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Evaluation of bio products as seedling treatment for development and invasion of root knot nematode in tomato

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Abstract
Root knot nematode is most destructive plant pathogen. It contributes considerable losses in the yield of crop plant. The evaluation of bio products at 5% concentration (Vampire Axiom and Fline) for their effectiveness on the development and invasion of root knot nematode was studied. Seedlings of tomato were dipped in Vampire, Axiom and Fline @ 5% concentration for 1 hour. Treated seedling with effective concentration of bio products were transplanted into pots. After one week of transplanting these plants were inoculated with @500 *J2*/ pot. This experiment was conducted in two sets. One set was harvested after 7, 14, 21, 35 days and other was harvested after 60 days. Fline gave less no. of Root kont nematodes *J2* population per root system 711 C as compared to control 2316. Almost all the treated plant showed good growth as compared to the control.

Keywords: Bio-products, Seedling treatments, Root knot nematodes, *Meloidogyne* species, concentration, inoculation

Introduction
The nematode attack 1332 hosts plants of economic importance [5] and this number has increased to 2,000 species [3] but presently potential host range encompasses more than 3000 plant species [1]. They have been found associated with severe root damage in large number of crops. However, *Meloidogyne incognita*, *M. javanica, M. arenaria, and M. hapla* are of outstanding economical importance, being responsible for at least 90% of all damage caused by root knot nematodes [13]. Root knot nematode (RKN) (*Meloidogyne* spp.) stand out as the dominant group of plant parasitic nematodes in almost all vegetable fields and cause enormous losses [8].

RKN cause direct and indirect effects on plants. Direct effects of RKN include restriction of water and nutrient supply by direct feeding, restriction of xylem and phloem vessels by formation of giant cells and death of root tips. They show indirect effects by breaking the host resistance mechanism, creating physical entry sites for pathogens and increasing susceptibility to foliar disease [9]. Bhatti and Jain (1977) [4] calculated yield loss of 59%, 27.3% and 46.2% in Okra, Brinjal and tomato respectively due to *M. incognita*. Taking into account the worldwide distribution for root knot nematodes, their affinity with other pathogens and serious destruction to vegetables and field crops, it is necessary to find out the most effective and feasible control measure because the use of chemicals for nematode control is an expensive and un practicable operation. In Pakistan the prices of nematicides are also very high and only small number of farmers can afford the use of nematicides. This situation demands the search for cheaper and pollution free alternative management strategies which can be made available to small growers.

Material and Methods
Collection of root samples
The plants showing the symptoms of root knot nematode infestation were collected with soil around the roots from Vegetable Research area of Plant Pathology, University of Agriculture Faisalabad. Root samples with galls were carefully lifted with the help of trowel up to one feet depth from the rhizosphere of tomato plants. The samples were collected in polythene bags and immediately brought to laboratory and processed for isolation. Processing was made by washing the roots carefully under gentle stream of water.

Isolation of nematodes

Juveniles (*J2*) population of Root Knot nematodes were obtained from severely infested roots of tomato. J2 were isolated from infested roots by modified White-head and Hemming tray method [15]. In this method, the infested roots with egg masses were washed thoroughly under tap water. The roots were kept in tray lined with tissue paper having sufficient water and after 24 hours, the water was poured off in a beaker and allowed to settle for one hour. When the juveniles had settled, the excess of water was siphoned off until about 100 ml remained.

Identification of root knot nematode on the basis of perineal pattern

Galls with mature females were selected and placed in a Petri dish with tap water, root tissues were teared apart with forceps and half spear to remove adult females. Neck of female was cut off with the help of half spear to remove the interiors out. Then cuticle was placed in a drop of 45% lactic acid on a plastic Petri dish. Similarly, 5-10 cuticles were collected in the drop and allowed them to stand for 30 minutes. Then cuticle was cut in half (equatorially) with the help of blade and scalp and portion of cuticle with perineal pattern was transferred to another drop, after this it was trimmed around the perineal pattern to square shape. The trimmed perineal pattern was placed back in the 45% lactic acid and cleaned it free from debris. After cleaning, the perineal pattern was transferred to a drop of glycerin on a clean micro slide and aligned in such a way that anus was oriented downward. A warm cover slip was placed on the glycerin drop, sealed with nail polish and labeled [6].

Mass culturing of root knot nematode

Three weeks old tomato seedlings were removed from seedlings trays and transplanted into pots filled soil. After one week of transplanting these plants were inoculated with 1000-1500 freshly hatched juveniles. These plants were not watered just after inoculation and they were kept in individual dishes to avoid cross contamination. Throughout the experiment the culture plants were kept in greenhouse where temperatures ranged 22-35ºC. These plants were regularly watered.

Extraction of *Meloidogyne javanica* eggs to obtain its infective stage juveniles (*j2s*)

Eight to nine weeks-old root knot nematodes infected seedlings of tomato cultivar were cleaned of debris by gently washing under a stream of water. The roots were cut into 2-3 cm segments and shaken vigorously (manually) for 3-4 min in a coffee jar (one liter) with a tightly fitting lid, containing 200 ml of 0.5 % (a.i.) NaOCl solution to dissolve the gelatinous matrix and to release the eggs from the egg masses. This suspension was quickly passed through 250 µm sieve nested over 300 µm sieve to collect root fragments on the former and freed eggs on the latter. Then 300 µm sieve with freed eggs was quickly passed under a stream of cold tap water to remove the residual NaOCl. Rinsing of eggs was done for several minutes. Then these freed eggs were collected in a beaker. This process was repeated twice, for removing additional eggs. The egg suspension was poured onto an extraction dish containing tap water. This extraction dish (10.5 cm diameter) with 1mm pore size, nylon mesh sieve was covered by a double paper (Kleenex) on the surface of water. It was covered with another dish of same size to prevent evaporation but allowing oxygenation to hatched juveniles (*J2*) and hatching eggs. The hatching juveniles passed through the tissue and eggs were held on the tissue paper. Eggs were incubated for 3-4 days at 28 °C. The nematode suspension was collected after every 24 h. The first collection done within 24 h was discarded, because: 1) it was mixture of few older juveniles and eggs, which passed through the tissue paper: 2) uniform and larger number of juveniles was available within the next 24 h. If a juvenile suspension was to be required to stay in the laboratory for more than 24 h before inoculation or evaluation, the volume was reduced and oxygen was provided by an air pump. Collecting of eggs by this procedure has several advantages: 1) it is simple and rapid procedure: 2) eggs are surface sterilized: 3) the inoculum is easily standardized and uniformly distributed around root system [7]. Care was taken that eggs should not be exposed to more than 1 % concentration of NaOCl and for more than 4 minutes.

Concentration of juveniles

Nematode suspension was settled for 3-4 h, and then excess water was siphoned off without disturbing the nematodes in the bottom. This method is slow but loss of nematodes is less. To concentrate the nematodes quickly the suspension was passed through 100 µm sieve and nematodes were collected from the sieve, this process was repeated 3-4 times to collect maximum number of nematodes.

Counting and standardization of nematodes

To estimate the inoculum's density the juvenile suspension was poured into a measuring cylinder. The suspension was mixed vigorously by blowing with pipette. The numbers of J2 were estimated in 3×1ml aliquots in a counting dish under a dissecting microscope at X3.5 magnification. The total population was estimated by multiplying the mean of three replications with total volume.

When the nematodes were difficult to count in one ml due to high concentrations, 0.25-0.5 ml nematode suspension was diluted with 1-2 ml water and then counting was done.

Inoculation of plants with root knot nematodes

Depending on the size of pots 4-6 holes up to middle of the pots around the plants were made with the help of pointed wood. Requisite number of juveniles was pipetted in these holes held in a small volume of nematode suspension. The holes were covered with soil to prevent drying. For ten days after inoculation these pots were watered carefully to prevent loss of nematodes through leaching or excessive drying.

Evaluation of bio products against root knot nematode as seedling treatment

Dipping of tomato seedlings in bio products (vampire, axiom and fline)

An experiment for the evaluation of various bio pesticides/ bio products was carried in green house, Dept. of Plant Pathology, University of Agriculture, Faisalabad. Fifteen seeds of Money maker variety of tomato were sown in pots without seed treatment. After 30 days of germination, the plants were uprooted from seedling tray and the seedlings were treated with bio nematicides (Vampire, Axiom, Fline). Seedlings of tomato were dipped in Vampire, Axiom and Fline @ 5% concentration. The seedlings were allowed to dip for 1 hour. After that seedlings were sown in pots and after one week, the plants were inoculated with 500 *J2* / pot. After sixty days of inoculation, the plants were uprooted and processed as mentioned earlier. This experiment was conducted in two sets. One set was harvested after 7, 14, 21,

35 days and other was harvested after 60 days. The parameters like number of galls, gall index, egg masses, egg masses index, eggs per plant, root weight, number of J2 per root system, number of females per root system, number of J2 per 100 cm³ of soil was recorded. After 60 days, plants were gently removed from the pots with soil, and roots and care fully washed in running water. Then data was recorded.

Data analysis

The data collected were analyzed by Complete Randomized Design (CRD). The analysis of variance (ANOVA) and the differences among means was analyzed by applying LSD test at 5% level of probability [14].

Result

Evaluation of bio products for their effectiveness on the development and invasion of root knot nematode

Development and invasion of root knot nematode after 7, 14, 21& 35 days

After 7 days the plants were uprooted and invasion of J2 was recorded. There were zero number of gall per root system. Table 1.After 14 days the plants were uprooted. There were zero no. of galls per root system on tomato plants treated with Fline product as compared to control tomato plant having four number of galls. Table 2. After 21 days, 3 number of females and 35 *J2* were observed on root system in Fline treated plants as compared to control contain 39 number of females and 296 *J2*.Table 3. After 35 days, 719 numbers of juveniles was observed in 100 cc soil of Fline treated plants root zone as compared to control having value 2027.Table 4. Similarly the data was recorded after 60 days, Fline gave less no. of Root kont nematodes *J2* population per root system 711 as compared to control 2316. Almost all the treated plant showed good growth as compared to the control. Table 5.

Discussion

Root knot nematode is among the most destructive plant pathogen. It contributes considerable losses in the yield of crop plant. The evaluation of bio products at 5% concentration (Vampire Axiom and Fline) for their effectiveness on the development and invasion of root knot nematode was studied which showed highly significant results. Fline gave the highest plant growth and the highest reduction in root knot nematode. Almost all the treated plant showed good growth as compared to the control. In root weight only Vampire 2.5% showed less growth which was also not significantly different. It reduced galling index and number of egg masses as compared with control [11]. Dipping root-knot nematoede infected tomato seedlings in extracts of neem leaf and cake had a significant effect on the number of females and egg masses on the root systems 30 d later. The fline treatment had no significant effect on the development of the nematode [12]. In our results the treatments of fline showed more significant results almost in all the parameters of our study. Various products (oils, cakes, extracts, etc) prepared from the leaves and seeds of the neem plant (*Azadirachta indica*) (Family *Meliaceae*) are effective protectants against nematode pests when used as root-dips treatment [2].

Javed *et al*. (2008a) [10] stated that second stage juveniles of *M. javanica* when exposed to aqueous extracts of neem crude formulations (leaves and cake) at 10%, 5% and 2.5% w/v and a refined product, Aza at 0.1% w/v. The 10% extracts of neem leaf and cake caused 83% and 85% immobility and 35% and 28% mortality, respectively. Aza caused neither immobility nor mortality of juveniles. When egg masses were placed in extracts of these formulations, hatching did not occur at all the concentrations (10%, 5%, 2.5% and 1.25% w/v) of the crude formulations. When the treated egg masses were returned to water, the eggs resumed hatching. Aza did not affect the nematode hatching. In glasshouse experiments, soil application of neem formulations significantly reduced the invasion of tomato roots by root- knot nematodes but once the nematodes managed to invade them, no effect detected on their development. Soil applications of Aza at 0.05% and 0.1% w/v significantly reduced the invasion and delayed development of nematodes within tomato roots whereas 0.025% did not. There were significantly fewer egg masses on tomato roots exposed to single egg mass in neem amended soil as compared to control.

Our results indicated that application of bio products (Vampire, Axiom and Fline) with an integrated control program could provide effective control of *Meloidogyne incognita*. Various strategies are used to manage nematodes but the use of bio pesticides as biological control of plant parasitic nematodes is most effective, economical and environment friendly method.

From the present study it may be suggested that the bioproducts (Vampire, Axiom, Fline) could be adjusted in integrated management of tomato. This method could prove economical and environment friendly method.

Table 1: Comparison of mean values of Different parameters of invasion of RKN after 7 days

	Root length(cm)	Shoot length(cm)	Root weight(gm)	Shoot weight(gm)	Galling index	No. of galls/ root system	No. of females/root system	J2/root system	J2/100 cm 3 of soil	egg mass
Vampire	6.2 B	5.3	0.2	0.3	0	0	0	13	520	0
Axiom	7.3 A	5.3	0.16	0.6	0	0	0	10	612	0
Fline	7.8 A	6.2	0.19	0.9	0	0	0	6	106	0
Control	7.9 A	7.4	0.11	0.9	0	0	0	16	1042	0
LSD	1.56							6.23		

Values with the same letters do not different significantly.

Table 2: Comparison of mean values of Different parameters of invasion of RKN after 14 days

	Root length(cm)	Shoot length(cm)	Root weight(gm)	Shoot weight(gm)	galling index	No. of galls/ root system	No. of females/root system	J2/ root system	J2/100 cm^3 of soil	egg mass
Vampire	6.8	6.3 A	0.17	1.22	1 B	2 B	3AB	60A	1001 B	2 B
Axiom	6.0	6.0 B	1.21	1.60	1 B	1 B	1 B	25B	658AB	1 B
Fline	5.8	6.1 B	2.17	1.68	0 C	0 C	1 C	11C	418 C	1 C
Control	6.1	7.4 B	2.11	1.66	2 A	4 A	7 A	96A	1596 A	3 A
LSD		1.656			0.7932	0.684	2.173	12.62	109.25	0.99

Values with the same letters do not different significantly.

Table 3: Comparison of mean values of Different parameters of invasion of RKN after 21 days

	Root length(cm)	Shoot length(cm)	Root weight(gm)	Shoot weight(gm)	galling index	no. of galls/ root system	no. of females/root system	J2/ root system	J2/100 cm^3 of soil	egg mass
Vampire	6.9	7.9 A	1.10	3.1	3B	11 B	20AB	105A	1223 B	10 B
Axiom	6.2	6.3 B	2.1	3.9	3 B	12 B	21 B	156B	930 AB	11 B
Fline	6.0	6.5 B	3.11	4.1	1 C	2 C	3 C	35C	291 C	2 C
Control	6.3	7.5 B	3.01	4.2	4 A	31 A	39 A	296A	1425 A	19 A
LSD		1.656			0.7932	3.684	4.173	52.62	131.25	6.99

Values with the same letters do not different significantly.

Table 4: Comparison of mean values of Different parameters of invasion of RKN after 35 days

	Root length(cm)	Shoot length(cm)	Root weight(gm)	Shoot weight(gm)	galling index	No. of galls/ root system	No. of females/ root system	J2/root system	J2/100 cm^3 of soil	egg mass
Vampire	8.1	8.5 A	2.18	7.15	4B	32 B	38 AB	715A	1031B	20 B
Axiom	8.9	8.6 B	3.19	7.75	4 B	31 B	25 B	756B	1025AB	18 B
Fline	7.1	7.9 B	4.15	8.07	2 C	8C	12 C	213C	719C	16C
Control	7.5	7.8 B	4.35	8.0	5 A	102 A	63 A	1296A	2027A	49 A
LSD		1.656			0.7932	19.684	12.173	113.60	309.25	16.99

Values with the same letters do not different significantly.

Table 5: Comparison of mean values of Different parameters of invasion of RKN after 60 days

	Root length(cm)	Shoot length(cm)	Root weight(gm)	Shoot weight(gm)	galling index	No. of galls/ root system	No. of females/ root system	J2/root system	J2/100 cm^3 of soil	egg mass
Vampire	14.2	15.4 A	5.3	10.2	4B	81 B	112B	1606B	3025 B	111 B
Axiom	14.0	13.4 B	6.1	11.1	4 B	90B	120B	1713B	3441B	118B
Fline	13.8	12.8 B	6.5	12.7	3 C	29 C	37C	711C	961C	35C
Control	13.4	12.6 B	7.0	12.0	5 A	165 A	166 A	2316A	4076A	132A
LSD		1.656			2.7932	29.684	45.173	713.60	909.25	31.99

Values with the same letters do not different significantly.

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