

Biocontrol Potential of Neem leaf Extract on Mortality of *Meloidogyne incognita*

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(Received 13 August, 2022; Accepted 12 October, 2022)

ABSTRACT

Root-knot nematode *Meloidogyne incognita* is one of the widely distributed in India and most economically damaging genera in vegetable crops. Plant products are the safer alternative approaches to control root knot nematodes. An experiment was carried out *in vitro* to test the Neem leaf extract of aqueous, acetone and methanol extract. Different concentrations of shade-dried leaves of Neem were tested on mortality of *Meloidogyne incognita* juveniles. Increase in concentration and exposure period resulted in increased mortality rate of Juveniles of *M. incognita*. Methanol Neem leaf extract (5 ml concentration) proved to be the most effective among used aqueous, acetone and methanol neem leaf extract. After 48 hours exposure aqueous and methanol leaves extract showed 100% mortality while acetone neem leaves extract showed 100% mortality after 72 hours exposure of 5 ml concentration. Neemleaves 0.25 ml con. was the least effective among all con.

Key words: Aqueous, Acetone, Methanol, Mortality, *Meloidogyne incognita*, Plant extract

Introduction

The root-knot nematode *Meloidogyne incognita* is one of the major problems for the vegetable production area. Brinjal is every infected by root-knot nematodes. Chemical controls of nematodes are very expensive as well as developed problem of residual toxicity environmental pollution public health hazard even depletion of stratospheric zone (Wheeler *et al.*, 1979). Nematode management of this dreadful nematode through chemicals has adverse effects on the environment and hazardous to all living entities on the earth. As an alternative to chemical pesticides specially for the purpose of protecting crops against nematodes and also for the conservation of biodiversity, botanicals may stand as the most

promising source of bioactive products of plant origin. Extract of plant products contain nematicidal and antifeedant compounds. Thousands of plants possessing insecticidal properties are known today (Banerji *et al.*, 1985). Use of neem and neem products has been advocated for the management of root-knot nematodes by many workers (Dekha and Rehman, 1998; Jain and Gupta, 1988). Plant extracts having the nematicidal properties and they not only reduce the nematode population but also enhance the plant growth (Hussain *et al.*, 2011). The present investigation to study the nematicidal effect of neem plant (*Azadirachta indica*) leaves aqueous, acetone and methanol extracts on juveniles of *M. incognita*.

Prasad *et al.* (2002) observed that some plant extract were toxic to root-knot nematode and further

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application in pots enhanced plant growth significantly with reduction in the nematode population.

Materials and Methods

Preparation of Extracts – The Neem (*Azadirachta indica*) plant leaves were shade dried and then oven dried at 60°C overnight, powder of plant parts was prepared by using a mixer or blender. Five gm powder of neem leaves were soaked in 50 ml water, acetone and methanol for 7-9 days for soak and evaporation after that acetone and methanol evaporated leaves washed with distilled water and absolute organic solvents. Extract were filtered through four ply muslin cloth and then passed through Whatman filter paper no. 1, filtered extract were made up 50 ml with required amount of distilled water and then centrifuged at 4000 rpm for 10 minutes then again filtered through Whatman filter paper no.1. The obtained extract was put in conical flasks and steam sterilized in an autoclave. The plant part neem leaves extract designated as stock solution for preparing different dilution viz. (5 ml, 2.5 ml, 2 ml, 1.5 ml, 1 ml, 0.50 ml, 0.25 ml) of each aqueous, acetone and methanol extracts were prepared by adding the required amount of distilled water. The sterilized distilled water served as control.

Effect of acetone extracts on juveniles Mortality of *M. incognita* – Seven dilution viz. (5 ml, 2.5 ml, 2 ml, 1.5 ml, 1 ml, 0.50 ml, 0.25 ml) were prepared from stock solution. Five ml of each neem leaves of aqueous, acetone and methanol extracts were prepared by adding the required amount of distilled water were taken in sterile beaker of 50 ml capacity. Two hundred freshly hatched second stage juveniles (J_2) of *M. incognita* were transferred in beaker. Equal number of J_2 was also transferred to separate beaker containing sterilized water to serve as control. Three replicated were taken completely randomized design (CRD). Observations of both live and dead nematodes were made at 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs after inoculation.

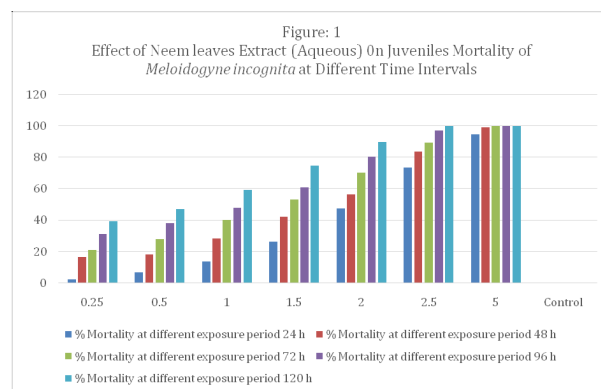
Results and Discussion

Result presented in Tables 1–3 revealed the maximum mortality was recorded in 5 ml concentration which increased with increase in aqueous, acetone and methanol extract concentration. More number or percentage of juveniles mortality with increase in exposure period. Minimum mortality per cent was

observed in neem leaves aqueous extract (Table 1 and Figure 1) there was 94.7 per cent mortality at its highest 5 ml concentration and 24 hrs exposure period. Only 39.6 per cent mortality recorded at its lowest concentration (0.25 ml) after 120 hrs of exposure period. Highest concentration of Neem leaf extract (5 ml) revealed that cent percent mortality after 72 hrs of exposure period and 40.33 per cent mortality recorded at it lowest concentration (0.25 ml) after 120 hrs of exposure period (Table 2 and Figure 2). Neem leaves methanol extract on the other hand was found to be the most effective injuvenile's mortality (Table 3 and Figure 3) the highest concentration (5 ml) were found to be most effective caused 96.6 per cent mortality from 24 hrs exposure and 100% mortality after 48 hrs exposure, It's lowest concentration (0.25 ml) had only 41.6 per

Table 1. Effect of Neemleaves Extract (Aqueous) on juveniles Mortality of *Meloidogyne incognita* at different time intervals
(Observations are mean of three replicates)

Extract Con. ml	% Mortality at different exposure period				
	24 h	48 h	72 h	96 h	120 h
0.25	2.4	16.7	21.2	31.4	39.6
0.50	6.9	18.2	28.1	38.2	47.3
1.0	13.8	28.6	40.2	47.9	59.5
1.5	26.4	42.4	53.2	61.1	75
2.0	47.7	56.7	70.2	80.6	90
2.5	73.5	83.8	89.4	97.2	100
5.0	94.7	99.4	100	100	100
Control	0	0	0	0	0
SEM	0.9	0.4	0.7	0.2	0.8
C.D (P=0.05)	2.1	1.1	1.6	0.6	2.0

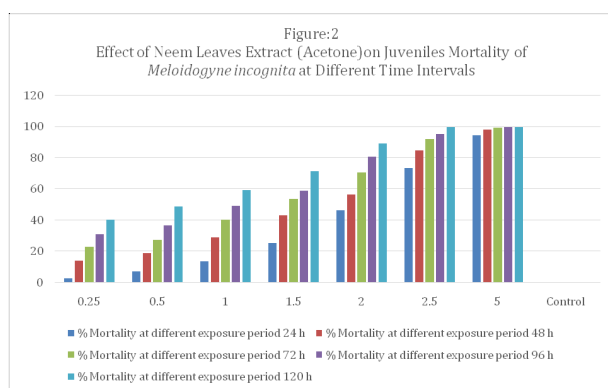


cent mortality after 120 hrs of exposure period.

Cumulative per cent mortality of *M. incognita* was maximum in neem leaves methanol extract and minimum in neem leaves acetone extract. Neem

Table 2. Effect of Neem Leaves Extract (Acetone) on Juveniles Mortality of *Meloidogyne incognita* at different time intervals
(Observations are mean of three replicates)

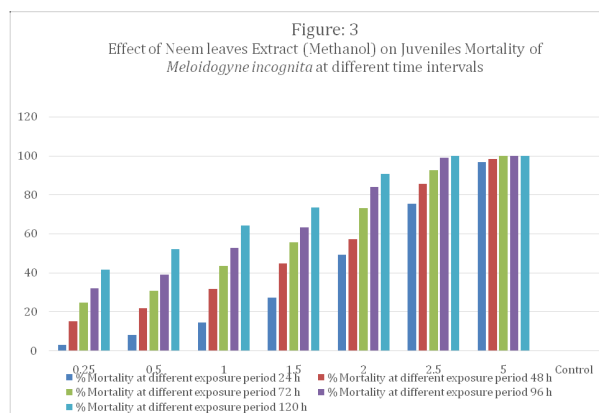
Extract Con. ml	% Mortality at different exposure period				
	24 h	48 h	72 h	96 h	120 h
0.25	3.0	14.0	23.0	31.0	40.3
0.50	7.1	19.0	27.3	36.6	49.0
1.0	13.6	29.0	40.6	49.3	59.3
1.5	25.6	43.3	53.6	59.0	71.6
2.0	46.6	56.6	70.6	81.0	89.3
2.5	73.6	85.0	92.0	95.3	100.0
5.0	94.6	98.3	99.3	100.0	100.0
Control	0.0	0.0	0.0	0.0	0.0
SEM	1.4	1.8	1.2	1.5	1.1
C.D (P=0.05)	3.3	4.2	2.8	2.6	2.6



leaves and neem seed having more concentrated nematicidal/nematostatic properties. Neem leaves and neem seed having more concentrated nematicidal/nematostatic properties. The present investigation is in confirmation with the finding of Gowda and Setty (1979) who observed that hatching of larvae from fresh eggs of *M. incognita* soaked for 24 hrs in water extract of *A. indica* cake was significantly reduced with 1: 1 dilution of extract. Acetone extract of leaf, flower, root and stem of five different varieties of tagetes were tested against egg hatching and mortality of second stage juveniles of *M. incognita* (Meena *et al.*, 2010). Leaves extract of *A. indica* Vijayalaxmi *et al.* (1979) significantly increased the *M. incognita* juvenile Mortality. Haseeb *et al.* (2007) reported that *A. indica* seeds powder were found effective against *M. incognita*. Neem productes (NeemKernel, Neemleaf, Neembark extract) are known top nematicidal activity against nematode population (Zaki and Bhatti, 1989; Derkar *et al.*, 1990). Differentcon. of shade-dried leaves, bark, and

Table 3. Effect of Neemleaves Extract (Methanol) on Juveniles Mortality of *Meloidogyne incognita* at different time intervals
(Observations are mean of three replicates)

Extract Con. ml	% Mortality at different exposure period				
	24 h	48 h	72 h	96 h	120 h
0.25	3.0	15.0	24.6	32.0	41.6
0.50	8.0	21.6	30.6	39.0	52.0
1.0	14.3	31.6	43.3	52.6	64.0
1.5	27.0	44.6	55.6	63.3	73.3
2.0	49.3	57.0	73.0	84.0	90.6
2.5	75.3	85.6	92.6	99.0	100.0
5.0	96.6	98.3	100.0	100.0	100.0
Control	0.0	0.0	0.0	0.0	0.0
SEM	1.2	2.0	1.7	1.2	1.6
C.D (P=0.05)	2.8	4.7	3.9	2.9	3.7



seed of neem were tested for mortalityof *M. incognita* juveniles increases in concentration and exposure period resulted inincreased mortality rate of juveniles (Nayak and Sharma, 2013). The present study also revealed that the mortality rate was increased with increased expose period and concentration as reported by Siddiqui and Alam (1988).

Conclusion

Azadirachtin is the principal active ingredient from neem leaf and seed kernel are a well known nematicidal action. The neem bitters (Triterpenoid) such as epinimbin, salannin, deacetylsalannin, azadirachtin, and many active principle like nimbidin, thionemone and limonoide from leaves have been reported the be highly nematicidal and nematostatics these neem chemicals are known to act against plant parasitic nematode (Mojumder, 2002). In view of general awareness about the hazardous effect of chemical pesticides, there is now an

emphasis on the non chemical nematode management technologies.

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