Biocontrol (Formulation of *Bacillus firmus* (BioNem)) of Root-knot Nematode, *Meloidogyne incognita* on Tomato Plants in the Field

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Abstract

Bacillus firmus, *commercial WP formulation (BioNem) was evaluated against root-knot nematode, Meloidogne incognita on tomato plants under field conditions of Dire Dawa and Haramaya University (Ethiopia). Studies were conducted with the objectives to find out the effect of BioNem on nematode infestation and plant growth/yield of tomato and to compare two methods of application of BioNem for the control of root-knot under field conditions. Soil application of BioNem at transplanting in the field @ 200 and 400 kg/ha provided control of root-knot nematode and increased crop growth and yield over untreated control significantly. Dip treatment of tomato nursery in 2% and 4% BioNem suspension for 2 hrs just before transplanting also reduced root-knot nematode infestation and improved crop growth and yield, though it was less effective than soil application of BioNem. In general, our result indicated, BioNem seems to be effective for the management of root-knot nematode and has the potential to be a vital component of integrated management for root-knot nematode.*

Key words: *Bacillus firmus, Biological control, Meloidogyne incognita, Tomato*

Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crops grown in the world, also one of the most important vegetable crops grown in Ethiopia. Despite its importance, the overall fruit yields of tomato in Ethiopia are often very low compared to the yields in many tomato producing countries of Africa and the world average. Production of tomato is influenced by various abiotic and a biotic factors. In Ethiopia about 13 diseases caused by different fungal, bacterial pathogens and viruses and 6 economically important insect and mite pests attacking tomato have been identified (Tsedeke, 1986). Earlier studies indicated that the major and economically important tomato diseases include early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), septora leaf spot (*Septoria lycopersici*) and viruses. Occurrence of root-knot nematode *Meloidogyne incognita* on tomato in Ethiopia was reported for the first time by Stewart and Dagnatchew (1967). In the course of time, some diseases, which were considered of minor importance, have become more important now. These diseases include root-knot nematode (*Meloidogyne* spp), powdery mildew (*Leveilla taurica*) and bacterial wilt (*Ralstonia solanacerum*). Root-knot nematode attacks tomato worldwide and can cause losses ranging from 28 to 61% in yield (Nagnathan, 1984).

Three species of root-knot nematode viz., *M. incognita, M. javanica* and *M. ethiopica* have been reported to occur in Ethiopia, but *M. incognita* is most widespread (Wondirad and Kifle, 2000). In eastern Ethiopia, widespread occurrence of *M. incognita* on tomato was reported by Tadele and Mengistu (2000). In central and western Ethiopia also, root-knot nematode is the major problem in tomato cultivation (Wondirad and Tesfamariam, 2002). In spite of wide distribution of root-knot nematode on many crops in Ethiopia, little work has been done on its management.

A number of methods for the management of root-knot nematode like chemical control, organic amendments, use of resistant varieties, soil solarization, and biological control have been tried with different levels of successes in tomato (Randhawa *et al.*, 2001). Chemical management is effective, but expensive and may lead to residue and soil pollution problems. Tomato varieties resistant to root-knot nematode have been developed in some countries, but are not very popular due to their lower yields. No such varieties are available in Ethiopia. Biological control is self perpetuating and usually free from residual and adverse environmental effects (Sumeet and Mukerji, 2000). A few bacteria like *Pasteuria penetrans* directly parasitize nematodes, while many bacteria like species of *Bacillus, Agrobacterium, Azotobacter, Pseudomonas* and *Clostridium* produce toxins that kill nematodes (Walia *et al*., 2000).

BioNem WP has been developed as a commercial bionematicide by Agro-green Minrav group of Israel. It contains the bacteria *Bacillus firmus* and has been reported effective against, *M. incognita, M. hapla, Heterodera* spp*., Tylenchulus semipenetrans, Xiphinema index* and *Ditylenchus dipsaci* (Keren-Zur *et al*., 2000). BioNem is currently introduced to Ethiopia to be used against root knot nematode in vegetables. In order to utilize for practical pest control registration as a micriobial pesticide is required. Therefore, these studies were conducted to test the efficacy of BioNem WP against root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, in tomato under Ethiopian field conditions with the objectives of to assess the effect of different levels of biocontrol formulation, BioNem on root-knot nematode infestation and plant growth / yield of tomato and to compare methods of application of biocontrol formulation, BioNem for the control of root-knot nematode in tomato.

Materials and Methods

Description of the experimental site

Field experiments were conducted at experimental field station of Haramaya University (HU) and Dire-Dawa (Tony Farm). HU is located at 9°26'N, 42°3'E, 1980 meter above sea level, Eastern Hararghe, Oromiya, Ethiopia. The site has a bimodal rain fall distribution pattern and is representative of a sub-humid, mid-altitude agro-climatic zone. The average annual rainfall is 760 mm and the mean annual temperature is 17 °C with a range of 14 °C to 18 °C. The Tony Farm is located at an altitude of 1197 meter above sea level and lies at 9°6 'N and 41°8'E longitude in the eastern part of Ethiopia. The station lies in the semi- arid belt of the eastern rift valley escarpment with a long-term average rain fall of 612 mm. The mean annual rainfall is 520 mm and mean maximum and minimum temperature range from 28.1 °C to 34.6 °C and 14.5 °C to 21.6 °C, respectively (Belay, 2002).

Determination of root-knot nematode population in soil samples

Soil sample collection

The soil samples were randomly taken from spots in a field with a history of root knot nematode at Haramaya University and Dire-Dawa (Tony Farm) in a "X" fashion with the help of auger at a depth of 10-15 cm and the samples were placed in polythene bags. The samples were brought to the Plant Pathology Laboratory for analysis.

Isolation and enumeration of nematodes from soil

For determination of initial and final population of root-knot nematode for both locations Baermann funnel technique (Southy, 1970) was used. A glass funnel was taken and a rubber tube was fixed to its stem then the tube was closed with a pinch cock to make it airtight and placed the funnel in a funnel stand. Water was filled in the funnel and run out a little through the rubber tube to remove air bubbles. One hundred cc of soil was taken in a 250 ml beaker, covered with muslin cloth and tied with a rubber band. The beaker containing the soil was kept gently (upside down) in the funnel containing water. After waiting for 1-2 hours, more water was added to the funnel so that it touched the lower level of muslin on the beaker. The assembly was kept undisturbed for 24 hours. The clip was then loosened slightly and about 10 ml of nematode suspension was collected in a beaker. The suspension containing the nematode juveniles were examined using a stereomicroscope at magnification of 50 X using a counting dish.

Effect of BioNem on root - knot nematode (*Meloidogyne incognita)* infestation and plant growth/yield of tomato

Experiment was conducted in a nematode sick field at Haramaya University and Dire Dawa, Tony Farm (Ethiopia) to evaluate the effectiveness of the BioNem against root knot nematode in tomato under field conditions in 2008/2009 cropping season. The plot size was 7.5 m^2 (3 m x 2.5m). Row to row and plant to plant spacing was 0.75 m and 0.5 m, respectively. Seedlings of tomato, variety Marglobe, were raised at Haramaya University and Tony Farm in nematode free nursery site. Forty five days old seedlings were transplanted to both locations with a size 7.5 m^2 (3 m x 2.5 m). Watering and hand weeding were done as required.

Experimental design and treatments

There were a total of 10 treatments and the experiment was laid down as a randomized complete block design (RCBD) with 4 replications. BioNem was applied by two methods namely soil application and seedling dip treatment. Soil application was done @ 50, 100, 200 and 400 kg/ha. Seedling dip treatment was done in 1, 2, 4, and 8 % aqueous suspension of BioNem for 2 hrs. Phenamiphos (Nemacur 10 G) was applied @ 11kg/ha as treated control. Untreated plots served as control. In the case of aqueous suspension, tomato seedlings were dipped into each of the suspension for 2 hours. However, in the powder formulation, the respective dosage of BioNem was applied to soil at transplanting. In addition, Nemacure 10% dust was used as a treated control; while untreated plots were used as untreated control.

Field data collection

Prior to establishment of the treatments initial and final nematode population density at harvest period from each plot were determined using the method of Baermann funnel technique (Southy, 1970). The plant height was measured by selecting five randomly plants per plot from each plot as procedure developed by AVRDC (2001). The main stem length from ground level to the tip of the longest stem of a plant was measured. Number of fruits / plant was calculated by counting total number of fruits in individual plant at harvest. Whilst, average fruit weight/ha and marketable fruit yield / ha were conducted from total weight of fruit harvested/plot and the weight of the cleaned and un damaged fruits after sorting from the total fruit harvested, respectively. For determining root-knot index destructive sampling was used, samples of 10 plants/plot were selected and the root-knot index was assessed using from 1-5 scale, where: 1= no-galls, 2= 1-25 %, 3= 26-50 %, 4=51-75%, 5= > 75 %.

Data analysis

The data on the plant height, average fruit weight, marketable fruit yield, root-knot index, were not transformed and directly analyzed according to the standard analysis of variance (ANOVA) procedures using Statistical Analysis System (SAS); whilst, number of fruits per plant and final nematode population were transformed to square root transformation to normalize variances by using the formula $(x + 0.5)^{1/2}$ before analysis where as x is the number of fruits per plant and final nematode population and 0.5 is constant number to be added as described by Gomez and Gomez (1984). The transformed data were subjected to ANOVA procedures using SAS (version.6.12, SAS. Institute Inc., Cary, NC, USA). The treatment means were separated using the least significant differences (LSD). Back transformed values were presented.

Results and Discussion

Effect of BioNem on root-knot nematode (*Meloidogyne incognita*) infestation and plant growth/yield of tomato

Effect of BioNem against root-knot nematode infestation and plant growth/yield of tomato was studied under field conditions of Dire Dawa (Tony Farm) and HU by applying as band treatment at transplanting and as seedling dip treatment just before transplanting. In the present field studies, BioNem application by both methods provided significant nematode control and improved plant growth and yield significantly over untreated control.

Root knot index

Root-knot nematode is manifested by the formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigor of the plant (Babu *et al.,* 1999). There were significant differences ($P < 0.05$) between different soil applications rates of BioNem in affecting the formation of root galls on tomato over untreated control (Table 1 and 2). At Dire Dawa, the soil application of BioNem at the rates 200 and 400 kg/ha proved the most effective in reducing galling as reflected by root-knot index. The reduction by the two dosages was at par and was also at par with treated control (phanamiphos) and reduction of root knot nematode ranged from 30 to 53.5 % (Table-1). However, at HU BioNem applied at the rates of 400 kg/ha had similar effect as comparison to treated control (phanamiphos) and reduction of root knot nematode ranged from 30 to 52 % (Table 2). The lower dosages of 50 and 100 kg/ha were also significantly effective over untreated control and at par with each other for the study sites. *Bacillus* has ecological advantage because it forms endospores, which are tolerant to adversely high temperature and moisture stress and remain viable for long period of time. *Bacillus* species produce different types of antibiotics and enzymes, which are strongly antagonistic to several pathogens (Saxena *et al.,* 2000).

Seedling dip treatment of nursery plants also proved significantly effective in reducing root-knot index over untreated control and maximum protection by dip treatments were provided by 4 and 8% concentration and were at par with soil application of 50 and 100 kg/ha and all rates of seedling dip treatment (Table 1 and 2). Soil application of BioNem at 200 and 400 kg/ha provided significant reduction in root-knot index over untreated control. Both of these dosages managed root-knot nematode at par and were better over lower dosages of 50 kg and 100 kg/ha. They also gave comparable results with the treated control (phenamiphos 11 kg/ha). Metasebia *et al.* (2008) also reported in nursery, application of BioNem at the rate of 200 kg and 400 kg decreased the formation of galls and increased plant height and biomass. Dipping of tomato nursery in 2% and 4 % BioNem suspension for 2 hrs under shade provided significant protection from root-knot nematode, though it was lesser effective than soil application. Production of *Bacillus* spp has been successful at mass scale and is routine, economical and ionic surfactants are mixed in formulations so that product easily mixes with water. BioNem WP has been developed as a biological nematicide, based on a unique strain of *Bacillus firmus*. It can be conveniently applied in field through irrigation systems. BioNem WP is effective against important phytopathogenic nematodes, and the product is registered and commercially used in Israel for the control of root-knot nematodes in vegetable crops such as tomatoes, cucumber, pepper, eggplant and herbs and in perennial crops such as peaches, olives, ornamentals (Keren *et al.,* 2000).

Final population of the nematode

For both study sites, BioNem soil and root dip application and phenamiphos significantly reduced the nematode population over untreated control at termination of experiment (P < 0.05) (Table 1 and 2). Metasebia *et al.* (2008) reported that application of BioNem at the rate of 2.5 % concentration caused 100% inhibition of egg hatching of *M. incognita* and BioNem at 2.5 % and 3% concentrations caused 100 % inhibition in motility of juveniles 24 h after treatment application and none of the juveniles recovered from the paralysis caused by BioNem at 2.5 % and 3 % concentrations, 24 and 48 h after treatment with water. At Dire Dawa site, maximum reduction (80%) was observed with phenamiphos (treated control), followed by soil application of BioNem @ 200 and 400 kg/ha (76 and 79%, respectively) also they were at par in affecting reduction of nematode population. No significant differences (P < 0.05) were observed in the soil application of BioNem treatments, applied at the rates 50 and 100 kg/ ha and both were significantly better over control, but lesser effective than the two higher dosages (Table 1). Whilst, there were significant differences (P<0.05) among the soil application of BioNem treatments, applied at the rates 50, 100, 200 and 400 kg/ ha in the study site of HU. Maximum reduction (78 %) of nematode population was recorded from soil application of BioNem applied at the rate of 400 kg/ha, followed by phenamiphos (treated control) (76 %) (Table 2). The study site of HU, the two treatments i.e. 50 and 100 kg/ha proved at par in reducing nematode population.

At both sites, root dip treatment at all the four concentrations reduced the final nematode population over untreated control significantly (Table 1 and 2). Maximum reduction (46 and 42.2%) was occurred at 8 and 4% respectively, and both concentrations were at par. On the other hand, least reduction (29 and 34 %) was recorded from BioNem applied at the rate of 1 and 2 % respectively and both concentrations gave similar effect on reduction of final nematode population (Table 1).

However, significant differences (P<0.05) among root dip treatment was observed in reducing final nematode population. Maximum reduction (45%) was recorded at 8%, and the least reduction (25%) from BioNem applied at the rate of 1 % respectively and one per cent concentration proved significantly less effective than 2%, which was less effective than 4 and 8% (P < 0.05) (Table 2). Soil application of BioNem at 200 and 400 kg/ha provided significant reduction in final nematode population over untreated control. Saxena *et al*. (2000) reported the toxin producing rhizobacteria hold a good promise in reducing the damage caused by phytonematodes. These bacteria are easy to culture and can be produced in large quantity. Rhizobacteria genera like *Bacillus, Pseudomonas, Arthrobacter, Alcaligenus, Azotobacter, Escherichia coli, Burleholderia* often colonize roots aggressively and have shown potential for biological control of plant pathogens both under *in vitro* and *in vivo* conditions *Bacillus* species like *B. subtilus* have been reported to be antagonistic against a number of pathogens. In recent years, a potentially antagonistic effect of the symbiotic complex on plant-parasitic nematodes has been reported by Lewis *et al.* (2001). Further investigation demonstrated that the symbiotic bacteria seemed to be responsible for the plant-parasitic nematode suppression via the production of defensive compounds. Three types of secondary metabolites have been identified as the nematicidal agents: ammonia, indole and stil bene derivative. They were found toxic to second-stage juveniles of root-knot nematode and to fourth-stage juveniles and inhibited egg hatching of *M. incognita* (Hu *et al*., 1999).

Table 1: Effect of soil application and seedling dip treatment with BioNem on infestation of root-knot nematode in tomato plants cv. Marglobe under field conditions of Dire Dawa in 2008/2009 cropping season

Treatment	Root-knot index	Reduction in RKI over UC (%)	Final nematode population /100 cc soil	Reduction in final population over UC (%)
BioNem WP Soil application				
50 kg/ha	3.0±0.2bc	30.0	467(22)* ±0.8** d	60.0
100 kg/ha	2.8±0.04c	35.0	433(21) ±0.3d	63.0
200 kg/ha	2.2±0.14ed	49.0	283(17) ± 0.4e	76.0
400 kg/ha	2.0±0.16e	53.5.0	250(16) ± 1.7e	79.0
Seedling dip treatment				
1%	3.4±0.2b	21.0	833(29) ±0.26b	29.0
2%	3.2±0.2bc	26.0	767(28) ±1b	34.0
4%	3.0±0.26bc	30.0	667(26) ±0.4c	42.5
8%	2.7±0.18cd	37.0	633(25) ±0.2c	46.0
TC	1.9±0.3e	56.0	233(15) ±0.3e	80.0
UC	4.3±0.18a	-	1167(34) ± 0.3a	-
LSD	**0.56**	**-**	**89.7 (2.24)**	**-**
CV	**13.6**	**-**	**10.79 (6.67)**	**-**

TC= treated control (phenamiphos @ 11 kg a i /ha) and UC= Untreated control

Initial population nematodes were 633 juveniles /100 cc soil

*Figures in parenthesis are the transformed mean values (square root transformation were used with the formula $(x + 0.5)^{1/2}$ and ** Standard error of mean (SEm±). Means within the same column with a common letter are not significantly different (P< 0.05).

CV =Coefficient of variance and LSD=Least significance difference

Table 2: Effect of soil application and seedling dip treatment with BioNem on infestation of root-knot nematode in tomato plants cv. Marglobe under field conditions of Haramaya University in 2008/2009 cropping season

Treatment	Root-knot index	Reduction in RKI over UC (%)	Final nematode population /100 cc soil	Reduction in final population over UC (%)
BioNem WP Soil application				
50 kg/ha	3.2 ±0.2 bcd	30	487 (22) * ± 0.8**f	61
100 kg/ha	3± 0.04cd	35	460 (21) ± 0.3g	63
200 kg/ha	2.5± 0.14ef	46	312 (18) ± 0.4h	75
400 kg/ha	2.2±0.16fg	52	275 (17) ± 1.7j	78
Seedling dip treatment				
1%	3.6± 0.2b	22	925 (30) ±0.26b	25
2%	3.3± 0.22bc	28	798 (28) ±1c	36
4%	3±0.26 cd	35	695 (26) ±0.4d	44
8%	2.9±0.18 de	37	680 (26) ±0.2e	45
TC	2±0.31 g	57	298 (17) ±0.3 i	76
UC	4.6± 0.18a	-	1237 (35) ± 0.3a	-
LSD	**0.4**	**-**	**8.3 (0.24)**	**-**
CV	**9.3**	**-**	**0.9 (0.7)**	

TC= treated control (phenamiphos @ 11 kg a i /ha) and UC= Untreated control , Initial population nematodes were 671 juveniles /100 cc soil

*Figures in parenthesis are the transformed mean values (square root transformation were used with the formula $(x + 0.5)^{1/2}$ and ** Standard error of mean (SEm±) . Means within the same column with a common letter are not significantly different (P< 0.05). CV =Coefficient of variance and LSD=Least significance difference

Plant height

In the present studies increase in plant height was observed as the dosage of BioNem soil application increased. Root-knot nematode is well known to reduce the plant height (Olabiyi, 2008). Melakeberhan and Webster (1993) stated that root-knot nematodes affect plant growth due to their consumption of host assimilates, through interfering with water and nutrient uptake by deforming roots thus diminishing and blocking water and nutrients absorption and translocation; nematode infection may also negatively influence the production of phytohormones. Significant differences were observed (P < 0.05) between soil application and root dip treatments of the BioNem applied at different rates in affecting plant height and yield, as compared to treated and untreated control in both study sites (Table 3 and 4). In Dire Dawa study site there were no significant differences (P<0.05) between soil application applied at 200 and 400 kg/ha and had similar result with treated control (phenamiphos). Maximum increase (26 %) of plant height was recorded from BioNem applied at the rate of 400 kg/ha and the least (13.5 %) was recorded from at the rate of 200 kg/ha besides, from root dip treatments maximum increase (8%) was observed from 2 % and the least (2 %) from 8 % concentration. Parallel with these, Metasebia *et al*. (2008) in his green house study, BioNem applied at 16 g/pot was phytotoxic and resulted in seedling mortality. The phytotoxic symptoms may be developed due to the additives of the product.This product contains 3 % lyophilized *Bacillus firmus* spores and 97 % additives (plant and animal extracts) (Giannakou and Prophetou, 2004). There were no significant differences (P < 0.05) between the rates of BioNem applied as root dip treatment at the rate of 1, 2, 4 and 8 % concentration. However, BioNem applied at rate of 4 and 8 % gave the same result with untreated control. BioNem applied at the rate of 1 and 2 % had similar result with soil application applied at the rate of 50 kg/ha in affecting plant height (Table 3).

On the other hand, on study site of HU, there were no significant differences (P<0.05) between soil application of BioNem applied at the rate of 50 and 100 kg/ha also no significant differences between 200 and 400 kg/ha. Although, the rate of 400 kg/ha had similar effect with treated control (phenamiphos) in affecting plant height. In soil application maximum increase (27 %) of plant height was observed from BioNem applied at the rate of 400 kg/ha and the least (13 %) was recorded from at the rate of 50 kg/ha as well, from root dip treatments maximum increase (9 %) was observed from 2% and the least (0 %) from 8% concentration and in root dip treatment at the rate of 1, 2 and 4 % gave similar result on plant height and BioNem applied at the rate of 4 and 8 % were not significantly different (P < 0.05) from each other and from untreated control in increasing plant height (Table 4).

In Dire Dawa, the highest plant height (62 cm) was recorded from treated control and the least (48 cm) from the untreated control. In soil application treatments of the BioNem treatments ranged from 54.5- 60.5 cm, while, in seedling dip treatments in BioNem it ranged from 49-51.7 cm (Table 3). On the other hand HU, the highest plant height (60 cm) was obtained from treated control and the least from the untreated control (46 cm). Plant height in soil application treatments of the BioNem ranged from 52 – 58.5 cm, whereas; in seedling dip treatments in BioNem ranged from 46-50 cm (Table 4).

Number of fruits per plant

There were significant differences (P < 0.05) between soil application and root dip treatment of the BioNem rates in affecting, number of fruits as compared to treated and untreated control, higher number of fruits was formed on tomato plants in plots treated with BioNem in the study site of Dire Dawa and HU (Table 3 and 4). In Dire Dawa, no significant differences (P < 0.05) between soil applications applied at the rate of 50, 100, 200 and 400 kg/ha in forming number of fruits. Highest number of fruits was recorded with phenamiphos (30 fruit / plant), followed by BioNem @ 100, 200 and 400 kg/ha (25 each) in comparison to 18 fruits/plant in untreated control. Williamson and Hussey (1996) reported that nutrient and water uptake is substantially reduced because of the damaged root system attacked by root-knot nematode, resulting in weak and poor-yielding plants. However significant differences were observed between root dip applications over untreated control in affecting fruits and no significant differences (P < 0.05) between rates of 2 and 8% concentration. On the other hand, BioNem applied at the rate of 2 and 4 % gave similar outcome as compared with the four different dosages of soil applications (Table 3).

In HU, significant differenceswere not observed (P < 0.05) of soil application with respect to number of fruits formed in comparison to untreated control. Nevertheless, soil application applied at the rate of 400kg/ha (25 fruit/plant) at par with treated control phenamiphos (27.5 fruit / plant). There were no significant differences (P < 0.05) between root dip application at the rate of 1, 2 and 8 %. One percent concentration (19 fruit/plant) had similar effect in forming fruit as compared with untreated control (16.5 fruit/plant) applications (Table 3). Soil application of BioNem at 200 and 400 kg/ha provided significant increased number of fruits, over untreated control.

Tomato yield

As compared to treated and untreated control both study sites, there were significant differences (P < 0.05) between soil application and root dip treatments of the BioNem applied at different rates in forming yield (Table 3 and 4). Giannakou and Prophetou-Athanasiadou (2004) found in their extensive testing on vegetable crops such as tomato, that BioNem preplant applications significantly reduced nematode populations and root infestation (galling index), resulting in an overall increase in yield. In Dire Dawa soil application of the BioNem at different rates increased the tomato yield significantly (P < 0.05) over untreated control. Among the BioNem treatments the highest increase in yield (77 %) over untreated control, occurred with 200 and 400 kg/ha dosages. Dosages of 100, 200, and 400 kg/ha were found to be at par in increasing the yield and significantly lesser effective than the treated control (phenamiphos @11 kg/ha.). Root dip treatment of seedlings in different concentrations also increased the yield by 7-30 % over untreated control, but the rate of 8 % was statistically not significant with the untreated control. Highest concentration of 8% resulted in lesser yield as compared to 1, 2 and 4 % concentration (Table 3).

In HU, there were no significant differences (P < 0.05) between soil application of 200 and 400 kg/ha in affecting yield of tomato. Maximum increase in yield (100 and 104 %) over untreated control occurred with 200 and 400 kg/ha dosages respectively and the yield ranged from 41-50 t/ha. In root dip treatments BioNem applied at the rate of 2 and 4% concentration statistically gave the same result but improves the yield over untreated control. Maximum increase (120 %) of yield was obtained from treated control and in root dip treatment of seedlings the yield ranged from 28-30 t/ha (Table 4).

Soil application of BioNem at 200 and 400 kg/ha provided significant increased plant height, number of fruits, total yield and marketable yield over untreated control. Both of these dosages managed root-knot nematode at par and were better over lower dosages of 50 and 100 kg/ha. They also gave comparable results with the treated control (phenamiphos 11 kg/ha).

Marketable fruit yield

Soil and root dip application of BioNem increased the marketable yield over untreated control significantly (P < 0.05) in Dire Dawa and HU sites (Table 3 and 4). The growth and nutritive value of tomato is reduced by many pests and diseases and influence ultimately leading to poor yield production. *Melodogyne incognita* is economically important as they affect the crop quantitatively and qualitatively. The nematode has got a wide range of host plants and cause economic damage to many agricultural crops (Sasser *et al.,* 1989). Maximum marketable yield (50 t/ha) was recorded from the treated control (phenamiphos). The three highest dosages of BioNem viz., 100, 200 and 400 kg/ha gave statistically the same result in marketable fruit yields. There were no significant differences ($P < 0.05$) among root dip treatments rates of BioNem at 1, 2 and 4 % in affecting marketable fruit yield (Table 3).

In HU, there were no significant differences ($P < 0.05$) between root dip treatments applied at different rates for improving marketable fruit yield. Maximum marketable yield (49 t/ha) were recorded from treated control. Though, no significant differences ($P < 0.05$) were observed between 200 and 400 kg/ha, they perform better as compared with untreated control in increasing marketable yield (Table 4). Soil application of BioNem at 200 and 400 kg/ha provided significant marketable yield over untreated control.

Table 3. Effect of soil application and seedling dip treatment with BioNem on growth and yield of tomato in root-knot nematode infested field in Dire Dawa (Tony farm) in 2008/2009 cropping season

Treatment	Plant height (cm)	Increase in height over UC (%)	Number of fruits/ plant	Increase in number of fruit over UC (%)	Yield (t/ha)	Increase in yield over UC (%)	Market. yield (t/ha)	Increase in market. yield over UC (%)
BioNem soil application								
50kg/ha	54.5±0.65bc	13.5	24(4.9)*±0.13**b	33	45±1c	50	40±1c	60
100kg/ha	55.0±0.9b	15.0	25(5)±0.09b	39	51±0.4b	70	45±0.7b	80
200kg/ha	60.0±0.9a	25.0	25(5)±0.08b	39	53±1b	77	46±1.9b	84
400kg/ha	60.5±0.6a	26.0	25(5)±0.03b	39	53±1b	77	46±2b	84
BioNem Seedling dip treatment								
1%	51.4±1.4cd	7.0	21(4.6)±0.09c	17	35.8±1d	19	35±1.8d	40
2%	51.7±0.6cd	8.0	23(4.8)±0.09bc	28	38±1.9d	27	36±1.5d	44
4%	50.0±1.8de	4.0	24(4.9)±0.08b	33	39±0.9d	30	37±0.7cd	48
8%	49.0±0.9de	2.0	21(4.60±0.07c	17	32±0.8e	7	31±0.9e	23
TC	62.0±0.9a	29.0	30(5.5)±0.07a	67	57±1.8a	90	50±1a	100
UC	48.0±1.3e	-	18(4.3)±0.04d	-	30±0.7e	-	25±1.6f	-
LSD	**3.2**	**-**	**2.5 (0.24)**	**-**	**3.3**	**-**	**3.8**	**-**
CV	**4**	**-**	**7.4(3.5)**	**-**	**5.3**	**-**	**6.7**	**-**

TC= Treated control (phenamiphos @ 11 a.i. kg/ha) UC= untreated control, ** Standard error of mean (SEm±)
Initial population nematodes were 633 juveniles /100 cc soil , CV =Coefficient of variance and LSD=Least significance difference
*Figures in parenthesis are the transformed mean values (square root transformation were used with the formula $(x + 0.5)^{1/2}$
Means within the same column with a common letter are not significantly different (P< 0.05).

Table 4: Effect of soil application and seedling dip treatment with BioNem on growth and yield of tomato in root-knot nematode infested field in Haramaya University in 2008/2009 cropping season

Treatment	Plant height (cm)	Increase in height over UC (%)	Number of fruits/ plant	Increase in number of fruit over UC (%)	Yield (t/ha)	Increase in yield over UC (%)	Market. yield (t/ha)	Increase in market. yield over UC (%)
BioNem soil application								
50kg/ha	52±0.65cd	13.0	23(4.8)± 0.13**bc	39.0	41±1d	67.0	36±1c	100
100kg/ha	53.5±0.9c	16.0	24 (4.9)±0.09 b	45.5	45±0.4c	84.0	38±0.7c	111
200kg/ha	57±0.9b	24.0	24.5 (5)± 0.09b	48.5	49±1b	100.0	41±1.9b	128
400kg/ha	58.5±0.6ab	27.0	25(5)±0.025ab	51.5	50±1b	104.0	41.5±2b	131
BioNem Seedling dip treatment								
1%	49±1.4e	6.5.0	19(4.4)±0.09ef	15.0	30±1fg	22.0	29±1.8ed	61
2%	50±0.62de	9.0	21(4.6)±0.09cde	27.0	32±1.9ef	37.0	29.5±1.5d	64
4%	47.5±1.8ef	3.0	22.5(4.8)±0.075bcd	36.0	33±0.9e	35.0	31±0.7d	72
8%	46±0.9f	0.0	20(4.5)±0.07de	21.0	28±0.8g	14.0	27±0.9e	50
TC	60±0.9a	30.0	27.5(5.2)±0.07a	67.0	54±1.8a	120.0	49 ±1a	172
UC	46±1.3f	-	16.5(4)±0.04f	-	24.5±0.7h	-	18±1.6f	-
LSD	**2.8**	**-**	**2.76(0.3)**	**-**	**2.3**	**-**	**2.3**	**-**
CV	**3.7**	**-**	**8.5 (4.3)**	**-**	**4.1**	**-**	**4.6**	**-**

TC= Treated control (phenamiphos @ 11 a.i. kg/ha) UC= untreated control, Initial population nematodes were 671 juveniles /100 cc soil CV =Coefficient of variance and LSD=Least significance difference, Means within the same column with a common letter are not significantly different (P< 0.05), ** Standard error of mean (SEm±)

In general, significant potential of the bionematicide BioNem against root-knot nematode in tomato. The study sites of Dire Dawa and HU indicated that soil application of BioNem proved significantly better over root dip treatment in reducing root-knot nematode and in increasing plant growth and yield of tomato over untreated control. Therefore, out of different dosages of BioNem evaluated the dosage of 200 kg/ha appeared to be the optimum dosage for nematode control and the crop yield. Seedling dip treatment at 2 % concentration also provided significant nematode control and increased marketable yield over untreated control. In conclusion, BioNem can be applied @ 200 kg/ha at transplanting for protection from nematode. Tomato seedlings can also be dipped in 2% BioNem aqueous suspension for two hrs before transplanting. BioNem seems to be effective for the management of root-knot nematode and has the potential to be a vital component of integrated management for root-knot nematode.

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