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Evaluation of Bio-Nematicides against the development of Root Knot Nematode (*Meloidogyne incognita*) in Tomato

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Abstract
Root knot nematodes(*Meloidogyne* spp.) are the major constraints for vegetable crop production in Pakistan. Objective of the present study was to determine the efficacy of most effective of bio-product (Vampire, Axiom, Fline) at 5% concentration on the invasion and development of root knot nematode by seed dip and seedling treatment. Nematode reproduction and their influence on plant growth were determined after 60 days. At harvest of data on population of J2 penetration and development, final J2 population in roots and soil, number of females/ gm of soil, root galling and egg masses was assessed on 0-5 scale. Vampire and Fline @ 5% showed best control of root knot nematode after 60 days(No. of root galls/root system) 50B,20B means value as compared to control 106A at seed dip treatment. similarly, Vampire and Fline @ 5% showed best control of root knot nematode after 60 days(No. of root galls/root system) 31B,08B means value as compared to control 102A at seedling treatment. These bio-product are seems to be the best in seed treatments as well as in seedling dip treatments economically.

Keywords: Bio-products, Seed treatments, Root knot nematodes (*Meloidogyne* spp.).

1. Introduction
There are various limiting factors in the production of crop but root knot nematodes (*Meloidogyne* spp.) are the most destructive one that tremendously reduce both quality and quantity of crop produce. In Pakistan major nematode problems that cause great damage to agricultural production are due to *Meloidogyne incognita* and *M. javanica.* They attack many vegetable crops, fruits and ornamental plants throughout the country [11]. The nematode attack many plants of economic importance include 1332 hosts plants [4] and this number has increased to 2,000 species [2] but presently potential host range encompasses more than 3000 plant species [1]. They have been found to be associated with severe root damage in large number of crops. However, *M. 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. Root knot nematodes (*Meloidogyne* spp.) stand out as the dominant group of plant parasitic nematodes in almost all vegetable fields and cause enormous losses [12]. *Meloidogyne* spp. cause direct and indirect effects on plants. Direct effects of 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 [7]. Bhatti and Jain (1977) [3] estimated yield loss of 46.2%, 99% and 27.3% in tomato, okra and brinjal respectively due to *M. incognita.* Various strategies have been used extensively over the years in Pakistan to manage nematodes but the use of bio-pesticides as biological control of plant parasitic nematodes is most effective and environment friendly method. So, the use of bio-products in Integrated Pest Management (IPM) is now accepted as an ecologically sound and economically viable alternative to chemical pest control. Thus, the present study was planned to investigate the most effective and economical bio-product in seed treatments as well as in seedling dip treatments.

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.

Raising of Tomato Nursery

"Money maker**"** a tomato cultivar is known to be highly susceptible to root knot nematodes infection. Therefore they were raised in large earthen pot. After 25 days tomato seedlings were ready for the transplanting. They were transferred in earthen pots (9", 12") containing formalin sterilized sandy loam soil

Soil Sterilization

The soil was sterilized with the help of formalin. Diluted formalin (1: 320) was poured on the small heap of soil and covered with polythene sheet completely, so that fumes should not come out. Soil was covered for 7 days. After 7 days, plastic sheet was removed; the soil was mixed thoroughly and then filled in pots.

Mass culturing of Root Knot Nematode:

Three weeks aged tomato seedlings at 5-6 leaves stage were removed from seedlings trays and transplanted into pots filled with 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 kept in individual dishes to avoid cross contamination. Throughout the experiment the nematode inoculated plants were kept in greenhouse where temperature ranged between 22–35 °C. These plants were regularly watered thereafter.

Evaluation of bio-products against root knot nematode as seed treatment

Seeds of tomato were dipped in Vampire, Axiom and Fline @ 5% concentration. The seeds were allowed to dip for 2 hours. After that seeds were sown in pots and after inoculation with 500 juveniles per pot and the plants were allowed to grow for 60 days.

This experiment was conducted in green house at 25°- 27°C. Firstly 5% concentrations Bio-products (Vampire, Axiom, Fline) were made. Ten seeds of tomato were dipped in the concentration for 2 hours and then seeds were sown in pots. After their germination, these plants were thinned to one plant per pot. After three weeks of germination, these plants were inoculated with freshly hatched second stage juveniles of *M. incognita* @ 500 per pot. Then after sixty days of inoculation, the plants were gently removed from the pots and the parameters like Root length(cm), shoot length(cm), shoot weight (gm),No. of galls/root system,J2/root system,J2/100 cm^3 of soil, egg mass was recorded.

Evaluation of bio products against root knot nematode as seedling treatment

Fifteen seeds 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. The parameters like Root length (cm), shoot length(cm), shoot weight(gm), No. of galls/root system,J2/root system, J2/100 cm^3 of soil, egg mass was recorded. After 60 days, plants were gently removed from the pots with soil, and roots and carefully washed in running water.

Root galling and egg masses were assessed on 0 to 5 scale, where 0 = no gall, 1 = 1-2 gall, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = > 100 galls per root system.

Data Analysis

The Complete Randomized Design (CRD) was used in experiment and the differences between treatments were determined by LSD 5% probability level [13].

Results and Discussion

For the control of root knot nematode (*Meloidogyne incognita*), comparative effect of bio pesticides was studied by seed and seedling treatment. At seed dip treatment, 5% concentration of each bio pesticides (Vampire, Axiom and Fline) was used. The effect of seed dip treatment of tomato seeds on growth and root knot development was studied. The parameters studied in this experiment were number of egg masses, number of females per root system, number of galls, root weight and shoot weight. The results were highly significant and proved that Fline, Vampire at 5% concentration gave the maximum reduction in root knot nematode as compared to Axiom (Table.1).

Fline as seedling dip treatment was effective against root knot nematode (*Meloidogyne incognita*) development. It reduced galling index and number of egg masses as compared with control [9]. Dipping root-knot nematode 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 [10].When the effect of seedling dip treatment of tomato seedling with bio products (Vampire, Axiom and Fline) on plant growth and nematode disease development gave highly significant results. Fline gave the highest plant growth and the highest reduction in root knot nematode at 5% concentration. Almost all the treated plant showed good growth as compared to the control (Table.2). Satisfactory protectant, curative and residual control was demonstrated when 100 to 500 lag of bio products was injected into plants [6]. Javed *et al*. (2005) [8] reported that neem (*A. indica*) products, i.e. crude (leaves, cake and seeds) and refined seed extract (azadirachtin) reduce the vigor of root-knot nematode (*M. javanica*) in the soil. The neem products significantly reduce the mobility of juveniles in the treated soil. Collectively, these data show that root-dip applications of Vampire may have potential to control nematodes on tomato [6].

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

Table 1: Invasion and development of Root Knot Nematode on tomato plant roots with bio-product application by seed dip treatment.

	Root length	shoot length	root weight	shoot weight	no. of galls/ root system	no. of females/ root system	J2/root system	J2/100 cm3 of soil	egg mass
Axiom	16.8a	17.0	0.8	1.36	60 b	85bc	629b	1506b	25bc
Vampire	15ab	14.0	0.86	2.1	55b	62b	601ab	1301ab	21b
Fline	13.2b	13.2	0.9	1.78	20b	29c	301c	902c	11c
Control	16.2a	14.8	1.12	1.76	106 a	145a	1302a	5819a	120a
LSD	2.380				7.991	31.303	305.374	911.322	12.947

Table 2: Invasion and development of Root Knot Nematode on tomato plant roots with bio-product application by seedling treatment.

Treatment	root length(cm)	shoot length(cm)	root weight(gm)	Shoot weight(gm)	no. of galls/ root system	no. of females/ root system	J2/ root system	J2/100 cm^3 of soil	egg mass
Axiom	8.1	8.5 a	2.18	7.15	32 b	38 ab	715a	1031b	20 b
Vampire	8.9	8.6 b	3.19	7.75	31 b	25 b	756a	1025ab	18 b
Fline	7.1	7.9 b	4.15	8.07	8c	12 c	213c	719c	16c
Control	7.5	7.8 b	4.35	8.0	102 a	63 a	1296a	2027a	49 a
LSD		1.656			19.684	12.173	113.60	309.25	16.99

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