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Efficacy of Different Seed Kernels against Root Knot Nematode *Meloidogyne incognita* in Mulberry

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Five seed kernel namely, Neem (*Azadirachta indica* A. Juss.), Pongamia (*Pongamia glabra* L. Pierre), Tamarind (*Tamarindus indica* L.), Mahua (*Madhuca indica* Gmel.) and Shikakai (*Acacia cancinna* De.) were tested against hatching of eggs and larval mortality of *Meloidogyne incognita* causing root knot disease in mulberry along with Furadan (Carbofuran) and Bionema (a bioformulation developed from *Verticillium chlamydosporium*) for comparison. Results revealed that highest hush-up of hatching was observed in Neem (77.40%) and Pongamia (75.99%) seed kernel extracts at 100% concentration over the check. Similarly, highest larval mortality was observed in Neem and Pongamia by 76.00% and 74.50%, respectively at 100% concentration after 72 hrs of exposure period. Pot culture studies revealed that pre-application of seed kernel powders (20 days before inoculation of nematode) found to be more effective in controlling the root knot disease than post application. In pre application of seed kernel powders, maximum reduction of root knots was observed in case of Neem seed kernel powder (54.85%) followed by Pongamia (51.9%). Similar trend was also observed in reduction of egg masses/plant and nematode population /250 cc soil. Rest of the seed kernel extracts was found to be less effective in suppression of hatching, enhancing the larval mortality and controlling the root knot disease. However, application of Furadan and Bionema tested for the comparison were found to be more effective than seed kernel powders. The generated information seems to be useful in developing an ecofriendly integrated approach for the control of root knot nematode disease in mulberry.

Key words: Seed kernel powder, Root knot nematode, Mulberry, Neem, Pongamia

Introduction

Mulberry (*Morus* sp.) provides food for silkworm (*Bombyx mori* L.), which is greatly influenced by various biotic and abiotic factors. Among the biotic factors, the diseases have always been major limiting factors in mulberry cultivation. The crop is affected by a variety of pathogens causing diseases to leaves, roots and stems and invariably reduces the yield and nutritive value of the leaf, and feeding silkworm with diseased leaves affects their growth, development, cocoon yield and quality (Philip *et al.*, 1994; Gupta, 2001). Among them, root knot caused by the nematode *Meloidogyne incognita* (Kofoid and White) Chitwood is serious one and wide spread in sandy soil under irrigated farming. Although application of Furadan (Carbofuran 3 G) and neem oil cake has been recommended for the control of root knot disease (Govindaiah *et al.*, 1993, 1994) the farmers are reluctant to use the chemical method because of its prohibitive cost and the possible toxicity to silkworm and it also affects the beneficial microbes in soil while alone application of neem oil cake is not so effective in controlling the disease. Recently, a bioformulation named as Bionema was also developed by *Verticillium chlamydosporium* for the control of root knot disease of mulberry (Sharma, 1999a). Considerable attention has been paid on application of oil cakes such as Neem, Pongamia, castor, groundnut mustard and sesanum against different nematode in various crops (Khan *et al.*, 1974). The oil cakes besides being non-toxic have no residual effect on plant growth. Various workers have tested extract of several seed kernels of medicinal plants

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and seed cakes for the control of phytonematodes including *Meloidogyne incognita* in agriculture crops effectively (Shivakumar and Marimuthu, 1986; Goswamy and Meshram, 1991; Alam, 1993; Khurana and Sushma, 1995). Hence in the present study various seed kernels were tested under *in vitro* on hatching of *M. incognita* eggs, mortality of larvae and impact on suppression of root knot disease *in vivo* (pot culture).

Materials and Methods

In vitro comparative efficacy of seed kernel extracts, Furadan and Bionema on hatching of *M. incognita* eggs and larval mortality

In vitro effect of seed kernel extracts of medicinal plants such as Neem (*Azadirachta indica* A. Juss.), Pongamia (*Pongamia glabra* L. Pierre), Tamarind (*Tamarindus indica* L.), Mahua (*Madhuca indica* Gmel.) and Shikakai (*Acacia cancinna* De.) were tested against hatching of *M. incognita* eggs along with Furadan (Carbofuran) and Bionema (a bioformulation developed from *V. chlamydosporium*) for comparison. The seeds collected from local market were sun dried and powdered in a mixi and sieved through 60-mesh sieve. 25 g of powder was soaked in 100 ml distilled water and filtered through Whatman filter paper No. 1. The extract was centrifuged at 6,000 rpm for 5 min. The supernatant was kept as stock solution and considered as cent percent concentration. In case of Furadan (Carbofuran), one gram was dissolved in 1000 ml sterilized distilled water to get 1,000 ppm concentration. In case of Bionema, culture filtrate of *V. chlamydosporium* was used. To obtain culture filtrate, *V. chlamydosporium* was grown on Potato Dextrose Broth for 10 days at 28°C. The culture filtrate was harvested through Whatman filter paper No. 42 and repeatedly centrifuged at 9,000 rpm until cell free culture filtrate was obtained. The supernatant was kept as stock solution of cent per cent concentration. Next grade of 50% made by dilution (V/V) with distilled water. Ten ml extract was taken in a Petriplates (5 cm diameter) for testing the hatching of nematode eggs along with check (distilled water). 1,500 eggs collected from infected mulberry roots were placed separately in Petriplates containing seed kernel extracts, Furadan, Bionema and check. The Petriplates were kept at 28 ± 2°C. The hatched larvae were counted every alternate day up to 10 days. The significance of value of number of larvae hatched as compared to check was tested at 5% level. Percentage inhibition of hatching over the check was calculated.

In another set of experiment, the mortality of *M. incognita* larvae was tested under *in vitro* condition. Egg masses from root knot samples collected from severely affected mulberry garden was handpicked and kept in distilled water in Petriplates for hatching. The Petriplates were incubated at 28 ± 2°C overnight. The hatched larvae were pipetted out at 500 larvae/Petriplates having different concentration of seed kernels extracts, Furadan solution and culture filtrates of *V. chlamydosporium*. The mortality was recorded after 24, 48 and 72 hrs in terms of dead larvae to calculate the mortality percentage. Apparently immobilized larvae were first transferred to distilled water for an hour to ascertain their mobility. If they failed to regain mobility they were considered dead.

Impact of various seed kernels on suppression of root knot disease in pots

Effect of seed kernel of different medicinal plants such as Neem, Pongamia, Tamarind, Mahua and Shikakai were tested against *M. incognita* affecting mulberry in pots along with Furadan and Bionema for comparison. The powdered seed kernels at 10 g/plant was applied to three months old potted (30 cm diameter) mulberry plants filled with 2 kg sterilized soil in the root zone as pretreatment. After 20 days of application of seed kernel powders larval suspension of *M. incognita* at 1,000 larvae/plant was inoculated to the root zone of potted plants. In another set, the post treatment of seed kernels powders was applied after 20 days of nematode inoculation at 10 g/ pot and plants were watered regularly. Similar trends were also maintained for an application of Furadan and Bionema. Furadan was applied at 800 mg/ plant while Bionema was applied after mixing with FYM (1:200) at 200 g mixture/ plant. The check (control) pots were kept without application of seed kernel powder. Each treatment was replicated 10 times. The similar quantity of FYM at 200 g/pot was applied in pots treated with seed kernel powders, Furadan and check.

After 60 days of inoculation the potted plants were carefully uprooted and washed free of soil and the number of root knot and egg masses were recorded. The egg masses/ plant was counted under binocular stereomicroscope. The nematode population/250 cc soil from each pot extracted by modified Cobbís sieving and Baermann funnel technique. The pooled data were subjected to analyze statistically for ANOVA test at 5% significance level.

Results

In vitro comparative efficacy of seed kernel extracts, Furadan and Bionema on hatching of *M. incognita* eggs and larval mortality

Results revealed that there was a significant reduction in

Table 1. *In vitro* comparative efficacy of different seed kernels with *V. chlamydosporium* (Bionema) and Furadan (nematicide) on hatching of *M. incognita* eggs

Sl. no.	Treatment	Concentration (%)	No of larvae hatched / 1,500 eggs on Days after					Total larvae hatched	Hatched larvae (%)	Inhibition over control (%)
			2	4	6	8	10			
1	Neem	100	140	96	45	25	00	306	22.59	77.40
		50	210	129	73	36	12	460	33.97	66.02
2	Pongamia	100	146	100	48	31	00	325	24.00	75.99
		50	216	136	79	39	15	485	35.81	64.18
3	Mahua	100	240	200	92	69	50	651	48.07	51.92
		50	300	240	110	82	62	794	58.64	41.35
4	Tamarind	100	244	209	99	72	54	678	50.07	49.92
		50	309	246	119	90	67	831	61.37	38.62
5	Shikakai	100	251	215	110	74	59	709	52.36	47.63
		50	319	211	121	93	71	815	60.10	39.80
6	*V. chlamydos-porium* (Bionema)	100	130	90	39	12	00	271	20.01	79.98
		50	190	115	60	26	09	400	29.54	70.45
7	Furadan (ppm olution)	1000	154	64	26	00	00	244	18.02	81.97
		500	165	78	55	26	00	324	23.92	76.07
8	Control (Distilled water)		319	443	276	181	135	1354	90.26	
Days (A)			0.40		1.13					
Treatment (B)			0.54		1.52					
Concentration (C)			0.25		0.71					
A × B			1.22		3.40					
A × C			0.57		1.60					
B × C			0.77		2.15					
A × B × C			1.76		4.79					
C V %			2.46							

hatching of *M. incognita* eggs in all the treatments and concentration over the check (Table 1). With the increase in concentration of seed kernel extract and incubation period, there was a corresponding decrease in hatching of eggs. Maximum hatching was seen on second day of incubation and it gradually decreased with an increase in incubation period up to 10 days. Among the two concentrations *i.e.*, 100% and 50%, maximum suppression of hatching was seen in 100% concentration of all the extracts. Maximum suppression of hatching was observed in Neem and Pongamia seed kernel extracts with 77.40% and 75.99% at 100% concentration, while at 50% concentration it was 66.02% and 64.18%, respectively over the check. With regards to percentage of larval mortality, it was directly proportional to the concentration of seed kernel extracts as well as period for which the nematode larvae were exposed. The mortality rate was low at beginning and increased gradually after 24 hrs of exposure. In general, significantly higher mortality was observed in 100% extract as compared to 50% concentration at all the exposure periods and maximum larval mortality occurred 72 hrs after exposure (Table 2). At 24 hrs exposure, none of the materials tested showed 100% mortality. However, more than 70% mortality was seen in Neem and Pongamia seed kernel extracts after 72 hrs of exposure period. Statistically, there was no significant difference between Neem and Pongamia seed kernel extracts on the suppression of hatching of eggs and larval mortality. Rest of the seed kernel extracts *viz.*, Mahua, Tamarind and Shikakai were found to be less effective to increase the suppression of hatching and larval mortality. However, in case of the Furadan and culture filtrates of *V. chlamydosporium*, which have been taken for the comparison, the suppression of hatching of nematode eggs and larval mortality were significantly higher as compared to seed kernel extracts.

Impact of various seed kernels on suppression of root knot disease in pots

Data revealed that pre-application of seed kernel powder 20 days before inoculation of nematode found to be more effective than post application (Table 3). In pre-applica-

Table 2. *In vitro* comparative efficacy of different seed kernels with Bionema and Furadan on mortality of *M. incognita* larvae

Sl. no.	Treatment	Duration (hour)	Mortality of larvae (%)	
			Concentration (%) of seed kernels/cultural filtrates	
			100	50
1	Neem	24	49.90 (44.94)	39.50 (38.93)
		48	72.00 (58.06)	50.00 (48.04)
		72	76.00 (60.69)	58.00 (49.61)
2	Pongamia	24	47.50 (43.60)	34.80 (36.14)
		48	68.00 (55.57)	45.70 (42.53)
		72	74.50 (59.70)	54.00 (47.29)
3	Mahua	24	34.80 (35.73)	19.50 (26.18)
		48	36.50 (37.16)	24.80 (29.85)
		72	38.00 (28.04)	29.00 (32.58)
4	Tamarind	24	26.30 (30.84)	16.90 (24.21)
		48	31.50 (34.16)	21.50 (27.57)
		72	35.00 (36.26)	26.00 (32.65)
5	Shikakai	24	23.00 (28.65)	15.50 (23.20)
		48	27.00 (31.29)	19.00 (25.80)
		72	32.00 (34.44)	22.00 (27.95)
6	V. chlamydosporium (Bionema)	24	52.00 (46.14)	40.50 (39.50)
		48	74.50 (59.68)	52.00 (46.14)
		72	77.80 (61.89)	60.50 (51.06)
7	Furadan	24	53.80 (47.18)	44.00 (41.93)
		48	73.00 (58.71)	58.00 (49.61)
		72	79.00 (62.72)	66.50 (54.63)
8	Control (Distilled water)	24	0.0 (4.05)	–
		48	0.0 (4.05)	–
		72	2.10 (8.39)	–
			SE ± CD at 5%	SE ± CD at 5%
Treatment (A)			0.92 2.62	1.11 3.15
Duration (B)			0.53 1.51	0.68 1.93
A × B			1.60 4.54	1.92 5.47
CV %			6.10	

Numbers in parentheses are angular transformed values.

Table 3. Comparative efficacy of different seed kernels with Bionema and Furadan on suppression of root knot disease in pots (pre inoculation of treatments)

Sl. no	Treatment	No of knots/pant	% reduction over control	No of egg masses/plant	% reduction over check	N. population/ 250 cc soil	% reduction
1	Neem	45.15	54.85	33.20	57.43	111.24	58.80
2	Pongamia	48.10	51.9	35.10	55.00	116.10	57.00
3	Mahua	54.60	45.4	39.78	49.00	136.35	49.50
4	Tamarind	60.00	40.0	44.46	43.00	149.85	44.50
5	Shikakai	63.00	37.0	46.02	41.00	151.74	43.80
6	Furadan	44.10	55.9	31.20	60.00	101.25	62.50
7	Bionema	46.20	53.8	32.76	58.00	108.00	60.00
8	Control	100.00	–	78.00	–	270.00	–
SE ±		0.64		0.61		2.81	
CD at 5 %		1.85		1.80		8.30	
CV %		2.75		3.06		2.65	

Table 4. Comparative efficacy of different seed kernels with Bionema and Furadan on suppression of root knot disease in pots (post inoculation of treatments)

Sl. no.	Treatment	No of knots/pant	% reduction over control	No of egg masses/plant	% reduction over check	N. population/ 250 cc soil	% reduction
1	Neem	49.00	51.00	36.47	53.24	121.50	55.00
2	Pongamia	51.50	48.50	39.00	50.00	130.14	51.00
3	Mahua	60.00	40.00	44.46	43.00	152.55	43.50
4	Tamarind	66.50	33.50	48.36	38.00	162.54	39.80
5	Shikakai	69.00	31.00	49.29	36.80	162.54	39.00
6	Furadan	47.20	52.80	33.93	56.50	111.24	58.80
7	Bionema	48.50	51.50	35.10	55.00	117.45	56.50
8	Control	100.00		78.00		270	
SE ±		0.77		0.67		3.21	
CD at 5 %		2.20		1.91		9.58	
CV %		3.07		3.62		3.96	

tion of seed kernel powders, the number of knots, egg masses and nematode population were significantly reduced over control. Among seed kernels, maximum reduction of root knots was observed in case of Neem (54.85%) followed by Pongamia (51.90%). Similar trend was also observed in reduction of egg masses /plant and nematode population /250 cc of soil. The percentage reduction of egg masses and nematode population in Neem was 57.43 and 58.80 while in case of Pongamia it was 55.00 and 57.00, respectively. Post application of seed kernel powders, 20 days after inoculation of nematode found to be less effective as compared to pre application. The trends in reduction of number of knots, egg masses and nematode population in post application of different treatments were observed similar to pre application (Table 4).

Rest of the seed kernel powders *viz.*, Shikakai, Tamarind and Mahua found to be less effective with less reduction in number of knots, egg masses and nematode population in pre and post application methods. However, application of Furadan and Bionema recommended for control of root-knot disease and taken for the comparison in the present study were found to be more effective than that of seed kernel powders.

Discussion

Among different seed kernel extracts, maximum suppression of hatching and larval mortality was seen in Neem and Pongamia seed kernel extracts. This might be due to presence of strong nematicidal compounds/alkaloids. Khan *et al.* (1974) were reported that oil cakes of Neem, Pongamia, *etc.* produce strong volatile/alkaloid compounds, which are responsible for inhibiting the hatching of nematode eggs and cause larval mortality considerably.

Confirmatory studies under pot culture revealed that pre application of seed kernel powders 20 days before inoculation of nematode found to be more effective than post application. This may be due to early absorption of alkaloids by mulberry roots released during decomposition of seed kernel powders, which reduce the nematode penetration and multiplication. Application of seed kernel powders of Neem and Pongamia significantly reduced the number of knots, egg masses and nematode population might be due to presence of strong anti-nematicidal alkaloids such as nimbidin, theonemone and phenolic compounds, which are toxic to nematodes (Alam, 1993). The present results are also in conformity with the findings of Darekar and Jagdale (1989) who found that application of neem oil cake reduced the nematode population considerably in betel vine. The present study also supports the findings of Govindaiah *et al.* (1994) who have recommended application of neem oil cake at 2 tones/ha/yr in four split doses for the control of root knot disease in mulberry. Seed kernel extracts *viz.*, Shikakai, Tamarind, Mahua found to be less effective in suppression of hatching, larval mortality and disease control as compared to Neem and Pongamia. This clearly indicates that these seed kernel extracts have poor sources of active principles in exhibiting the strong anti-nematicidal effect against nematode.

However, nematicide Furadan and Bionema taken for the comparison in the present study were found to be more effective than seed kernels in suppression of hatching of eggs, larval mortality and reducing disease severity. This might be due to that *V. chlamydosporium* known to produce certain toxic metabolites and antibiotic sub-

stances, which have been found lethal to the nematode eggs and larvae. Further, *V. chlamydosporium* parasitize the eggs of *M. incognita*, which stopped the further hatching and development of nematodes. Many workers have reported that *V. chlamydosporium* contains toxic metabolites, which are lethal to nematodes in various crops including mulberry (De Leij *et al.*, 1993; Martens and Stirling, 1993; Sharma, 1998, 1999b). De Leij *et al.* (1991) reported that *V. chlamydosporium* is a nematode egg parasitic fungus and prevented further hatching of nematode eggs and Sharma (1999a) reported that application of Bionema reduced the severity of root knot disease in mulberry effectively. Similarly, maximum suppression of hatching and reduction of root knot in pot culture with Furadan might be due to strong nematicidal properties of the chemical as reported by Govindaiah *et al.* (1993) in mulberry.

In the present study, both Neem and Pongamia were found to be effective and can be used against *M. incognita* in the form seed kernel powder/oil cakes with an integration of other recommended methods to develop an ecofriendly integrated approach for the control of root knot nematode disease in mulberry as an alternative to chemical method.

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