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Control of root-knot nematode *Meloidogyne incognita* by Nimin (Triterpenes) on green gram *Vigna radiata* (L.)

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Abstract

Studies on pathogenicity of *Meloidogyne incognita* on the pathophysiology of green gram, *Vigna radiata* (L.) were carried out in relation to different concentration of Nimin with reference to growth parameters, root-knot development, nematode population build-up, host plant metabolites such as protein and carbohydrate, inhibition of egg hatchability and larval mortality of *M. incognita*. All the concentration of Nimin resulted in reduced infection in the host plant there by increasing the length and weight of root and shoot. Among the different concentration of Nimin the significant increase in plant growth was found at 2g/kg level. However at higher rate (4g/kg level) the plant growth reduced due to the phytotoxicity of Nimin. Higher treatment shows reducing the gall formation and improved growth in host plant. In treated plants shows the highest values of protein and carbohydrates. A negative correlation found between concentrations of Nimin and *M.* incognita egg hatchability and 80 ppm concentration of Nimin shows maximum inhibitory action in egg hatchability. The 10 ppm concentration of Nimin shows cent percent larval mortality of *M. incognita* after 24 hours exposure. From the present investigation it can be concluded that soil application of Nimin 2 kg/ ha is adequate to protect the sunflower plants from the potential damage caused by root-knot nematode.

Keywords: *Meloidogyne*, root-knot nematode, Nimin, *Vigna radiata*

Introduction

Plant parasitic nematodes, are capable of reproducing on over 2,000 species of plants (Sasser and Freckman, 1987) [21] and are responsible for approximately 50% of overall damage (Abbasi *et al.*, 2008) [2, 3]. The root knot nematodes (*Meloidogyne incognita*) produce galls on the roots of many vegetable crops, pulses, some of the fruit crops, tobacco, ornamental crops and causes severe losses. This nematode is known to attack more than 3,000 separate host plants. *M. incognita* causes 33 percent loss in vegetables in Bihar and 60 percent loss in New Delhi.

The symptoms of nematode infection include formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plants (Abad *et al.*, 2003) [1]. Although the application of chemical nematicides has been found to be an effective measure for the control of nematodes, the highly toxic residual effect of chemical on the environment and particularly on non-target organisms (Anastasiadis *et al.*, 2008) [8], require an urgent need to develop alternative strategies for the control of nematodes.

Crops and weeds may show biochemical mechanisms to counteract the activity of nematodes. Numerous plant species, on behalf of 57 families, have been shown to encompass nematicidal compounds (Sukul, 1992) [22]. There is a need to develop naturally occurring nematicides, which may be less toxic to man and animals but as effective against nematodes of various crops as synthetic ones. The toxicity of root extracts of different plants against nematodes has been reported previously (Onifade and Egunjobi, 1994; Adegbite and Adesiyan, 2005) [17, 4]. Worldwide voluminous crops were damaged by *Meloidogyne incognita* (Askary, 2017; Mukhubu *et al.*, 2021) [10, 14]

This research was undertaken to evaluate the nematicidal efficiency of Nimin (triterpenes) as soil amendment treatment against *M. incognita* infecting Bhendi.

Materials and Methods

Hatchability test

Eggs of *M. incognita* were collected by the method of Whitehead and Hemming (1965) [24]. A suspension of eggs in distilled water was prepared. 1 ml of egg suspension (40-55 eggs/ml) and 1 ml of leaf extract was transferred in glass cavity blocks and kept at 270C. Every treatment was replicated thrice. The glass cavity containing 1 ml egg suspension and one ml distilled water served as control. After four days of acquaintance, the number of hatched eggs was counted with a help of low power (6X) stereomicroscope. The toxicity of plant extract was evaluated as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Whitehead and Hemming, 1965) [24].

Mortality test of nematode larvae

Eggs and eggs masses of M. incognita were placed in distilled water and incubated at 28±2^{0}C. After hatching, the J2 larvae were collected and a suspension of juveniles in distilled water was prepared. 1 ml of egg suspension (50-56

juveniles/ml) and one ml of root extract for each plant were transferred into different glass cavity blocks and kept at 27^0C. Every treatment was replicated thrice. The glass block containing 1 ml distilled water served as control. Percentage mortality was calculated after 12, 24 and 48 hours of exposure, the number of killed juveniles was counted with low power (6X) stereomicroscope. The toxicity of root extract was evaluated based on the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle. (Whitehead and Hemming, 1965) [24].

Preparation of Nimin coated soil

1000 gms of sterilized sand soil mixture was taken in an enamel tray to which 0.5, 1.2, 4gm of Nimin was added separately and mixed. Throughly and the Nimin soil mixture was kept in polythene bags.

Seedling Propagation

Seeds of green gram were surface sterilized with 0.1% mercuric chloride (wt/v) and then washed with distilled water. Fine surface sterilized seeds of green gram were sour in 15 cm polythene bags containing 1 kg of sand soil mixture and also in Nimin (different concentration) coated soil mixture. One week after germination, the seedlings were thinned to one seedling per pot. The pots were watered at regular interval.

Source of inoculum: naturally infected *Acalypha indica*

Standard procedures were followed for estimation of protein, chlorophyll content, and total phenols. Results obtained in the present studies were subjected to the S.D and ANOVA statistical analysis.

Results and Discussion

The plants were uprooted after 35 days period of infection, it was found that there was considerable reduction in plant growth characters when compared to uninfected control plants. Such reduction in shoot length was 23.15%; in root length 13.57%; shoot dry weight 30 %; root dry weight 30%. Amendment application of Nimin boosted the plants growth and even the increment in growth commenced at 0.5g/Kg treatment and increment progressively increased as the concentration of Nimin increase from 0.5g to 2g of host plants (Table.1).

Impact of different concentration of Nimin on root-knot development (galls), root nodules, root branches and leaf numbers with the increase of concentration of Nimin there was decrease in root galls in host plant. Statistical analysis of C.D. revealed that the numbers of galls were significantly reduced by all the concentration of Nimin. It was found that 37% reduction occurred when compared to unifected root. Except the T_4 treatment all the other treatments of Nimin significantly increase the number of leaves and the numbers of root branches. The quantity of protein and carbohydrate of shoot tissue of uninfected, infected and infected treated plants were analysed. The increase of 38% in protein 26% in carbohydrate was found when compared with uninfected plants (Table.2).

Asif *et al*., (2017) [9], observed that there was a plodding decrease in egg hatching with an increase in the concentration of aqueous extracts of weeds. *A. aspera, S. xanthocarpum* and *A. spinosus* were found to be most effective in reducing egghatching and increase in mortality of J2 juveniles of M. incognita. It seems to be the efficacy of treatments improved with increase in their concentration and exposure period. Hatching of larvae and juvenile mortality were sturdily influenced by concentration of botanical extract.

The inhibitory effect of different concentration of Nimin shows that linear relationship between the egg hatch and concentration of Nimin maximum inhibition (74.31%) of larval emergence was observed at 50 ppm and the minimum of (42.11%) at 10 ppm over check (Table. 3). A significant negative correlation between the concentration of Nimin and the number of larvae emerged. Similar report observed in Mohanapriya *et al*, (2019) [15], that humic acid granules inhibit the egg hatching at 1% concentration after 24 hrs which showed the highest inhibition of hatching over control. Among the oil cakes, neem oil cake recorded the highest inhibition at 100% concentration after 24 hrs. In mortality test, humic acid liquid formulation (1% concentrations) at 72 hrs recorded 100% mortality, in neem oil cake at 100% concentration after 96 hrs showed 96% mortality (Table.4). In penetration study, among the different formulation of humic acid, humic acid liquid formulation recorded the J_2 penetration on 2^{nd} day after inoculation and only 6% of nematode penetrated into roots. Among the oil cakes neem oil cake recorded the J_2 penetration on 2 days after inoculation only 13% of nematode penetrated into roots compared to control (Premachandra, 2020) [20].

Various neem products including neem cake, its oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts, have been found to be nematicidal against several species of parasitic nematodes attacking vegetables and legumes (Haseeb *et al*., 2005) [12] and banana (Musabyimana and Saxena, 1999) [16]. However, soil amendment with neem seems to be the most practical method for nematode control (Alam, 1993) [6]. The roots of plants raised in neem-cake amended soil appear to undergo physiological changes that render them unsuitable for nematode penetration and development, thus inducing a certain degree of resistance in plants against nematode infestation (Alam, 1993) [6].

Increased growth of the plant might be due to toxic effect of root-knot nematode. Many chemical compounds are known to occur in plants. Some of them like fatty acids, Phenols, Phenolic glucosides, Terpenoids, alkaloids, flavonoids, (Afzal *et al*., 2021, Krishnamoorthy *et al.*, 2014) [5, 13].

Similar reports revealed that the presence of Nimbidan and thionemone (Alam *et al*., 1992) [7]; Azadirachtin and Limnoid comounds (Devakumar, 1975) present in NOE might have been responsible for the killing of larvae of *M. incognita*. Juvenile mortality increased corresponding to an increased time of exposure. The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several authors (Adegbite and Adesiyan, 2005; Opareke *et al.*, 2005; Orisajo *et al.*, 2007; Abbasi *et al*., 2008, Mohanpriya *et al*., 2019) [4, 18, 19, 2, 3, 15]. Wani and Yaqub Bhat (2012) [23] reported that Soil amendment with urea coated with different doses viz., 0.02g, 0.04g and 0.06g/pot of nimin and oils of neem, castor rocket-salad significantly reduced the development of the roo t-knot nematode and thereby improved plant growth and increased chlorophyll content of mung leaves at 1% and 5% level of signicicance.

From these findings, it may be conclude that *M. incognita*

resulted in the disturbance of metabolism of protein and phenol as well as chloroplast pigments in infected plants of tomato.

Conclusion

From the present experiment it can be concluded that soil application of Nimin 2 Kg/ha is adequate to protect the host plant from damage due to *M. incognita*. In view of low cost of application and non pollutary effects it is hoped that vegetable oil extracts will be further tested and used as effective protectants against nematode infection.

Table 1: Influence of different concentration of Nimin on the growth characteristics of Green gram infected with Root-knot nematode *Meloidogyne incognita*.

Nimin treatment (g)	length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Un infected	20.30 ±0.66	28.00±0.96	2.42±0.18	0.98±0.02	1.20±0.15	0.30±0.03
0	15.60±1.48	24.20±0.78	2.30±0.26	0.72±0.02	0.84±0.06	0.21±0.01
0.5	18.83±1.15	28.00±0.85	3.22±0.26	0.97±0.03	1.01±0.09	0.28±0.01
1.0	23.00±0.42	31.33±0.86	4.58±0.28	1.28±0.12	1.66±0.06	0.39±0.02
2.0	25.00±1.08	33.17±0.47	5.17±0.56	1.49±0.24	1.88±0.12	0.42±0.04
4.0	16.00±0.86	24.00±0.26	3.04±0.13	0.82±0.03	0.84±0.03	0.26±0.02
C.D. at 5%	1.335	2.386	0.474	0.354	0.972	0.055
C.D. at 1%	1.899	3.393	0.674	0.504	1.380	0.078

Each value (Mean ± SD) represents an average performance of 5 observations.

Table 2: Influence of different concentration of Nimin on No. of leaves, No. of branches of Root, No. of Galls and No. of Branches of Galls of Green gram.

Nimin Treatment (g)	No. of leaves	No. of branches of root	No. of galls	No. of branches of galls
Un infected	12.00 ± 1.73	18.33 ± 3.00	-	-
0	7.67 ± 1.00	14.00 ±1.53	22.00 ± 3.00	13.00 ± 1.15
0.5	9.33 ± 1.00	16.67 ± 2.00	11.00 ± 1.52	4.66 ± 1.00
1.0	13.66 ± 3.20	27.33 ± 2.65	9.00 ± 2.65	5.00 ± 1.53
2.0	16.33 ± 2.50	32.33 ± 2.52	5.00 ± 0.58	2.67 ± 1.15
4.0	10.00 ±1.73	14.67 ± 0.57	4.00 ± 1.53	2.33 ± 0.58
C.D at 5%	23.35		71.63	11.05
C.D at 1%	33.21		101.89	15.65

Each value (Mean ± SD) represents an average performance of 5 observations.

Table 3: Cumulative emergence, Percent emergence (overcheck) and Percent reduction in emergence of *Meloidogyne incognita* as influenced by different concentration of Nimin.

Concentration (ppm)	Mean cumulative emergence	Percent emergence over check (%)	Percent Reduction of emergence over check (%)
Distilled water	95	-	-
10	70.6 ± 1.15	74.31	25.69
20	60.3 ± 0.58	63.47	36.53
40	50.6 ± 0.58	53.26	46.74
80	40.0 ± 0	42.11	58.00

Each value (Mean ± SD) represents an average performance of 5 observations.

Table 4: Influence of different concentration of Nimin on Root-knot nematode *Meloidogyne incognita*.

Treatment (ppm)	(%) Mean percent mortality			
	6 hours	12 hours	18 hours	24 hours
10	11.0 ± 1.00	35.6 ± 1.15	72.6 ± 1.53	100%
20	17.3 ± 0.58	50.0 ± 1.00	80.3 ± 1.53	100%
40	21.3 ± 1.53	57.3 ± 1.15	100%	-
80	49.0 ± 1.00	100%	-	-

Each value (Mean ± SD) represents an average performance of 5 observations.

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