Integrated management of root-knot nematode (*Meloidogyne incognita*) for tomato production and productivity

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ABSTRACT

Root-knot nematode, *Meloidogyne incognita,* causes significant economic losses to tomato production. Aqueous suspension of rapeseed cake and BioNem WP were evaluated for their effect on root-knot nematode in the laboratory, pot house, nursery and field conditions. In the laboratory, three concentrations of rapeseed cake extracts (5, 10 and 20%) and two concentrations of BioNem extracts (5 and 10%) significantly reduced ($p<0.05$) the nematode egg hatching as well as juvenile motility over the untreated control. Higher concentration of rapeseed cake (20%) proved most effective in reducing hatching and affecting motility. The application of 0.6 and 0.4 g/pot of BioNem both ten days before transplanting and at the time of transplanting resulted in less gall formation, number of eggs per egg mass and final nematode population over the untreated control in the pot house experiment. The incorporation of 600 kg/ha and 300 kg/ha of rapeseed cake and 150 and 75 kg/ha of BioNem in the nursery beds reduced the nematode infestation and improved tomato seedling growth as compared to the untreated control. The effects of higher dosages BioNem (150 kg/ha) and rapeseed cake (600 kg/ha) were at par with the chemical treated control (Fenamiphos at 3 kg/ha).

Key words: BioNem, *Meloidogyne incognita*, Rapeseed cake, Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important food and cash crop of the farmers and is widely cultivated throughout the country (Lemma Dessalegn, 2004). It has very high potential for expansion under wide range of agro-ecologies of the country, provided that certain production constraints are alleviated (Fekadu Mariame *et al*., 2003). Tomato can be produced by direct sowing of the seed in the field or transplanted from seed beds. Transplanting has the advantage of economic use of seeds, selecting superior and vigorous seedlings, easiness for field establishment and early harvest (Lemma Dessalegn, 2002). The current average national yield of tomato is 7.1 t/ ha (CSA, 2009) which is significantly lower than the current world average yield of 27 t/ ha (FAOSTAT, 2007). Diseases and insect pests are the major bottlenecks in production in major tomato growing areas of Ethiopia (Lemma, 2002).

Root-knot nematodes (*Meloidogyne* spp.) are among the major pathogens of vegetables especially in tomato and eggplants throughout the world (Sakhuja and Jain, 2001). In Ethiopia, tomato is heavily attacked by root-knot nematode, *Meloidogyne* spp. and the species *Meloidogyne incognita* is the dominant species in Rift Valley (Lemma Dessalegn, 2002), central and eastern Ethiopia (Tadele Tefera and Mengistu Hulluka, 2000; Wondirad Mandefro and Tesfamariam Mekete, 2002). Soils with higher sand content and near neutral pH in lower altitude in eastern Ethiopia were fund to be suitable to inhabit more nematode population (Tadele Tefera, 1998). Vegetable crops grown in warm climates can

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experience severe losses from root-knot nematodes (Sakhuja and Jain, 2001). Symptoms of root-knot nematode include galling on roots, stunted growth, wilting, a decline in yield of the crop (Sasser, 1980). Root-knot nematodes can severely restrict all the vital functions of plant roots, including the absorption and transfer of water and nutrients (Agrios, 2005). Besides, they provide easy entrance for secondary infection causing microorganisms (Romy and Robert, 2008). Therefore, to avoid damage due to root-knot nematode, appropriate management options should be applied timely.

Crop rotation with non host or less susceptible crop varieties, soil solarization, organic soil amendments, cultural and tillage practices, use of nematode free transplants, and pre-planting nematicide treatments and biological control are among different options of its management (Donald and Brent, 1998; Sakhuja and Jain, 2001). Root-knot nematodes can be successfully managed by soil organic amendments with oil cakes or by integrating oil cakes with other management components (Khan and Sexena, 1997; Goswami *et al*., 2006). Glucosinolates which are the constituent of rapeseed cake have nematicidal and nemestatic effects on root-knot nematode due to its hydrolyzed substance like isothiocyanates (Mojatahedi *et al.*, 1993). BioNem WP is a biological nematicide, derivatives of the bacterium *Bacillus firmus*, supplemented with certain additives which are of plant and animal origin. The product is commercially used in Israel for the control of root-knot nematodes (*Meloidogyne* spp.) in vegetables (cucumber, tomatoes, pepper, eggplant), herbs, in perennial crops (stone-fruits) and ornamentals (Keren-Zur *et al*., 2000).

Although many options have been tested for the management of root-knot nematode on different crops in different parts of the world, little work has been done in Ethiopia. There is need of determining some management options which can be practically feasible.The objectives of this research were to evaluating the effects of extracts of rapeseed cake and BioNem on egg hatching and motility of root-knot nematode, find out the effect of time of application of BioNem on root-knot nematode infestation in tomato and Determine effect of rapeseed cake and BioNem applications in nursery on root-knot nematode and tomato seedling performance

MATERIALS AND METHODS

Determination of root-knot nematode population in the soil

Determination of population of root-knot nematode was done based on Baermann funnel technique (Southy, 1970). One hundred cc of soil sample was added in a 250 ml beaker. The beaker containing the soil was kept gently (upside down) in the funnel containing water and then about 20 ml of nematode suspension was collected in a beaker after 24 hours. The suspension was made to 60 ml by adding the 40 ml of tap water. It was bubbled with pipette and 5 ml of it was transferred to the nematode counting dish. The number of juveniles of root-knot nematode was counted under a stereomicroscope and multiplied by the dilution factor (12) for estimation of nematode population in the soil sample.

Identification of *Meloidogyne* species

Root galls on an infested tomato root collected from Tony farm were teased apart with forceps to separate out the adult females after stained with acid fuchion. The adult females were collected and kept overnight in a Petri dish with lactopheno. The perineal region of females was cut and was trimmed to square shape and placed to a drop of glycerine on a clean glass slide with the interior surface of the cuticle being placed against the glass. The cover slip was gently placed on the glycerin drop and sealed with nail polish and the slide was labeled. Examination of 20 perineal patterns for species identification was

performed with compound microscope. All the perineal patterns resembled to those of *M. incognita* as described by Jepson (1987) and Sasser and Carter (1985).

Raising of stock culture of root-knot nematode and collection of egg masses

Severely infected tomato plant roots were collected from Tony farm which is the main research site of Haramaya University in Dire Dawa. Using dissecting needle and forceps, the egg masses were separated from the infected root tissues after washing and then kept in the sterile water for hatching for 5 days. The hatched juveniles were inoculated to the sterilized soil in pots on which the tomato seedlings were transplanted to raise stock culture of root-knot nematode (Randhawa *et al.*, 2002). This was sub cultured to maintain sufficient culture of root-knot nematode for subsequent experiments in the laboratory and pot house. With similar procedure some egg masses were collected from the stock culture and kept in refrigerator at 10 ^{o}C to prevent hatching before application of treatments for hatchability experiment. Others were kept on laboratory bench at room temperature for 5 days till hatching of the juveniles for motility evaluation experiment.

Effect of Rapeseed Cake and BioNem Extracts on Egg Hatching

Extracts were prepared separately by taking 100 g of rapeseed cake and BioNem each soaked in one liter of water for 12 hours (Metasebia Terefe *et al*., 2009). The suspensions were filtered through the muslin cloth and then centrifuged at 3000 rpm for 5 minutes to obtain the supernatants which were designated as standard 100 % solutions. Six treatments (5, 10 and 20% rapeseed cake extract; 5 and 10% BioNem extract and untreated control) were laid out in completely randomized design (CRD) with four replications for each. Three ml of extracts of each concentration were added in sterile Petri dish (5 cm diameter). Five almost equal sized egg masses were picked up using forceps and placed in each Petri dish containing the extracts. Egg masses kept in sterilized distilled water were used as control. All the Petri dishes were covered to avoid possible contamination from open air and kept on the laboratory bench for hatching. The maximum and minimum temperatures during the experiment were 24.2 and 16.0 ^{o}C, respectively.

Effect of rapeseed cake and bionem extracts on motility of juveniles

Six treatments (5, 10 and 20% rapeseed cake extract; 5 and 10% BioNem extract and untreated control) were evaluated in completely randomized design (CRD) with four replications. Three ml of extracts of 10, 20 and 40% rapeseed cake and 10 and 20% BioNem were added. Three ml of nematode suspension containing 42 juveniles/ml on average was added. The resultant concentration of extracts in the Petri dishes became 5, 10 and 20% of rapeseed cake and 5 and 10% of BioNem. The Petri dish with 3 ml of nematode suspension 3 ml sterilized distilled water was used as control.

Effect of bionem application on root-knot nematode infestation in tomato

Pots were prepared. One month old tomato seedlings (cv. Melkashola) were transplanted to each pot containing sterilized soil with the proportion of 1: 2: 3 of sand, compost and clay, respectively. The soil near to the seedling root system was inoculated artificially with freshly hatched *M. incognita* at 1000 juveniles per 1200 cc of soil 3 days after transplanting. Eleven treatments comprising different times (at transplanting, 10 days before and 10 days after transplanting) of application of different rates (0.2, 0.4 and 0.6 g per pot) of BioNem were evaluated in randomized complete block design (RCBD) with three replications in greenhouse. There were uninoculated and inoculated controls. In

uninoculated control neither the nematode juveniles nor BioNem was applied. In case of inoculated control, the nematode juveniles were applied.

Effect of rapeseed cake and bionem on root-knot nematode and seedling growth in nursery

Rapeseed cake at the rates of 300 and 600 kg/ ha and BioNem at 75 and 150 kg/ha were evaluated in a randomized compete block design (RCBD) with five replications. Chemical treated control (Fenamiphos (Nemacur 10 G) at 3 kg/ha) and an untreated control check were included. The selected site for nursery was infested with root-knot nematode. However to create better and uniform infestation, well chopped 15 kg of severely nematode (*M. incognita*) infested roots of tomato were incorporated in to the soil in a uniform manner before raising the beds. The rapeseed cake was applied and incorporated in the soil three weeks before sowing of tomato seeds and the beds were watered as required to keep the soil moist and facilitate the decomposition of oil cake, whereas BioNem and Fenamiphos were applied at the time of sowing in furrows.

Data Analysis

Three days after treatment application, the hatched juveniles were looked and counted using stereoscopic microscope (50X) and this was done every three days interval, till hatching ceased in the control. The data on hatched juveniles were square root transformed to normalize variances by using the formula ($\sqrt{(x+0.5)}$). The transformed data were subjected to analysis of variance (ANOVA) using SAS programme (SAS Institute, 2004). The data on motile and non-motile juveniles were also recorded after 12, 24, 36 and 48 hours in percentage and subjected to ANOVA (SAS Institute, 2004).

The data on plant height, fresh shoot weight, fresh root weight, dry shoot weight, number of galls per plant, number of eggs per egg mass and final nematode population per pot were collected at 70 days after transplanting. These collected data were analyzed using SAS (version 9) (SAS Institute, 2004). Number of galls per plant, final nematode population and number of eggs per egg mass were square root transformed ($\sqrt{(x+0.5)}$) to normalize variances before analysis.

Table 1. Effects of rapeseed cake and BioNem extracts on cumulative egg hatching of M. incognita under laboratory condition

Treatments (extracts)	No. of juveniles hatched/ 5 egg masses (Mean+SE)	% inhibition of hatching over untreated control
5% rapeseed cake	9.09+0.12 (82.25)* b	76.52**
10% rapeseed cake	6.08+0.43 (37.50) cd	84.30
20% rapeseed cake	4.84+0.21 (23.25) d	87.50
5% BioNem	7.24+0.14 (52.00) c	81.30
10% BioNem	4.96+0.32 (24.75) d	87.19
Untreated control	38.72+0.82 (1502.00) a	-
CV	9.46	-
LSD	1.66	-

SE = standard error, CV=coefficient of variation, LSD=least significant difference
Means within a column followed by the same letter are not significantly different ($p<0.05$)
*Figures in parentheses are non-transformed data
** the percentage was calculated from transformed data

Prior to application of rapeseed cake initial nematode population density was determined (i.e. 573.8 juveniles/100 cc soil). Number of gall per seedling, seedling height and biomass growth were recorded from randomly taken seedlings of the inner rows in each bed. The data were analyzed according to the standard ANOVA procedures using SAS (version 9) (SAS Institute, 2004). The treatment means were separated using the least significant differences (LSD).

RESULTS AND DISCUSSION

The effect of rapeseed cake and bionem extracts on egg hatching of root-knot nematode

Rapeseed cake and BioNem extracts significantly inhibited ($p<0.05$) the cumulative hatch of *M. incognita* eggs over the untreated control (Table 1). made after three days of treatment application and hatching went on decreasing more with time period (Figure 1) whereas more hatching was observed in free water (control).

The effect of rapeseed cake and bionem extracts on motility of juveniles

The highest concentration of rapeseed cake extract (20%) affected significantly ($p<0.05$) the juvenile motility within 12 hours (first observation) (Table 2). The lower concentrations of extracts which initially appear less effective but later on their effect increased and showed as equal effect as the higher concentrations at the last observation (48 hrs) and more than 95% of juveniles were affected at all concentrations of the treatments.

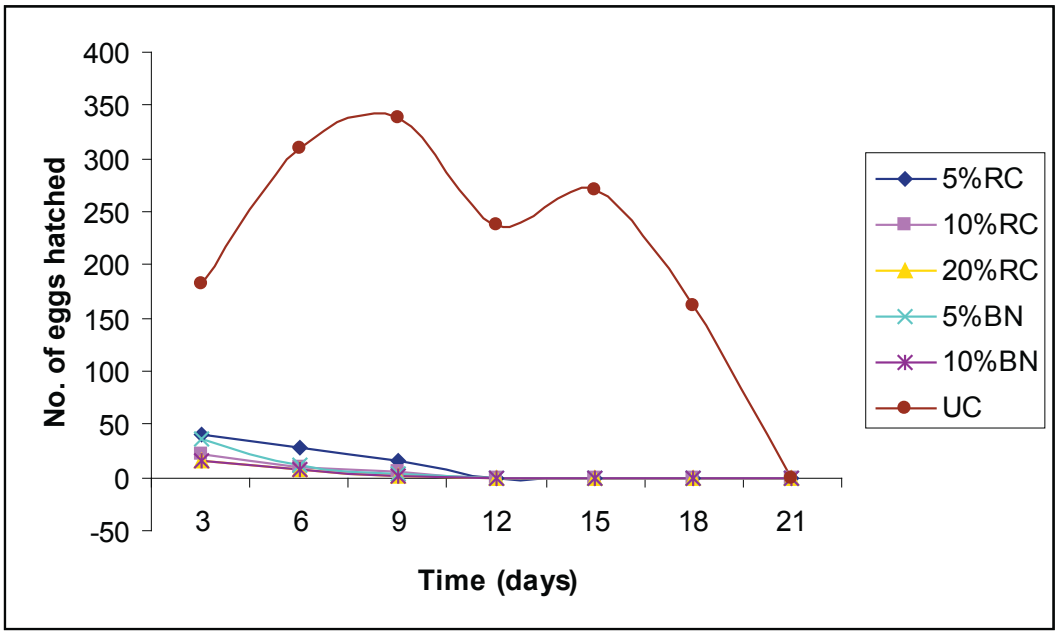

Figure 1. Effect of rapeseed cake and BioNem extracts on *M. incognita* egg hatching

The maximum inhibition percentage was observed with the extract of rapeseed cake at the highest concentration (20%). Rapeseed extract at 5% was least effective among the different treatments but it also significantly reduced cumulative egg hatch over the untreated control.

Inhibitions in hatching by rapeseed cake and BioNem extracts were observed from the first observation

The results obtained from the two experiments indicated that extracts of rapeseed cake as well as BioNem adversely affected the egg hatching and juvenile motility of *M. incognita*. Singh *et al*. (1978), reported that the oil cake extracts adversely affected hatching of root-knot nematode (*M. incognita*) in India. The inhibitory effect with rapeseed cake extracts may be due to the toxic substances released

Table 2. Effects of rapeseed cake and BioNem extracts on motility of M. incognita juveniles

Treatment (extract)	% of motile juveniles*			
	12 hours	24 hours	36 hours	48 hours
5% rapeseed cake	66.6+2.87 b	41.07+4.91 b	20.55+3.88 b	3.33+1.56 cb
10% rapeseed cake	38.52+3.60 c	18.10+2.57 c	4.68+6.72 cd	1.82+0.36 cb
20% rapeseed cake	29.14+1.86 d	4.72+0.88 d	1.21+0.53 d	0.00+0.00 c
5% BioNem	62.87+2.72 b	25.97+1.63 c	8.90+1.65 c	4.42+1.10 b
10% BioNem	37.53+1.87 c	9.29+2.09 d	1.71+0.77 d	0.56+0.36 c
Untreated control	97.77+0.73 a	93.12+1.95 a	91.17+1.99 a	89.99+1.99 a
CV (%)	8.85	16.59	18.07	13.73
LSD (α=0.05)	7.28	7.9	5.74	3.4

CV=coefficient of variation, LSD=least significant difference
Means with in the same column with a common letter are not significantly different ($p<0.05$)
*Percentage of the motile juveniles are used for analysis

during decomposition. Lazzari *et al*. (2004) reported that the hydrolysis products of glucosinolates (essentially isothiocyanates) present in Brassica oil cakes resulted in highly different biocidal activities. In the presence of myrosinase, glucosinolates are toxic to *Heterodera schachtii* (Lazzeri *et al*., 1993). Rapeseed which contains glucosinolates has been found effective in suppressing *M. Chitwoodi* and the second stage juveniles were more sensitive than egg masses (Mojtahedi *et al*., 1993).

Mendoza *et al.* (2008) proved that *B. firmus* inhibit egg hatchability of *M. incognita.* The effect of BioNem extract on egg hatching may be attributed to the dual effect of the product through direct toxicity of the plant and animal origin additives as well as the toxic substance (secondary metabolites) produced by *B. firmus*. BioNem is a formulated commercial product of 3% *B. firmus* and 97% of animal and plant origin additives (Giannakou and Prophetou-Athanasiadou, 2004). Similar result was reported by Metafer Terefe *et al*. (2009) from study on egg hatching inhibitory effect of BioNem at different concentration on *M. incognita* in Dire Dawa. Different culture filtrates of bacteria are also known to affect the motility of juveniles of root-knot nematodes. The inhibitory effect on juvenile mortality of the root-knot nematode, *M. javanica,* due to *Pseudomonas polymyxa* and *Pseudomonas* spp. was reported by Ali *et al*. (2002).

The effect of bionem application on root-knot nematode infestation in tomato

Application of BioNem 10 days before transplanting, at transplanting and 10 days after transplanting at different rates were found to adversely affect root galls, final nematode population and number of eggs per egg mass of root-knot nematode (*M. incognita*) and improve the performance of tomato plant (Table 3, 4 and 5). Significantly higher gall reduction was recorded at 0.6 g/pot (62.88%) followed by 0.4 g/pot (52.98%) when applied at the time of tomato transplanting (Table 3). In contrast, more gall formation was recorded with 0.2 g/pot when applied at 10 days after transplanting. The inoculated control had the highest number of galls.

There was the least population density with 0.6 g/pot used at the time of transplanting followed by the pot treated with 0.6 g/pot before ten days of transplanting

Table 3. Effect of time and rate of application of BioNem on M. incognita infestation in tomato plants in the pot house

BioNem/pot (g)	Galls/plant* (Mean+SE)	Reduction in galling over the inoculated control (%)	Final nematode population* (Mean+SE)	Reduction in final nematode population over inoculated control (%)	Number of eggs/egg mass* (Mean+SE)	Reduction in number of eggs/ egg mass over inoculated control (%)
0.2BT	18.74+0.36 b(351.00)**	40.14	10.68+0.19 bcd(113.67)	53.41	19.86+0.44 bc(391.83)	21.05
0.4BT	17.86+0.42 bc(318.67)	45.65	10.36+0.21 cd(107.00)	56.15	18.34+0.64 d(336.83)	32.14
0.6BT	17.19+0.28 bc(295.00)	49.69	9.37+0.22 e(87.33)	64.21	17.88+0.14 d(319.33)	35.66
0.2ST	18.66+0.56 b(348.33)	40.59	10.64+0.12 bcd(112.67)	53.82	17.92+0.69 d(321.67)	35.19
0.4ST	16.61+0.47 c(275.67)	52.98	10.24+0.32 d(104.67)	57.1	17.75+0.23 de(314.67)	36.6
0.6ST	14.75+0.61 d(217.67)	62.88	9.11+0.27 e(82.67)	66.12	16.46+0.48 e(270.83)	45.43
0.2AT	18.84+0.51 b(355.00)	39.45	11.12+0.31 b(123.33)	49.45	20.27+0.13 b(410.50)	17.29
0.4AT	18.77+0.42 b(352.33)	39.91	11.05+0.22 bc(121.67)	50.14	18.65+0.80 cd(348.50)	29.78
0.6AT	18.42+0.72 b(340.00)	42.01	10.42+0.31 bcd(108.33)	55.6	18.55+0.28 cd(343.83)	30.73
Inoculated control	24.17+1.12 a (586.33)	-	15.63+0.42 a(244.00)	-	22.29+0.25 a(496.33)	-
Uninoculated control	0.71+0.00 e(0)	-	0.71+0.00 f(0)	-	0.71+0.00 f(0)	-
CV (%)	6.04	-	4.33	-	4.5	-
LSD (α=0.05)1.73	-	0.73	-	1.31	-	

BT=before transplanting, ST=at the same time of transplanting, AT=after transplanting, SE = standard error, CV=coefficient of variation, LSD=least significant difference
Means within the same column with a common letter are not significantly different ($p<0.05$).
* The data on number of galls, final nematode population and number of eggs were square root transformed ($\sqrt{(x+0.5)}$)
**figures in parentheses are non-transformed data

among BioNem treatments. BioNem at 0.6 g/pot at the time of transplanting resulted in the least number of eggs/ egg mass compared to that of the inoculated control as well as BioNem used at different times and rates (Table 3).

BioNem at 0.6 g/pot used at the time of transplanting improved the plant height which was comparable with the nematode free control (Table 4). No significant difference in plant height was observed with time of application of the particular rate although relatively better plant height was observed when applied at the time of transplanting.

In contrast to the previous trends, the lowest fresh root weight was recorded from the nematode free control whereas the nematode inoculated control resulted in the largest fresh root weight followed by

Table 4. Effect of time and rate of application on BioNem on plant height and fresh root weight of tomato plants in *M. incognita* infested soil in the pot house

BioNem/pot (g)	Plant height (cm) (Mean±SE)	Increase in height over inoculated control (%)	Fresh root weight (Mean±SE)	Decrease in fresh root weight over inoculated control (%)
0.2BT	47.67±2.33 ^{c}	2.89	52.30±1.97 ^{bc}	13.45
0.4BT	53.67±3.48 ^{abc}	15.84	47.97±1.68 ^{cd}	20.62
0.6BT	57.00±2.52 ^{ab}	23.03	46.50±1.31 ^{cd}	23.05
0.2ST	54.67±4.06 ^{abc}	18.00	48.53±2.27 ^{cd}	19.69
0.4ST	59.33±1.45 ^{ab}	28.06	46.33±2.07 ^{cde}	23.33
0.6ST	62.00±4.16 ^{a}	33.82	43.90±2.19 ^{de}	27.35
0.2AT	47.00±2.52 ^{c}	1.45	56.73±4.57 ^{ab}	6.12
0.4AT	52.00±2.00 ^{bc}	12.24	49.00±3.19 ^{bcd}	18.91
0.6AT	54.33±2.33 ^{abc}	17.27	48.63±3.14 ^{cd}	19.53
Inoculated control	46.33±2.60 ^{c}	-	60.43±3.59 ^{a}	-
Uninoculated control	61.33±1.86 ^{a}	32.38	38.43±1.11 ^{e}	36.41
CV (%)	9.06	-	9.65	-
LSD (α=0.05)	8.35	-	8.05	-

BT = before transplanting, ST = at the same time of transplanting, AT = after transplanting, SE = standard error, CV = coefficient of variation, LSD = least significant difference and
Means with in the same column with a common letter are not significantly different ($p<0.05$)

0.2 g/pot used at ten days after transplanting because diseased root tissue were heavier than the healthy tissue. Pots with 0.6 g/pot BioNem at the time of transplanting gave relatively healthy root system and lower root weight as compared to other treatments.

The root-knot nematode infestation adversely affected the plant growth showing that the nematode infestation was the limiting factor. The highest foliar biomass was recorded with 0.6 g/pot BioNem used at the time of transplanting followed by nematode free control (Table 5). The lower dose of BioNem (0.2 g/pot) applied ten days after transplanting resulted in less increase in shoot growth over the inoculated control.

Dry shoot weight also showed similar trends to the fresh shoot weight. BioNem significantly improved ($p<0.05$) the dry shoot weight ranging from 20.97 to 27.07 g (Table 5). The nematode inoculated control resulted in the least dry shoot weight (19.47 g) while the nematode free control resulted in the highest (28.27 g) dry shoot weight.

The root-knot nematode *M. incognita* is responsible for large yield losses in several horticultural crops (Lazzeri *et al*., 2004). From this study, different times of application and rates of BioNem reduced the *M. incognita* infestation at different level. However, it was noticeable that 0.6 g/pot at transplanting was found better than others affecting galling and egg production per egg mass followed by 0.4 g/pot BioNem application at the time of transplanting. Different rates of BioNem utilization showed different extent of damage. Metasebia Terefe *et al.* (2009) also proved on their study on rate of BioNem against *M. incognita* infestation and the effect was higher with increased rate. BioNem used before and at the time of transplanting were better compared to the delayed applications. This may be happened due to the direct effect of BioNem to the inoculated juveniles before they established themselves in

Table 5. Effect of time and rate of application on BioNem on fresh and dry shoot weight of tomato plants in *M. incognita* infested soil in the pot house

BioNem/pot (g)	Fresh shoot weight (g) (Mean±SE)	Increase in fresh shoot weight over IC (%)	Dry shoot weight (g) (Mean±SE)	Increase in dry shoot weight over IC (%)
0.2BT	147.87±2.15 ^{de}	5.72	21.23±0.88 ^{cde}	9.04
0.4BT	170.00±2.47 ^{cd}	21.54	25.20±1.06 ^{abc}	29.43
0.6BT	175.40±0.92 ^{abc}	25.4	26.17±1.20 ^{ab}	34.41
0.2ST	170.23±2.64 ^{bcd}	21.71	25.27±1.66 ^{ab}	29.79
0.4ST	172.97±5.97 ^{abc}	23.66	25.57±1.90 ^{ab}	31.33
0.6ST	195.87±4.93 ^{a}	40.04	27.07±1.55 ^{ab}	39.03
0.2AT	141.17±14.39 ^{e}	0.93	20.97±1.75 ^{de}	7.7
0.4AT	147.77±3.70 ^{de}	5.65	23.77±0.74 ^{bcd}	22.09
0.6AT	159.17±7.05 ^{cde}	13.8	25.13±1.22 ^{abc}	29.07
Inoculated control (IC)	139.87±19.56 ^{e}	-	19.47±2.09 ^{e}	-
Uninoculated control	195.00±2.76 ^{ab}	39.42	28.27±0.17 ^{a}	45.2
CV (%)	8.91	-	9.71	-
LSD (α=0.05)	24.99	-	4.03	-

BT = before transplanting, ST = at the same time of transplanting, AT= after transplanting, SE = standard error, CV = coefficient of variation, LSD = least significant difference and
Means within the same column with a common letter are not significantly different ($p<0.05$)

the tomato root. Moreover, the bacterium may release nematoxic or nemastatic substances to the soil environment. Khan and Tarannum (1999) also proved that the use of *Bacillus subtilis* in root dipping and soil application showed a significant reduction of root galling and multiplication of *M. incognita* and hence improved the plant growth and yield. Siddiqui and Shaukat (2004) also showed on their study that the application of *P. aeruginosa* and *P. fluorescens* against root-knot nematode in tomato significantly lowered the root-knot development and final nematode population densities. *P. penetrans* has been identified as an important biological control agent of root-knot nematodes in suppression of nematode survival (Chen *et al*., 1997).

It is apparent from the present study that the application of BioNem before or at the time of transplanting is better for healthy plant growth in terms of fresh and dry shoot weight and fresh root weight since the infestation of the nematode was suppressed. However, there were no significant differences in plant height.

Effect of rapeseed cake and bionem on root-knot nematode and seedling growth in nursery

There were significant differences ($p<0.05$) in gall formation on seedlings among rapeseed cake (300 and 600 kg/ha), BioNem (75 and 150 kg/ha), chemical treatment (Fenamiphos at 3 kg/ha) and untreated control (Table 6). There was significantly more gall formation in the untreated control compared to all other treatments applied. Fenamiphos treated beds had the lowest number of galls followed by higher rate of BioNem (150 kg/ha) and rapeseed cake (600 kg/ha) treatments.

The heights of the tomato seedlings at the time of uprooting for transplanting were significantly different ($p<0.05$) among treatments (Table 6). The seedling height was maximum in the chemical treated control which was followed by 150 kg/ha BiNem treatment. The lowest seedling height was recorded in the untreated control, though it was not significantly different from the application of the

Table 6. Effect of soil application of rapeseed cake and BioNem on *M. incognita* infestation and growth of tomato seedlings in nursery

Treatments (kg/ha)	Galls/plant (Mean±SE)	Reduction in galling over UC (%)	Seedling height (cm) (Mean±SE)	Increase in height over UC (%)	Seedling biomass (g) (Mean±SE)	Increase in weight over UC (%)
300 rapeseed cake	3.41±0.05 ^{b}	23.54	16.33±0.64 ^{bc}	5.15	14.99±0.64 ^{bc}	12.45
600 rapeseed cake	2.73±0.14 ^{bcd}	38.79	17.60±0.76 ^{bc}	13.33	15.99±0.71 ^{ab}	19.95
75 BioNem	3.33±0.16 ^{bc}	25.34	15.94±0.52 ^{cd}	2.64	13.95±0.26 ^{cd}	4.65
150 BioNem	2.65±0.26 ^{cd}	40.58	18.24±0.73 ^{ab}	17.45	15.99±0.44 ^{ab}	19.95
Nematicide	2.54±0.39 ^{d}	43.05	19.62±0.91 ^{a}	26.34	16.72±0.46 ^{a}	25.43
Untreated control (UC)	4.46±0.27 ^{a}	-	15.53±0.75 ^{d}	-	13.33±0.27 ^{d}	-
CV (%)	17.3	-	8.86	-	7.27	-
LSD (α=0.05)	0.73	-	2.01	-	1.45	-

SE = standard error, CV=coefficient of variation, LSD=least significant difference and
Means with in the same column with a common letter are not significantly different ($p<0.05$)

lower rate of BioNem (75 kg/ha) as well as rapeseed cake (300 kg/ha). The higher rates of BioNem and rapeseed cake also ensured the largest seedling biomass next to the Fenamiphos treated control.

The damage of *M. incognita* is more sever if the seedlings are infested. Therefore to minimize yield losses it is necessary to manage the nursery to have relatively healthy transplants for the main field. The results obtained from the present study showed that BioNem at 150 kg/ha and rapeseed cake at 600 kg/ha applications reduced the number of galls formed and improved the seedling growth. Comparatively the soil treated with different rates of oil cake significantly reduced root-knot development and nematode multiplication, hence, increased the plant growth and yield (Pandey, 1994). Rapeseed cake has been used as a soil amendment to control nematodes (Brown and Morra, 2005). Its incorporation in to soil before planting served as effective nematicide against *Meloidogyne* spp. (Ameen and Bondox, 2006). The adverse effect of mustard oil cake on root-knot nematode has also been reported by Goswami *et al*. (2006).

The improvement in plant growth through the incorporation of oil cake may be attributed to the addition of nutrients to the soil resulting from the decomposition and the decomposed material may have direct killing or inhibiting effect on nematodes. Moreover, it may also create conducive environment for the naturally existing microorganisms which parasitize nematodes (Oka *et al*., 2007). The ability of *P. lilacinus* to control the nematodes increased when it was integrated with the organic matter (Ahmad and Khan, 2004). The decomposition of organic matter released nematicidal principle and the residual organic matter increased fungal activity and persistence (Khan and Saxena, 1997).

The present study proved that the application of BioNem at 75 and 150 kg/ha adversely affects the root-knot nematode (*M. incognita*) infestation. This effect might be due to direct toxicity of the product to the juveniles. Metasebia Terefe *et al*, (2009) reported that the application of BioNem at different dosage gave better and healthy plant growth and reduced the gall formation over the uninoculated control.

The effectiveness of Bionematicidal formulation (*B. firmus*) affecting hatching juvenile mortality was reported by (Giannakou *et al*., 2007).

CONCLUSION

The aqueous extracts of rapeseed cake (20% concentration) and BioNem (10%) preferably inhibited the egg hatching as well as juvenile motility of *M. incognita*. This highest concentration of rapeseed extracts was the best, although other treatments also significantly inhibited both the egg hatching and juvenile motility.

BioNem at all rates per pot was effective in reducing the nematode infestation and improvement of the tomato plant growth in the pot house. Of all the treatments, the higher rate (0.6 g/pot) applied at the time of transplanting showed significantly better suppression in gall formation, final nematode population density and number of eggs per egg mass of *M. incognita* and also improved plant height, fresh and dry shoot weight and it was followed by the application done ten days before transplanting.

The application of 150 kg/ha BioNem and 600 kg/ha rapeseed cake gave results comparable with the Fenamiphos in reducing gall formation, improving seedling height and weight in the nursery trial. Management in the nursery is very important since the pest is aggressive in the early stages of the crop. Treating the main field on which the seedlings are transplanted ensures more protection against *M. incognita* as far as infection occurs in the main field also. Therefore, combination of treatments in the nursery and main field can be applied to fields suffering from root-knot nematode infestation.

Generally, the application of higher dosage of rapeseed cake and BioNem has given better results on the suppression of nematode infestation and improvements in plant performance. The time of incorporation of rapeseed cake should allow better decomposition of the cake since the availability of more decomposed substance give better control and avoids chances of phytotoxicity.

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