 ml of distilled water, which was then rapidly blended in a kitchen blender. The resultant suspension was filtered using filter paper (Whatman No. 1). The same process was followed with the remaining leaves. The filtrate was centrifuged in a clinical centrifuge at 1000 rpm for ten minutes. The extract was removed, and the pellet was then dissolved in 1 ml of distilled water. The stock solution for this preparation was 100%. This stock solution was diluted five, ten, and twenty times using distilled water. Without employing any plant extracts, a control experiment was run using just distilled water. With the appropriate controls, each experiment was repeated three times.

Assessment of anti-nematode Potential. The anti-nematode potential of selected plant leaves was investigated *in vivo* in laboratory conditions. The egg masses (15 egg masses per sterile petriplate with 10 ml of the chosen concentration of the leaf extracts) were diluted by 5%, 10%, and 20% using the stock solution. The incubation was carried out at 28^0C in a biological incubator. The nematode egg hatch at the end of the sixth day. The hatching process was observed at 24hrs, 48hrs, 72hrs, 96hrs and on day 6. Each experiment was carried out three times. After the predetermined time intervals, the quantity of juveniles was counted using nematode counting slides. The delayed hatching of nematode eggs served as evidence of the leaf extract's anti-nematode potential. Also, the number of deceased juveniles in the batch that was preserved until the sixth day were counted and noted. The degree of anti-nematode potential from the selected leaves was determined by the level of juvenile nematode activity at the time of monitoring. In each group, the number of dead juveniles was counted.

Juvenile Mortality Study. The J2s were placed in a 30 mm glass pertiplate that was filled with various leaf extract concentrations. Their movement was originally observed under a light microscope. Later, after 96hrs of incubation period, mortality of the juveniles was noted.

Statistical Study. Following statistical analysis using the Student's t-test and one-way ANOVA (Dunnett's Multiple Comparison Test), the results were expressed as the mean standard error (S.E.). A conventional pattern was followed where $p<0.05$ was taken as evidence of significant variance, $p<0.01$ was defined as highly significant and $p>0.05$ was not significant (Monte Carlo approach). Using the required controls, each experiment was carried out three times.

RESULTS AND DISCUSSION

The anti-nematode potential screening shows that the anti-hatching active compounds may be present in the chosen leaves at varying levels. The number of juveniles that have emerged from the egg masses suggests the presence of the active component found in the leaf (15 egg masses were used with each concentrate in triplicate). Reduced egg hatching is indicative of the presence of potent nematicidal activity. When the egg masses were kept in the presence of *Ricinus* leaf extract, egg hatching process was significantly low both during the incubation phase and also with the increase in the leaf extract concentrations. Moreover, the juvenile population substantially fell in 20% of the leaf extract concentration. Similar declining patterns were observed in the case of *Anagallis* and *Peristrophe* leaf extracts where the decline was highest at 20% concentrations (Table 1). The data in the table reveals that the leaves of the chosen plants exert nematode inhibition that prevents *Meloidogyne incognita* eggs from developing into larval form. The anti-nematode action of the *Ricinus* plant extract was notable, and it intensively slowed the egg hatching process. Moreover, a significant amount of nematicidal activity was discovered in the *Ricinus* extract.

The screening of a particular leaf extract for anti-nematode potential indicated towards the presence of the anti-nematode chemicals in these leaves. With varied dosages of leaf extract, *Meloidogyne incognita* egg hatching was significantly hindered. To support the results of the screening experiment, *M. incognita*

juveniles (J2) were tested through mortality experiments. Juvenile mortality rates were 15.4%, 24.5%, 61.4%, and 84.3% for *Boerhavia, Achyranthes, Peristrophe* and *Ricinus* respectively (Fig. 2). The death rate in the case of highest concentration of *Anagallis* leaf extract was 40.3%. At of 5 gm/100 ml concentration, the percent juvenile mortality was 4.9%, 6.5%, 11.8%, 37% and 23.4% for *Anagallis, Boerhavia, Achyranthes, Peristrophe* and *Ricinus* respectively. With the increase in the concentration, the percent juvenile mortality in the presence of different leaf extract increased gradually. The graph shows the percent mortality of juveniles at different concentrations (5%, 10%, 20%) after exposure to the leaf extracts. According to the aforementioned results, *Peristrophe* and *Ricinus* leaf extract demonstrated most potent anti-nematode properties.

It is obvious that all of the selected plants' leaf extracts exerted anti-nematode effects. *Ricinus* leaf extract prevented nematode eggs from hatching to a high level in comparison to the untreated control groups (Table 1). At a dose of 20 gm/100 ml, this action showed its highest manifestation. *Ricinus* leaf extract was also discovered to have anti-nematode potential at doses of 5 and 10 gm/100 ml, but the impact was best at 20 gm/100 ml. The statistical significance of the experiment was established using all the required parameters in the context of the data's screening analysis and regression analysis (Table 2).

To decrease the danger caused by nematodes that attack plants, scientists have experimented with many metabolites from living organisms and also researched about the possible anti-nematode properties of compounds like serpentine, tannic acid, fungal poisons, fatty esters, sea weeds, mustard oil and the action of phenolic complexes (D'Addabbo *et al.*, 2020; Guo *et al.*, 2017; Oliveira *et al.*, 2019). The effects of essential oils and secondary metabolites has also been investigated (Hugot *et al.*, 2001). Manure-based products have also been researched as management possibilities. Moreover, the power of microbes has also been used to control nematodes (Bhat *et al.*, 2023). Cultivars of resistant crop plants provide organic defences against nematode invasion. The plants having active compounds have all shown resistance in one form or another. Detailed explanations of nematode management techniques that work both alone and in combination with other diseases are also reported (Fleming *et al.*, 2017). Since last decade, transgenic methods have also been investigated in an effort to reduce plant-parasitic nematode infection (Iqbal *et al*., 2020). The basic necessity for transgenic technology is an improved comprehension of nematode biology.

Many studies conducted over the years have shown that a variety of techniques can be used to manage plant-parasitic nematodes (Anjali *et al.*, 2022; Aloria *et al.*, 2023). Control by the use of chemicals is widely accepted and practiced by farmers. The harm caused by continuous use and enormous amount of chemicals added to the soil have necessitated the exploration of new methods for PPN management. A great deal of research has been done on the biological control of PPN in order to scale up the product for future use. Among biological methods of control, the common plant varieties and weeds should be tested as they may offer a potential solution. Waste products are also among these resources (Topalovic *et al.*, 2020). Prior investigations on plant-based nematode control agents have shown varied results (Firew *et al.*, 2020; Sheshma *et al*., 2023). They prove to be economic and easy to produce.

The study plants are known for medicinal properties. Active compounds present in the plants may lessen the nematode burden in the test plant and thus, improve the crop yield. *Ricinus communis* have always been known for their biological activities such as antiviral, antibacterial, antifungal, antioxidant, cytotoxic, insecticidal) (Elkousy *et al.*, 2021; Abbas *et al.*, 2018; Sotelo-Leyva *et al.*, 2020; Owoseni and Faboro 2010; Awatif and Mohammed 2018). The results of the current study can be corelated with the presence of toxic substances released by the extracts such as alkaloids, saponins, flavonoids and amides including benzamide and ketones, which have juvicidal and ovocidal potentials and work singly or in combination (Waris *et al.*, 2020).

Table 1: Number of juveniles (J2) observed after incubating for specified time intervals in different (5%, 10%, 20%) of leaf extracts of five selected plants.

Sr. No.	Name of the plants	Concentration of leaf extracts											
		5%				10%				20%			
		24h	48h	96h	144h	24h	48h	96h	144h	24h	48h	96h	144h
1 .	*Boerhavia*	125	215	306	406	115	154	215	246	95	133	119	96
2 .	*Anagallis*	84	126	216	294	104	124	118	106	61	84	54	57
3 .	*Achyranthes*	106	176	246	316	114	142	176	221	106	149	224	265
4 .	*Peristrophe*	68	107	159	226	52	64	68	69	49	52	59	43
5 .	*Ricinus*	77	83	103	119	48	47	35	34	45	46	22	11

Table 2: The LC_{50}, LC_{90}values of different leaf extracts.

Sr. No.	Name of the plant	LC_{50}(mg/ml)	LC_{90} (mg/ml)	Regression	Regression equation
1.	*Boerhavia*	42.04	86.98	0.88	Y = 0.97+2.15X
2.	*Anagallis*	17.58	53.12	0.16	Y = 0.23+3.17X
3.	*Achyranthes*	24.25	62.76	0.24	Y = 0.39+3.22X
4.	*Peristrophe*	15.68	36.32	0.45	Y = 0.44+3.83X
5	*Ricinus*	14.75	31.26	1.75	Y = 1.89+0.79X

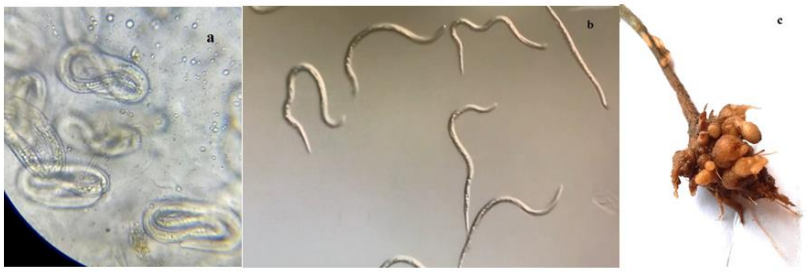

Fig. 1. The various stages of root knot nematode (a) showing juvenile stage (J2) inside the egg, (b) juveniles hatched out of the eggs, (c) galled root of tomato plant.

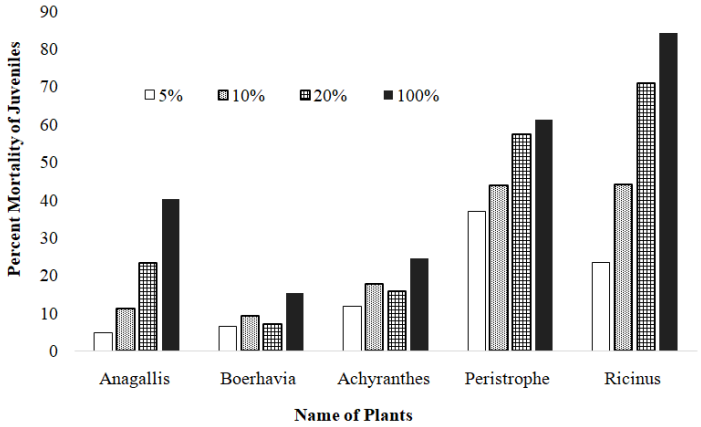

Fig. 2. Percent mortality of juveniles (J2) in the presence of different concentrations (5%, 10%, 20% and 100%) of leaf extracts of selected plants.

CONCLUSIONS

The main source of global food supply comes from agricultural products. Loss of crop yield results in a threat to food security, increased food shortage, malnutrition and starvation. It is, therefore, necessary to produce ample amount of good quality and nutritious food crops. Among other factors, plant parasitic nematodes are a major group that hinder the crop yield. Chemical nematicides are prohibited in several nations due to their environmental harm. In an effort to find alternative control mechanisms, a variety of solutions, from waste materials to cutting-edge molecular procedures, have been researched. Further characterisation of the chemical moieties in the selected leaf extract is necessary for the large-scale synthesis of bioactive compounds. It is safe to say that plant extracts offer an efficient and reasonably cost source for reducing the threat of RKN in fields based on the findings of this screening of anti-nematode capabilities in five selected plants. This experiment shows that *Ricinus*, followed by *Peristrophe*, *Anagallis*, *Achyranthes* and *Boerhavia*, has the strongest anti-nematode power against root-knot nematode. Root-knot nematode management is made simple with plant-based remedies. A list of plants that are naturally anti-nematode requires significant research involving a larger variety of plants and numerous nematode species (depending on their feeding habits). The products that contain *Ricinus* as a main component have shown to be the most reliable.

FUTURE SCOPE

The promising nematicidal potential of aqueous *Ricinus* leaf extract will be further extrapolated. The histology studies will be carried out to observe the effect of extracts on the roots of the tomato plant. *In vitro* evaluation of leaf extracts will be carried out at various concentrations for determination of least effective dose for environment friendly management of root knot disease.

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Conflict of Interest. None.

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