International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; SP6: 463-466

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(Special Issue -6)
3rd National Conference
On
PROMOTING & REINVIGORATING AGRI-HORTI, TECHNOLOGICAL INNOVATIONS
[PRAGATI-2019]
(14-15 December, 2019)

Management of root-knot nematodes, *Meloidogyne incognita* on chillies using botanicals

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Abstract
Investigations were carried out to study the nematicidal potential of botanicals against Root-Knot Nematode, *Meloidogyne incognita* in Chilli. The aqueous extract of *Azadirachta indica* had the highest level of inhibition on hatching of nematode eggs (60.50 and 71.5% after 72 hr and 120 hr respectively). Significant increase in egg hatching inhibition as compared to *A.indica* was also observed at 120 hr interval (66.5 and 54.25 %) with *Ocimum sanctum* and *Calotropis gigantea* respectively. In control 100 % of egg hatching was found upto 48hrs.The number of fruits was higher (4.75) in carbofuran treated plant followed by plants with *A.indica* (4.10), *O. sanctum*(4.0) and *C.gigantea* (3.75). The highest reduction of 81.87 per cent in the population of infective juveniles was recorded in the treatment of carbofuran followed by *A. indica* (78.94), *O. sanctum* (74.73), and *C. gigantea* (74.26). The gall index ranged from 2 to 3 in treated plants whereas untreated plants recorded 4 as gall index with more than 33 galls per plant.

Keywords: Chilli, Root knot nematode, Botanicals, Nematicide

Introduction
Chilli (*Capsicum annum* L.) is considered as one of the most important commercial spice crops and is widely used universal spice, named as wonder spice. India is the world leader with a production of 13.76 million tons of chillies contributes 36 per cent to world's production followed by China, Thailand and Pakistan. Tamil Nadu is one of the major producers and consumers of chillies with a production of 29,390 tons from 44,610 ha contributing 3 per cent of total national production.

Root-knot Nematodes belonging to the genus *Meloidogyne* are considered the most important group of plant-parasitic nematodes worldwide attacking nearly every crop. About 5 per cent of the total world crop yield is destroyed due to root knot nematodes (Sasser, 1987) [4]. Chemical nematicide is one of the most fastest and effective nematode control methods, but they are detrimental to both humans and the environment and are relatively unaffordable to the average small scale farmers (Washira *et al*., 2009) [6]. Therefore, there is a need to develop alternative methods of control that are cheap, environmentally friendly and not harmful to humans. Botanical pesticides are often readily available, cheaper than the synthetic nematicides and their crude extracts and are easy to be prepared even by farmers. They reduce the chances of development of resistance or resurgence in pests. Thus, the present investigation was done to evaluate the nematicidal efficacy of botanical extracts *viz*., *Azadirachta indica Tephrosiapurpurea, Acacia auriculiformis, Calotropis gigantea and Ocimum sanctum* on hatching of *Meloidogyne incognita* eggs, nematode infectivity and growth of chilli plants under pot culture study.

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Materials and Methods

Multiplication of Root knot nematodes, *Meloidogyne incognita*

The root knot nematode, *M. incognita* (Kofoid and White) Chitwood required for this study was maintained as pure culture on Chilli cv. PKM 1 raised in pots with sterilized pot mixture (2:1:1 sand, loamy soil and farmyard manure respectively) at Agricultural College and Research Institute, Eachangkottai, Thanjavur.

Preparation of aqueous extracts of botanicals

Healthy leaves of *A.indicaT. purpurea, A. auriculiformis, C.gigantea and O. sanctum* were used for aqueous extracts preparation. It was prepared separately by grinding 50g of leaves with 200ml of distilled water. To obtain a clear and transparent extract, the aqueous extract was filter through a muslin cloth and then centrifuged at 4000 rpm for 10 minutes. The supernatant solution was considered as stock solution and stored it in a refrigerator for laboratory studies. However, for pot culture experiments centrifugation was not done and filtrate from muslin cloth was used as such.

Egg mass collection

Root-knot nematode infected Chilli plant (cv. PKM 1) from the pure culture pot was up-rooted and washed gently under running tap water. Egg masses of *M. incognita* were picked up from the root using dissecting needle and forceps. The collected egg masses were kept in water at 10˚C in a refrigerator to prevent hatching before application of treatments.

In vitro study

The effect of different botanical extracts on the hatching of *M. incognita* eggs was evaluated *in vitro* using the following procedure. The experiment was conducted in a 7.5 cm diameter petri plates taking 10 ml stock solution from each botanicals and maintaining 4 replications. Sterilized distilled water was taken as control. Five uniformly sized egg masses of *M. incognita* were transferred to each botanical extracts in sterilized petri plates (7.5 cm diameter), while egg-masses in distilled water only served as control. The petri plates containing the suspension and the egg masses were kept at room temperature on laboratory bench to allow eggs hatch. The number of hatched second stage juveniles was counted after 24, 48, 72 and 120 hrs.

Pot culture study

Seedlings required for pot culture study was grown in portray filled with steam sterilized coir pith by using PKM 1 variety. Two weeks old seedlings were transplanted in 5 kg capacity pots filled with sterilized pot mixture of 1:2:3 proportions of sand, compost and red soil respectively. The root knot nematode @ 1000 J2 / kg soil was inoculated two weeks after transplanting. The botanical extracts performed well under *in vitro* study were taken for pot culture study. The experiment was terminated at 90 days after transplanting. Growth parameter such as shoot length (cm), root length (cm), shoot weight (g), root weight (g) and no. of fruit per plant were recorded.

T1 – 100 ml of stock solution of aqueous extract of *C.gigantea*
T2 – 100 ml of stock solution of aqueous extract of *O. sanctum*
T3 – 100 ml of stock solution of aqueous extract of *A. indica*
T4 – Carbofuran 0.3 g/pot
T5 – Control

Estimation of final nematode population in soil and number of galls per root

Soil samples from the pots were collected in polythene bags and the final population density of root-knot nematode was assessed by Cobb's sieving and decanting method (Cobb, 1918) followed by modified Baermann's funnel technique (Christie and Perry, 1951). The chilli plant after harvest were carefully dug out and rated for galls using the scale of Taylor and Sasser (1978) by dissection microscope immediately after taking fresh root weight. 0 = No galls, 1 = 1-2 galls, 2= 3-10 galls, 3 = 11 – 30 galls, 4 = 31 – 100 galls, 5 = more than 100 galls.

Statistical analysis

The data were analysed by Anova and least significant differences were calculated at p = 0.1.

Results and Discussion

Nematicidal efficacy of botanical extracts on hatching of *Meloidogyne incognita* eggs

Nematicidal efficacy of botanical extracts *viz*., *A.indica, T. purpurea, A. auriculiformis, C.gigantea and O. sanctum* on hatching of *M. incognita* eggswas made *under in vitro* condition. The results of nematode egg hatching test showed that all tested botanical extracts were found to decrease the hatching rate in egg masses of *M. incognita.* The aqueous extract of *A. indica*had the highest level of inhibition on hatching of nematode eggs (60.50 and 71.5 per cent after 72 hr and 120 hr respectively). Significant increase in egg hatching inhibition as compared to *A. indica* was also observed at 120 hr interval (66.5 and 54.25 per cent) with *O. sanctum* and *C.giganteanr*espectively. In control 100 per cent of egg hatching was found upto 48hrs (Table 2).

Effect of botanicals on nematode infectivity and growth of chilli plants

Pot culture experiment was conducted to assess the effect of botanicals on nematode infectivity and growth of chilli cv. PKM 1. The results indicated that plant growth parameter was greater in plant treated with carbofuran. Similarly, the number of fruits was higher (4.75) in carbofuran treated plant followed by plants with *A. indica* (4.10), *O. sanctum* (4.0) and *C. gigantea* (3.75).

The highest reduction of 81.87 per cent in the population of infective juveniles was recorded in the treatment of carbofuran followed by *A. indica* (78.94), *O. sanctum* (74.73), and *C. gigantea* (74.26). In general all the treatments recorded reduced number of galls in the root. The effects of treatments namely, *O. sanctum* and *C. gigantea*were on par with each other, accounting for 65.90 and 65.15 per cent reduction in number of galls compared to untreated control respectively. The gall index ranged from 2 to 3 in treated plants whereas untreated plants recorded 4 as gall index with more than 33 galls per plant (Table 2).

Table 1: Effect of different botanicals on Inhibition of egg hatching under *in vitro* study

Treatments	Per cent Inhibition of egg hatching at different time interval			
	24h	48h	72h	120h
Calotropis gigantea	36.75	42.25	50.5	54.25
Ocimum sanctum	41.75	46.5	53	66.5
Azadirachta indica	43.5	53.75	60.5	71.5
Acacia auriculiformis	26.25	34.5	41.5	38.5
Tephrosia purpurea	26.75	38	47	45.25
Control	0.00	0.00	0.25	0.36
CD = 0.05	0.602	0.473	0.602	2.126
SEd	0.286	0.225	0.286	1.012

Table 2: Effect of different botanicals on nematode infectivity and growth of chilli plants

Treatments	Plant growth parameter					Nematode population		
	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	No. of fruits / plant	Juvenile population / 100 g soil	No. of galls / plant	Gall Index
Calotropis gigantea	52.02 (4.24)	22.55 (10.97)	15.15 (3.98)	8.42 (18.25)	3.75 (36.36)	11.00 (-74.26)	11.50 (-65.15)	3
Ocimum sanctum	54.42 (9.05)	24.95 (22.78)	15.82 (8.57)	8.55 (20.08)	4.00 (45.45)	10.80 (-74.73)	11.25 (-65.90)	3
Azadirachta indica	60.95 (22.14)	25.85 (27.21)	17.77 (21.96)	10.52 (47.75)	4.10 (49.09)	9.00 (-78.94)	8.75 (-73.48)	2
Carbofuran	62.12 (24.48)	27.32 (34.44)	18.77 (28.82)	11.75 (65.02)	4.75 (72.72)	7.75 (-81.87)	8.00 (-75.75)	2
Control	49.90	20.32	14.57	7.12	2.75	42.75	33.00	4
CD = 0.05	187.65	0.91	58.42	0.56	1.29	1.97	4.12	
SEd	88.03	0.43	27.41	0.26	0.60	0.92	1.93	

Figures in parentheses represent per cent increase or decrease over control.

A number of wild plants growing throughout the world produce compounds having an immobilizing effect on *M. incognita*. These compounds are likely secondary metabolic products and while not involved in primary metabolism contribute to the defense of plants. To assess the nematicidal properties of leaf extracts egg masses were transferred to *A. indica*, *T. purpurea*, *A. auriculiformis*, *C. gigantea*, *O. sanctum* and distilled water. Enhanced inhibition on egg hatching was obtained in *A. indica* extract followed by *O. sanctum* and *C.gigantea* indicating that they possessed nematostatic properties, presence of toxic chemicals in the botanicals might have acted as prohibitors inhibiting emergence of juveniles (Sarosh and Hussain, 1986) [3]. The effect of different extracts on egg hatching could be due to the presence of tannins, alkaloids and flavonoids which have been reported to kill nematodes.

The nematicidal effect of *O. sanctum* extract is attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock *et al*., 1989) [1].

Investigation on the effect of botanicals on infectivity of *M. incognita* on chilli variety PKM 1 pointed out that all the botanical extracts reduced the number of galls on plant roots and nematode population in soil as compared to infested control (Table 2). But the chemical nematicide carbofuran resulted in the maximum suppression of nematode infectivity on plant and soil probably because the population was reduced to a very low density within a short period (Poornima and Vadivelu, 1993) [2]. Of the different botanicals, *A. indica* and *O. sanctum* proved to be most effective followed by *C. gigantea* which may be due to release of some nematoxins from additives (Tiyagi *et al.*, 1991) [5].

Plate 1: Effect of different botanicals on nematode infectivity and growth of chilli plants under Pot culture study

Plate 2: Chilli Plants (PKM 1) treated with different botanical extracts

Conclusion

Experimental results from both *in vitro* as well as pot trials revealed that *A. indica*, *O. sanctum* and *C. gigantean* were highly effective in controlling *M. incognita* and possess nematicidal activity. Thus, the plant extracts can be used for the management of root knot nematodes in chilli and its application is expected to be cheap, easily available and eco-friendly.

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