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Use of Neem Extract in Controlling Root-knot Nematode (*Meloidogyne javanica*) of Sweet-gourd

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Abstract: Extract of neem (*Azadirachta indica*. L) seed was used to control the root knot nematode *Meloidogyne javanica* of sweet gourd. Extract of neem seed was found to be leathal to the juvenile of *M. javanica* compared to the extracts of bark and leaf of neem. Pot experiment with standard `S' concentration of all the extracts both in side drench and root-dipping methods appeared to give significant suppression in root galling, L_2 and L_3 population of the nematode. Identical response among the treatments with respect of plant growth characters was attributed to the synthesis of less toxic metabolites in the immature neem seeds used in the study. Positive correlation between gall number and eggmass indicated higher activity of the nematode allowing more adult females to develop with the production of increased number of eggmasses.

Key words: Sweet-gourd, neem extract, root-knot nematode

Introduction

Sweet-gourd (*Cucurbita moschata Duchesne*) is a vitamin A rich, popular, year-round but specially summer vegetable in Bangladesh. The production of cucurbits in different years is 7.78 to 9.66% of total vegetables production in Bangladesh (Meah, 1994). Though the popularity of sweet-gourd is increasing quite rapidly but its production remain limited due to many diseases caused by fungi, bacteria, virus and several species of nematodes especially root-knot (*M. javanica*). The common species of root-knot nematodes in Bangladesh are *M. javanica*, *M. incognita* and *M. graminicola* (Timm and Ameen, 1966; Mian, 1986). Page (1979) reported that 36 different crops that were sampled in different districts of Bangladesh, half of them were found susceptible to root knot nematodes. The nematode attack different crops including the vegetable in the Jessore region where *Meloidogyne* spp. occurs predominantly. The root-knot disease is of economic importance to the growers of this country as the loss is increased becouse root-knot nematode predisposes the plants by other pathogenes (Chester, 1950). But no specific control measures have yet been adopted in Bangladesh to save sweet-gourd and other common vegetables from the root-knot disease. Chemical control of root knot is costly and hazardous to agro-ecosystem and environment.

Many plant extracts have been reported to have nematicidal properties (Irshad ***et al.***, 1982 and Stephan ***et al.***, 1989). Neem is being used by the farmers in various ways in Sri Lanka (Ganesalingam, 1986). Neem seed kernel extracts have been effectively used to control field crop pests, in extension experimental plots as well as in fields of vegetable growers (Fagoone, 1986). Seed, bark and leaf of neem are easily available and cheap in this country. Since, it is not feasible to use expensive as well as harmful chemicals for the control of nemic diseases in Bangladesh, due to economic consideration. It was thought worthwhile to study the effect of certain less extensive materials such as indigenous plant extracts of neem against plant parasitic nematodes. The present research programme was undertaken to see the effect of extract of neem seed, bark and leaf on the incidence of root-knot of sweet-gourd caused by ***Meloidogyne javanica***.

Materials and Methods

The experiment was conducted both in the laboratory and glass house of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The efficacy of different concentrated solutions of neem seed, bark and leaf extract in reducing the incidence of root-knot of sweet-gourd was evaluated in two individual sets of pot experiments. One set was conducted with treatments applied as side dressing and soil drench after 5 days of inoculation and other set was conducted with seedlings root-dipping in liquid treatments defore 2 days of inoculation. Sand, sandy loam soil and well-decomposed cowdung (2:2:1) mixed and sterilized with formaldehyde solution at the rate of 30% and covered by polyethene for 72 h without disturbance. After 72 h the polyethene was removed and the soils were air-dried for 48 h in order to remove toxic gasses of formaldehyde. Earthen pot was filled with 5kg sterilized and dried soil. Experiment was set up in the glass house. One seedling was planted in each pot. There were seven treatments with 5 replications i.e. T_1 = control, T_2 = neem seed extract (side drench), T_3 = neem seed extract (root dipping at the time of transplanting), T_4 = neem bark extract (side branch), T_5 = neem bark extract (root-dipping) T_6 = neem leaf extract (side drench) and T_7 = neem leaf extract (root-dipping). Different growth characters of plants, gall incidence and the growth stages of the nematode within the treated as well as treated plants were studied.

Preparation of solution of neem extract for juvenile mortality test

The effect of four concentrated extracts of neem seed, bark and leaf along with a control (distilled water) were taken for the mortality test of juveniles of ***M. javanica*** in the laboratory. Extracts of neem seed (unripe), bark and leaf were prepared after pressing with a pestle and mortar, separately. Concentrated seed extract (25g) bark extract (25g) and leaf extract (25g) of neem as pastes were treated as standard(s). Subsequent dilutions of S/2, S/10 and S/100 were also prepared by adding distilled water in 6 cm diameter petridishes, separately. Petridish containing only distilled water served as control and each type of dilution as treatment was also replicated three times.

Counting of juveniles in the mortality test

Dead and surviving nematodes were counted after 12, 24, 48, 72 and 96 h with the help of

laboratory counter and mean percent mortality was calculated ignoring the fraction of the juvenile numbers. Mortality of the juveniles was assessed by touching the juveniles with a fine needle.

Preparation of inoculum and inoculation

Eggmasses were collected from the roots of eggplant, which were previously inoculated individually with a single eggmass of *M. javanica*. For inoculation, 10 reddish brown mature eggmass were placed in each pot around the standing plant in 2 holes (2.5 cm deep) five eggmass in each side of the plant.

Preparation of seed, bark and leaf extracts of neem

Extract of neem seed, bark and leaf were prepared from the fresh green seed, bark and leaf with help of a blender. A quantity of 25 g of each macerated seed, bark and leaf was mixed with 100 ml distilled water. Each type of the paste was then filtered and all the filtrates were collected separately for use as extracts in the pot experiments.

Application of extracts of neem seed, bark and leaf

Standard extracts of neem seed, neem bark and leaf were applied in two following method one as side drench and the other as root dipping. The extracts were used separately in 15 pots as side drench, five pots for each treatment. Five-milliliter extract was applied to each plant. The treatment was repeated for three times during the experimental period at ten days interval. In the other method of application, 15 seedlings of 12 days old were uprooted and roots of the seedlings were dipped in the extracts of neem seed, bark and leaf for 12 h from 6 a.m. to 6 p.m. in a shady place. Five seedlings were dipped into each extract and transplanted to five respective pots. Hence, a total number of 15 seedlings were used in root-dipping method.

After 60 days of inoculation, plants were uprooted carefully to study the length of shoot, fresh weight of shoot, length of root, fresh weight of root, Number of galls/g fresh root, Number of eggmasses/g fresh root and number of adult male or female nematode, eggmass, L_2, L_3 and L_4 stages in ten well developed galls per treatment.

Counting of galls

After washing, the roots were preserved in 5% formalin solution. The roots were cut into small pieces of 1 cm size and randomly 1g of root was taken from the bulk to count the number of galls formed.

Staining of galled-roots

To stain the galled-roots the following steps were followed: 1.Liquied phenol (500 ml), glycerine (100 ml) and distilled water (500 ml) were poured orderly into a two liter capacity conical flask and shaken thoroughly for mixing them well. Then cotton-blue was added to the content of the beaker to such an amount that turned the solution into the desired blue colour. 2. A quantity of 150-175 ml prepared lactophenol cotton-blue solution was taken in a beaker. 3.

The beaker containing 150-175 ml lactophenol cotton-blue solution was heated to the boiling point. 4. A number of 10 galls per treatment was taken in a special type of cloth bag. At the time of boiling of the liquid of the beaker, the cloth bag containing galls was rinsed for about 1 minute. Then the bag was lifted from the beaker with galls and washed under gentle running tap water to remove excess blue colour. 5. Fresh lactophenol was poured onto petridishes, six for each set, where galls were retained for 15 days for destining. 6. Ten galls from each treatment were crushed one after another with fine pointed needle and counted the number of adult male or female, eggmass, L_2, L_3 and L_4 stages, if any, under stereobimocular microscope. Data were analysed statistically to find out the level of significance.

Results

Effect of extracts of seed, bark and leaf of neem on juvenile mortality of *M. javanica*

In the mortality test, neem seed extract was found to be more toxic to the juveniles followed by neem leaf extract (Table 1). Standard concentration (S) of all extracts caused 100% mortality within 12 h. In case of bark extract a mortality range of 80-100, 82-100, 84-100, 85-100 and 90-100% observed among the tested solutions of S/2 after 12, 24, 48, 72 and 96 h, respectively between neem bark and seed. The juvenile mortality was found to increase with the increase of concentration and exposure period (Table 1). Similarly a mortality range 50-80, 52-80, 55-80, 58-80 and 60-95% were recorded among the S/10 solutions after 12, 24, 48, 72 and 96 h, respectively between neem bark and seed. Incase of S/100 solution, it ranged from 30-62%, 32-65%, 35-70%, 40-75% and 48-80% after 12, 24, 48 and 96 h, respectively. The results indicated that neem seed extract gave more toxic on the L_2 nematodes.

Effect of extracts of seed, bark and leaf of neem on plant growth, galling and development of nematodes

After 60 days of inoculation, effect of treatments was found to be significant on the different growth parameters, galling incidence and stages of nematode development studies.
The highest length of shoot and root was observed 546.6 cm and 55.0 cm, respectively and in case of neem seed extract as side drench white it was lowest 276 cm and 21.6 cm, respectively in control plots (Table 2). The highest and lowest fresh weight of shoot and root was observed 222.8 to 129.0g and 10.0 to 4.80g, respectively. Number of galls/g of root and number of eggmass/root was recorded 30.20 to 8.0 and 190.0 to 100.0 respectively (Table 2).

The control treatment was noted with the highest significant number 190.0 of eggmasses. This was followed by T_4 having 150.0 eggmasses. The rest treatments are identical in response.

Effect on the growth stages of *M. javanica*

In case of number of adult female, eggmass L_2 stage, L_3 stage and L_4 stage on the growth stages of *M. javanica* all treatments caused significant reduction in population of adult females. The highest effect was noted with T_7 followed by T_3, T_5, T_6, T_4 and T_2 all treatments except T_1

Table 1: Effect of extracts of seed, bark and leaf of neem on juvenile mortality of *M. juvanica*

		Mean percent mortality at different concentrations				
Plant extract	Exposure hours	Control	S	S/2	S/10	S/100
Neem seed	12	0	100	100	80	62
	24	0	100	100	82	65
	45	0	100	100	85	70
	72	1	100	100	95	75
	96	3	100	100	95	80
Neem bark	12	0	100	80	50	30
	24	0	100	82	52	32
	45	0	100	84	55	35
	72	3	100	85	58	40
	96	4	100	90	60	48
Neem leaf	12	0	100	95	65	50
	24	0	100	100	70	52
	45	0	100	100	72	55
	72	2	100	100	75	60
	96	3	100	100	80	68

Each value is an average of 3 replications; S = standard solution

Table 2: Effect of extracts of seed, bark and leaf of neem on the growth and galling incidence of sweet-gourd inoculated with *M. javanica*

Treatment	Length of shoot (cm)	Length of root (cm)	Fresh wt. shoot (g)	Fresh wt. root (g)	No. galls/g of root	No. eggmass/ root
T_1 = (control)	276.0b	21.60b	129.0b	4.80b	30.20a	190a
T_2 = (neem seed extract as side drench)	546.6a	55.00a	222.8a	10.00a	10.40bc	120b
T_3 = (neem seed extract as root-dipping)	410.0ab	46.60a	160.6ab	6.40ab	8.00c	100b
T_4 = (neem bark extract as side drench)	426.4ab	48.00a	177.0ab	8.00ab	14.40b	150ab
T_5 = (neem bark extract as root-dipping)	365.4ab	39.00ab	136.8ab	5.20b	10.00bc	112b
T_6 = (neem leaf extract as side drench)	450.8ab	54.60a	205.6ab	9.20ab	11.20bc	128b
T_7 = (neem leaf extract as root-dipping)	388.0ab	43.00a	149.2ab	6.00ab	9.00c	104b
LSD (0.05%)	177.1	19.28	80.50	4.026	4.395	58.07

Each value is an average of five replications. In a column, values having same letters do not differ significantly at P = 0.01 level

Table 3: Effect of extract of neem seed, bark and leaf on the population of root-knot nematode (*M. javanica*) in sweet-gourd

Treatment	No. adult female/10 galls	No. egmass/ 10 galls	No. L_2 stage/ 10 galls	No. L_3 stage/ 10 galls	No. L_4stage/ 10 galls
T_1 = (control)	9.20a	9.60a	17.30a	10.30a	8.80a
T_2 = (neem seed extract as side drench)	8.00ab	6.70bc	9.70b	6.70b	6.40ab
T_3 = (neem seed extract as root-dipping)	5.80b	5.40c	8.60b	6.50b	5.50b
T_4 = (neem bark extract as side drench)	8.00ab	9.00ab	10.40b	7.30b	8.00ab
T_5 = (neem bark extract as root-dipping)	7.00ab	6.50c	9.90b	6.60b	6.20ab
T_6 = (neem leaf extract as side drench)	7.80ab	7.30abc	9.80b	7.00b	7.80ab
T_7 = (neem leaf extract as root-dipping)	5.60b	6.40c	9.30b	6.40b	5.90b
LSD (0.05%)	2.381	2.308	4.295	2.931	2.534

Each value is an average of ten replications. In a column, values having same letters do not differ significantly at P = 0.01 level

gave significant decrease in number of eggmass. Treatment T_1 was found to give the highest significant number 17.30 of L_2 stage, L_3 stage (10.30) and L_4 stage (8.80) and lowest significant and identical response was found with the rest treatments. Overall, the treatment effect was found to be statistically identical in responses (Table 3).

Discussion

In the juvenile mortality test with the extracts of neem seed, bark and leaf, it was revealed that 100% mortality started within 12 h at concentration 'S'. Other than neem bark extract, almost similar trend of mortality was observed with S/2 concentration. At concentration S/10 of neem seed extract with 80-95% mortality in the study periods proved its superiority over the other extracts. Similarly, neem seed extract was found to be more lethal to the juveniles even at S/100 concentration. From the overall mortality test, it appeared that the extracts of neem seed, bark and leaf even at S/100 concentration were found to be toxic to the nematode (Table 1). While studying the antifeedant efficacy of the extracts of seed kernel, seed coat and fallen leaves of neem against desert locust Singh (1986) also observed that neem kernel extracts were found most active, seed coat extracts followed and fallen leaf extracts were last effective. Seed kernel extract yielded more extract than fallen leaves or seed coat.

In the pot experiment with `S' concentration of all the extracts were found to give statistically identical response in plant growth characters like length of shoot and root, fresh weight of shoot and root compared to the control treatment (Table 2 and 3). Similarly, no significant effect was observed on the development of eggmass, adult female and L_4 population of the nematode (Table 2 and 3). But the significant effect was observed with the all extracts of neem in both the methods of application in respect of number of galls per g of root and L_2 and L_3 populations of the nematodes compared to the non-treated control treatment (Table 2 and 3).

The increased galling incidence with the non-treated control treatment T_1 (Table 2) clearly indicated that extracts of neem seed, bark and leaf were effective in suppressing the nematodes activity, but their effect could not sufficiently act upon plant nematode physiology as evident with non-significant responses in plant growth characters and reduction of adult females which ultimately resulted in the non significant development of eggmasses and L_4 population. Incase of L_2 and L_3 populations, significant responses among the treatments with neem extracts compared to the control one revealed that the extracts were found to be effective in suppressing their development. But onward development to L_4 and adult females as well as eggmass their action was found to be receded.

While working with the leaf extract of margosa or marigold, Hussain ***et al.*** (1984) found that the development of ***Meloidogyne incognita*** was considerably reduced in root-dip treatment with extract as compared to treatment with piperazine citrate, chenopodium oil and ground-nut cake. They further observed that treatments with half-standard concentration S/2 of ***Artemiria maritina*** were better for plant growth, but for disease control, standard concentration `S' was more effective. Their results corroborate with out findings. Sharma ***et al.*** (1985) used powders of 9 plants to pot experiment. They observed that with increase of time (after 45 days of

application) there was decline in population. Alam (1987) similarly reported that chopped plant leaves when incorporated into naturally infested soil effectively suppressed [population of plant parasitic nematodes in tomato cv. marglobe. Sartaj Ali ***et al.*** (1986) observed that the different standard solutions of leaf extract of lemon grass (***Cymbopega flexuosus***) were found to be highly toxic to all the tested nematodes, ***Meloidogyne incognita***, ***Rotylenchulus reinformis***, ***Tylenchorhynchus indicus***.

Goswami and Vijaylakshmi (1986) reported that the number of galls per g of root was reduced by 9 different plant extracts especially with Euclipta alba, Shorea rubuta and Datura metal. Chabra ***et al.*** (1988) also noted that leaf extracts of Ricinus communis, Leucaena leucocephala, Populus deltoides, Azadirachta indica, Lantana camara and Eucalyptus hybrida were highly toxic to J_2 of ***M. incognita***. Leaf extracts of Ricinus communis was found the most toxic to ***M. incognita***. The lower populations of L_2 and L_3 stages in our experiment with all the neem extracts clearly indicated its harmful effect on the nematodes.

As the existing practice of chemical control is too costly, particularly for poor farmers of Bangladesh, as well as its harmful effect responsible for air, soil and water pollution as stated by Alam (1987), the use of extracts of neem seed, bark and leaf have been found encouraging in the control of root-knot of sweet-gourd caused by Meloidogyne javanica. The present research was conducted with unripe (immature) neem seed. The difference in result with respect to the growth characters of plants as observed with neem seeds by other workers (Rossner and Zebitz, 1987, Siddiqui and Alam, 1987 and Pathak ***et al.*** (1988) might be due to use of immature neem seeds in the present study. It is quite obvious that mature seeds of neem synthesize more metabolic substances like azadirachtin and other closely related metabolites-vepaol, isovepaol and nimibidin which have been stated to be antifeedant and growth inhibitor of insects by Sankaram ***et al.*** (1986). Such synthesized metabolites in mature seeds of neem accumulate in more concentrate from and are likely to be more lethal to the plant pathogen including nematodes allowing better plant growth.

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