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Eco-friendly management of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) chitwood using seed kernel extracts, cow urine and agniastra

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Abstract
Two indigenous plants aqueous seed kernel extracts *viz.*, Neem Seed Kernel (NSK) and Dharek Seed Kernel (DSK), cow urine and agniastra were evaluated for their effect on juvenile mortality and egg hatching inhibition of root-knot nematode, *Meloidogyne incognita*. Cow urine (93.76%) @ 10% concentration was most effective for the juvenile mortality of *M. incognita* followed by agniastra (91.81%) at 2% concentration. Cow urine (75.00%) was found to be most effective followed by agniastra at 2% and NSKE (66.67%) at 10% concentration for the egg hatching inhibition of *M. incognita*. Whereas, aqueous DSKE at 2% concentration was found least effective for juvenile mortality as well as egg hatching inhibition of *M. incognita.*

Keywords: *Meloidogyne incognita*, juvenile mortality, egg hatching inhibition, seed kernel extracts, cow urine, agniastra

1. Introduction
Root-knot nematodes (*Meloidogyne* spp.) are among the major pathogens of vegetables especially in tomato and eggplants throughout the world [1]. Plant parasitic nematodes, especially root-knot nematodes (RKNs) from the genus *Meloidogyne*, are widely distributed and cause significant yield loses in a wide range of crops [2]. Leading crops like tomato, brinjal, okra, chrysanthemum etc, suffers heavy yield losses due to various biotic factors *viz.*, insect pests, nematodes, fungi, bacteria and viruses etc. Among these, plant parasitic nematodes are the major factors responsible for the huge qualitative and quantitative losses to the crop. Among the various plant parasitic nematodes, genera *Meloidogyne incognita, M. javanica, Rotylenchulus reniformis, Belonolaimus longicaudatus, Longidorus* spp., *Hoplolaimus* spp., *Hemicriconemoid* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp. and *Paratylenchus* spp. are most common in occurrence. *Meloidogyne incognita* is major plant parasitic nematodes affecting the quality and quantity of the major crops. *Meloidogyne incognita* is one of the most widespread nematode pests of tropical and subtropical regions of the world [3]. It belongs to the family Heteroderidea. It is an endoparasite which affects so many species of crops some of its host are tomato, brinjal, okra, bell pepper, coconut, cucumber, carnation and banana. *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* are the most commonly found root-knot nematode species. About 2000 plants are susceptible to their infection and they cause approximately 5% of global crop loss [4]. The economic importance of root-knot nematode cannot be overestimated as farmers worldwide lose millions of dollars due to their attack on crops. Plants with root-knot nematode show signs like stunted growth, root galls and in chronic cases death of plant [5]. The use of botanicals possessing the anti-feedant and nematicidal properties, not only reduce the *nematode* population but also enhance the plant growth [6].

2. Materials and Methods
2.1 Nematode culture (*Meloidogyne incognita*)
Egg masses of *M. incognita* were isolated from brinjal roots collected from the experimental farm of College of Horticulture and Forestry, Neri, H.P and identified on the basis of perennial pattern. Egg masses were placed in Petri plates containing distilled water.

The second stage juveniles hatched from egg masses were inoculated separately on brinjal seedlings grown under aseptic conditions in 4 kg soil capacity plastic pots. The plants were maintained in greenhouse for nematode multiplication and the nematode culture was used for further experiments.

2.2 Collection/procurement of various seed kernels for extraction

Seed kernels of indigenous plants namely, dharek (*Melia azedarach*) and neem (*A. indica*) were collected from the surrounding locations of Hamirpur H.P and were shade dried before being pounded with a wooden stick to make a coarse powder and stored in cool and dry place in the room. Cow urine of indigenous cow was also collected from the nearby village of Neri, Hamirpur H.P.

2.3 Preparation of aqueous leaf and seed kernel extract

Seed kernels were powdered with the help of electric grinder/pestle mortar. 20 g powder was weighed and soaked in 200 ml of distilled water overnight in a 500 ml capacity glass beaker. The contents were filtered 3-4 times through a fine muslin cloth. This solution was designated as 10 per cent seed kernel extract (aqueous). The stock solutions prepared for the experiments were stored in the refrigerator for further use.

2.4 Preparation of doses

Ten per cent concentration of all indigenous seed kernels was prepared on v/v basis. In order to remove the dense inert matter, the water extracts were centrifuged at 1200 rpm for five minutes and filtered through Whatman's filter paper No. 1. Three different concentrations (2, 5 and 10% each) were prepared from stock solutions and stored in the reagent bottles for evaluating their effect on egg hatching and juvenile mortality.

2.5 Preparation of agniastra

Five ingredients namely, green chilies (50 g) + garlic (50 g) + neem leaves (500 g) + cow urine (1500 ml) + tobacco (50 g) were used to prepare a two liter of agniastra solution. The ingredients were ground into fine paste and were mixed with cow urine. This solution was boiled for four times, leaving it for 48 h, filtered and added some water to make two liter stock solution of agniastra [7].

2.6 For juvenile population

Concentrations of 2, 5 and 10 per cent were prepared from stock solution for each formulation in distilled water. One ml formulation of each concentration was poured in sterilized cavity blocks/Petri plates (2 ml capacity). Besides, one control treatment was also maintained (added distilled water only). All the treatments were replicated three times. Hundred freshly hatched juveniles of *M. incognita* were added into each replicate. All cavity blocks were kept at room temperature. Observations regarding per cent juvenile mortality were recorded at 12, 24, 36 and 72 h of exposure.

2.7 For egg mass

Methodology was similar to the former but in place of juvenile population each replicate was inoculated with two egg masses. Observations regarding egg hatching inhibition were recorded at 12, 24, 36 and 72 h of exposure.

3. Results and discussion

3.1 Effect on per cent juvenile mortality

Hundred freshly hatched juveniles of *M. incognita* per replicate were added into the cavity blocks/Petri plates containing 2.0, 5.0 and 10 per cent concentrations and their per cent mortalities were observed at the exposure periods of 12, 24, 36 and 72 h.

Data showed in Table 1 revealed the effect of different seed kernel extracts, cow urine and agniastra on per cent mortality of juveniles of *M. incognita* at different exposure periods. Highest mean per cent juvenile mortality was achieved in cow urine with the per cent juvenile mortality of 93.76 in 10 per cent concentration followed by agniastra with mean per cent juvenile mortality of 91.81 at 2.0 per cent concentration. Lowest mean per cent juvenile mortality was achieved by dharek seed kernel extract (DSKE) with per cent juvenile mortality of 51.70 in 2.0 per cent concentration. Mean per cent juvenile mortality increased with the increase in exposure period. Highest mean per cent juvenile mortality was attained at 72 h exposure period with mean per cent juvenile mortality of 74.28 followed by 67.64 at 36 h exposure period. Lowest mean per cent juvenile mortality was attained in 12 h exposure period with mean per cent juvenile mortality of 53.54. Further, in the interaction between the concentrations of different seed kernel extracts, cow urine and agniastra and per cent juvenile mortality, the highest mortality (100%) was achieved in agniastra at 2.0 per cent concentration within 72 h of exposure period followed by 98.78 per cent in cow urine at 10 per cent concentration within 72 h and least per cent mortality (39.88%) was achieved in DSKE at 2.0 per cent concentration within 12 h of exposure period.

3.2 Effect on per cent egg hatchability

Egg masses of *M. incognita* per replicate were added into the cavity blocks/Petri plates containing the above referred concentrations and per cent inhibition of *M. incognita* were observed at the exposure periods of 12, 24, 36 and 72 h.

Perusal of data presented in Table 2 revealed that mean per cent inhibition of egg hatching of *M. incognita* was highest (75.00) achieved in cow urine at 10 per concentration followed by 66.67 per cent in agniastra at 2.0 per cent concentration and NSKE at 10 per cent concentration. The mean inhibition rate achieved by cow urine at 10 per cent concentration (75.00) was significantly similar to that achieved by agniastra at 2.0 per cent concentration (66.67) and NSKE at 10 per cent concentration (66.67). Lowest (8.33) mean per cent inhibition was achieved in DSKE at 2.0 per cent concentration.

Table 1: Effect of different seed kernel extracts, cow urine and agniastra on mortality of second stage juveniles of *M. incognita* at different exposure periods

Treatment	Per cent mortality of juveniles of *M. incognita* at exposure period (h)				Mean
	12	24	36	72	
Aqueous NSKE @ 2%	45.04 (42.15)	49.73 (44.85)	59.55 (50.50)	64.81 (53.61)	54.78 (47.78)
Aqueous NSKE @ 5%	47.10 (43.36)	52.49 (46.42)	65.16 (53.84)	71.84 (57.96)	59.14 (50.39)
Aqueous NSKE @ 10%	49.36 (44.63)	56.80 (48.91)	64.43 (53.39)	74.35 (59.58)	61.23 (51.63)
Aqueous DSKE @ 2%	39.88 (39.16)	47.21 (43.40)	53.28 (46.88)	66.42 (54.63)	51.70 (46.02)
Aqueous DSKE @ 5%	45.02 (42.14)	51.26 (45.73)	62.74 (52.40)	72.56 (58.41)	57.90 (49.67)
Aqueous DSKE @ 10%	45.61 (42.48)	52.77 (46.59)	67.22 (55.08)	77.71 (61.84)	60.83 (51.49)
Cow Urine @ 2%	67.01 (54.97)	76.75 (61.22)	84.25 (66.68)	89.99 (71.62)	79.50 (63.62)
Cow Urine @ 5%	80.37 (63.82)	85.81 (67.94)	90.46 (72.53)	95.94 (78.52)	88.14 (70.70)
Cow Urine @ 10%	87.96 (69.74)	92.66 (74.35)	95.64 (78.08)	98.78 (83.71)	93.76 (76.47)
Agniastra @2%	80.17 (63.63)	88.96 (70.62)	98.13 (83.59)	100.00 (90.00)	91.81 (76.96)
Untreated control	1.46 (6.92)	2.07 (8.27)	3.21 (10.32)	4.76 (12.59)	2.87 (9.52)
Mean	53.54 (46.68)	59.68 (50.76)	67.64 (56.66)	74.28 (62.04)	

Figures in the parentheses are angular transformed values

Highest (81.82) mean per cent inhibition was achieved at 12 h exposure period followed by 65.15 per cent at 24 h exposure period while lowest (6.06) mean per cent inhibition was achieved at 72 h concentration. In the interaction between the exposure periods and concentrations, highest per cent inhibition was achieved in all the treatments except in both DSKE at 2.0 per concentration and at 5.0 per cent concentration at 12 h of exposure period. There was a decreasing trend in inhibition rate with increase in the exposure period.

The effective performance of *Azadirachta indica* may be as a result of some biological active substances like azadirachtin, nimbine, kemferol, thionemone etc. that are nematicidal in nature [8, 9]. Not much literature is available related to agniastra but its ingredients alone are quite effective on *M. incognita*. The literature ahead discussed is from some of the ingredients of agniastra:

Feyisa *et al.* (2016) reported in their studies that neem leaf exract alone accounted for maximum per cent juvenile mortality of *M. incognita* after 72 h of exposure period at 5.0 and 10 per cent concentration. Nimbalkar and Rajurkar (2009) reported from their findings that neem root extract attained maximum juvenile mortality after the exposure period of 48 h. Nimbalkar and Rajurkar (2009) reported from their findings that neem root extract gave the maximum egg hatching inhibition. Adegbite (2011) reported that *A. indica* was effective inhibitors of egg hatch of root-knot nematode *Meloidogyne incognita*. Feyisa *et al.* (2016) reported that neem leaf extract accounted for maximum inhibition over control after the exposure period of seven days. Haroon *et al.* (2018) repoted from their studies that leaf extract of *A. indica* extract was the most effective in preventing egg hatching. Ladi *et al.* (2019) reported that *A. indica* accounted for maximum egg hatch inhibition over the control.

Table 2: Effect of different seed kernel extracts, cow urine and agniastra at different exposure periods and concentrations on egg hatching inhibition of *M. incognita*

Treatment	Per cent inhibition at exposure period (h)				Mean
	12	24	36	72	
Aqueous NSKE @ 2%	100.00 (90.00)	100.00 (90.00)	16.67 (15.00)	0.00 (0.00)	54.17 (48.75)
Aqueous NSKE @ 5%	100.00 (90.00)	100.00 (90.00)	33.33 (30.00)	0.00 (0.00)	58.33 (52.50)
Aqueous NSKE @ 10%	100.00 (90.00)	100.00 (90.00)	50.00 (45.00)	16.67 (15.00)	66.67 (60.00)
Aqueous DSKE @ 2%	33.33 (30.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (7.50)
Aqueous DSKE @ 5%	66.67 (60.00)	16.67 (15.00)	0.00 (0.00)	0.00 (0.00)	20.83 (18.75)
Aqueous DSKE @ 10%	100.00 (90.00)	100.00 (90.00)	50.00 (45.00)	0.00 (0.00)	62.50 (56.25)
Cow Urine @ 2%	100.00 (90.00)	50.00 (45.00)	0.00 (0.00)	0.00 (0.00)	37.50 (33.75)
Cow Urine @ 5%	100.00 (90.00)	66.67 (60.00)	16.67 (15.00)	0.00 (0.00)	45.83 (41.25)
Cow Urine @ 10%	100.00 (90.00)	83.33 (75.00)	83.33 (75.00)	33.33 (30.00)	75.00 (67.50)
Agniastra @2%	100.00 (90.00)	100.00 (90.00)	50.00 (45.00)	16.67 (15.00)	66.67 (60.00)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	81.82 (73.64)	65.15 (58.63)	27.27 (24.54)	6.06 (5.45)	

Figures in the parentheses are angular transformed values

Conclusion

From the present investigation, we can conclude that cow urine and agniastra at 10 per cent and 2 per cent respectively, played an important role in juvenile mortality and also leads towards the reduction in egg hatching of *M. incognita*.

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