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EFFECT OF NURSERY BED SOIL DRENCHING WITH EMAMECTIN BENZOATE 1.9% WP, A NOVEL AVERMECTIN DERIVATIVE, TOWARDS ECO-FRIENDLY MANAGEMENT OF ROOT-KNOT NEMATODES IN CHILLI

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ABSTRACT
Three field experiments were carried out with the aim of detecting the efficacy of emamectin benzoate 1.9%WP as nematicide against root-knot nematodes and it shows that chilli seedlings treated at 2-3 leave stages in nursery bed with emamectin benzoate 1.9%WP @ 285.0g a.i./ha recorded highest reduction of *Meloidogyne* juveniles (J_2) population (in soil) and female(f) nematode population(in per g of root) at every time of observation *i.e.* 35 days after sowing (DAS) [140.4(J_2), 9.5(f)], 90 DAS [227.6(J_2)] and 180 DAS [251.9(J_2), 16(f)] respectively. Similarly, the same dose of emamectin benzoate (@ 285.0g a.i./ha), when applied at 7 days before seed sowing (DBS) and 15 days before seed sowing in separate nursery bed also evidenced of recording lowest number of juveniles population in the soil and female RKN populations per g of root in the nursery or in the main field, at every occasion at 35 DAS [123.5(J_2) and 10.0(f) in 7DBS, 135.0(J_2) and 10.3(f) in 15DBS] and 180 DAS [249.0(J_2) and 13.5(f) in 7DBS, 279.5(J_2) and 16.5(f) in 15 DBS] respectively. None of the treatments exhibited visual signs of phytotoxic effect on chilli crop.

INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the important vegetable and condiments crop having immense commercial dietary and therapeutic values and grown throughout the year (Meena *et al*., 2013). Its production suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also by abiotic stresses (Choudhary *et al*., 2013). Among them, root-knot nematodes (*Meloidogyne* spp.) are the most serious pests, which have been reported to cause about 10-15% yield loss in chilli (Jain *et al*., 2007) and also infect a wide variety of crops in open fields and protected cultivation systems (Barker *et al*., 1976; Dasgupta and Gaur, 1986; Gill and Jain, 1995). Besides that, Ghosh and Dutta (2014) reported that the aggregative effect of *Meloidogyne javanica* increase the wilt severity at higher population level and hastens the symptom development. It infests the nursery in the seedbed as well as the transplanted crop in the field. Early infection in the seedlings not only makes the plants weak and vulnerable to other infections but the seedlings also carry with themselves root knot nematode infestation to a healthy field. Since nurseries are the main source of inoculums to field; nematode free transplants need to be raised for better crop and yields (Dhillon and Sharma, 2012). Carbofuran was widely recommended for this purpose. Due to its widespread use in agriculture, contamination of food, water, and air has become imminent, and consequently adverse health effects are inevitable in humans, animals, wildlife, and fish (Gupta, 1994). Furthermore, these chemicals are limited and costly. Therefore, alternative environment friendly measures are needed to be developed to protect the seedlings from root-knot nematodes infestation very early in nursery bed. Emamectin benzoate can be proposed for this purpose. Nematicidal property of abamectin (a fermentation product from *Streptomyces avermitilis*) particularly in the form of its analog avermectin, as like emamectin, has been well documented by several authors. Authors like Jayakumar *et al*., 2005; blackbern *et al*., 1996; Faske and Starr, 2006; Chubachi *et al*., 1999 have tested avermectin for suppression of nematodes in bhindi, tomato and cucumber *etc*. Information received from Jansson and Rabatin (1998), Abbas *et al*. (2008) and Rehman *et al*. (2009) draw attention to the nematicidal property of emamectin. On the other hand application of emamectin in nursery bed reduces the exposure of toxicant to the environment. As it was derived from soil microbes offer promising solution to avoid environmental pollution and crop losses due to the pests and diseases and these metabolites are important component of sustainable agriculture. The diversity in structure and the activity, biodegrability and environmental friendly properties make these proposed microbial metabolites, agro active agents of future generation (Birtle *et al*., 1982). Here attempt has been made to evaluate the nematicidal property of emamectin benzoate 1.9% WP as soil drenching in nursery bed of chilli.

MATERIALS AND METHODS

Total three experiments were conducted during July-December in 2012 (Experiment-I) and Feb-July in 2013 (Experiment-II and III) in an established sick plot infested with *Meloidogyne* spp., at central research farm of Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal. In case of first experiment, soil drenching was done with different doses of emamectin benzoate 1.9%WP at two to three leaf stages of chilli seedlings in nursery bed. After completion of the 1st experiment following 2nd and 3rd experiments were conducted as to see the effect of emamectin benzoate1.9%WP as soil drenching on root-knot nematodes at 7 days before seed sowing and 15 days before seed sowing in nursery bed. The different treatments of emamectin benzoate 1.9% WP as mentioned bellow were imposed in Randomised Block Design. The total numbers of treatments were 5 including control in first experiment. Those were; T_1- emmamectin benzoate 1.9% WP @ 142.5g a.i./ha; T_2- emmamectin benzoate 1.9% WP @ 190.0g a.i./ha; T_3- emmamectin benzoate 1.9% WP @ 237.5g a.i./ha; T_4- emmamectin benzoate 1.9% WP @ 285.0g a.i./ha; T_5- Control. But there were 5 treatments and an untreated check in each case of second and third experiment. Among these five treatments of emmamectin benzoate 1.9% WP applied in second and third experiment, treatment T_1, T_2 and T_3 were the half doses of corresponding treatments T_1, T_2 and T_4 applied in the first experiment and these were; T_1- emmamectin benzoate 1.9% WP @ 71.3g a.i./ha; T_2- emmamectin benzoate 1.9% WP @ 95.0g a.i./ha; T_3- emmamectin benzoate 1.9% WP @ 142.5g a.i./ha; T_4- emmamectin benzoate 1.9% WP @ 190.0g a.i./ha; T_5- emmamectin benzoate 1.9% WP @ 285.0g a.i./ha; T_6- Control. Each treatment was replicated four times in all three experiments.

In each experiments, root-knot nematode susceptible chilli cultivar (Kajli bullet) was taken and the seeds were line sown in separate nursery beds of 4m^2 (2 m x 2 m) each. After germination, the seedlings were transplanted at 35 days after sowing (DAS), in the previously prepared main field. The plants were removed at 180 days after sowing (DAS), carefully with intact roots system washed free of soil. For estimating initial population of nematode, three composite samples each consisting of nine subsamples covering the entire experimental area, were collected from nursery bed and main field separately just before the land preparation (Mukhopadhyay and Roy, 2007) in each experiments. Then the soil samples (200 cm^3 from each of the composite samples) were processed according to Cobb's decanting and sieving technique combined with a modified Baermann's funnel method (Christie and Perry, 1951). Root populations of nematodes were assessed after differential staining by the NaOCl- acid fuchsin method (Byrd *et al*., 1983).

In each experiments, observation were made on juvenile (J_2) RKN population per 200 cc of soil in nursery bed at transplanting (35 DAS) as well as in main field at flowering and fruiting stages (90 DAS and 180 DAS). Number of free-living nematodes population in soil and female nematodes population of per gram of roots per replication at transplanting and at fruiting (35 DAS and 180 DAS) were recorded. Root-knot index at transplanting (35 DAS) as well as at fruiting stages (180 DAS) on a 1-5 scale (1 = no galls and/or egg masses, 2 = 1-10, 3 =11-30, 4 = 31-100 and 5 >100 galls and/or egg masses per plant) (Mukhopadhyay *et al*., 2006) of per gram of root system per replication were also determined. The critical difference (CD) at 5% level of significance was worked out from the data recorded during experiments.

RESULTS

Experiment-I

All the treatments performed significantly better over untreated control in reducing soil nematode population in nursery bed (Table 1). Emamectin benzoate 1.9%WP @ 142.5g a.i./ha (T_1) as soil drenching at 2 to 3 leaf stages reduced soil population from 525.3 (INP) to 200.4 per 200cc of soil as compared to 560.4/200cc of soil in control plot. Whereas, the highest dose of emamectin benzoate 1.9%WP @ 285.0g a.i./ha (T_4) effected maximum reduction of soil population to 140.4. Notably none of the treatments exhibited visual signs of phytotoxicity on

Table 1: Effect of emamectin benzoate 1.9%WP on the *Meloidogyne* spp., gall indexing and free living nematodes population applied as post sowing soil drenching at 2 to 3 leaf stages in chilli nursery bed

Treatments	Population of *Meloidogyne* spp.(j_2)/ 200 cc of soil			Number of females /g of root		Root gall index		Free living nematodes /200 cc of soil	
	35 DAS (Seedling)	90 DAS (Flowering)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)
T_1	200.4(64.2)*	408.7(23.8)	495.9(34.3)	14.3(26.7)	26.0(21.2)	2.4	3.6	640.3	721.4
T_2	185.1(67.0)	337.5(37.0)	445.6(45.9)	10.3(47.8)	24.5(25.8)	2.2	3.1	595.0	621.4
T_3	150.0(73.2)	328.7(38.7)	408.7(41.0)	10.0(48.7)	21.3(35.5)	2.1	2.8	675.1	848.4
T_4	140.4(75.0)	227.6(57.6)	251.9(66.6)	9.5(51.3)	16.0(51.5)	2.1	2.4	751.1	821.3
T_5	560.4	536.3	755.3	19.5	33.0	3.0	4.3	652.3	690.0
SEm ±	6.4	1.91	1.91	1.15	1.38	0.12	0.08	61.6	53.0
CD (0.05)	19.7	5.87	5.90	3.55	4.27	0.36	0.26	NS	NS

Note: T_1: Emamectin benzoate @142.5g a.i./ha, T_2: Emamectin benzoate @190.0g a.i./ha, T_3: Emamectin benzoate @237.5g a.i./ha, T_4: Emamectin benzoate @285.0g a.i./ha, T_5: Untreated control Initial nematode population (*Meloidogyne* spp.) per 200cc of soil was 525.3 in nursery bed and 494.3 in the main field. DAS- days after sowing; Gall index: 1-5 scale:1 = no galls and/or egg masses, 2 = 1-10, 3 =11-30, 4 = 31-100 and 5 >100 galls and/or egg masses per plant root system; *Figures in the parentheses indicate "per cent decrease over control".

Table 2: Effect of emamectin benzoate 1.9%WP on the *Meloidogyne* spp., gall indexing and free living nematodes population applied as pre sowing soil drenching (7 DBS) in chilli nursery bed

Treatments	Population of *Meloidogyne* spp.(j_2)/200 cc of soil			Number of females /g of root		Root gall index		Free living nematodes/ 200 cc of soil	
	35 DAS (Seedling)	90 DAS (Flowering)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)
T_1	286.4 (38.3)*	378.7(23.1)	453.7(18.6)	14.3(31.3)	25.5(12.1)	2.6	3.3	515.6	655.3
T_2	242.8 (47.7)	239.1(33.2)	405.8(27.2)	14.0(32.7)	21.0(27.6)	2.4	3.0	577.8	621.8
T_3	198.2 (57.3)	321.2(34.8)	295.8(47.0)	12.3(40.7)	18.8(35.2)	2.2	2.7	614.0	793.0
T_4	125.8 (72.9)	214.0(56.6)	249.8(55.2)	10.3(50.5)	13.8(52.4)	2.0	2.4	536.5	797.6
T_5	123.5 (73.4)	205.3(58.3)	249.0(55.4)	10.0(52.0)	13.5(53.4)	2.0	2.3	473.4	686.8
T_6	464.0	492.6	557.7	20.8	29.0	3.8	4.0	514.7	705.3
SEm ±	3.02	1.89	1.98	0.89	1.26	0.08	0.11	45.7	49.1
CD (0.05)	9.09	5.70	5.96	2.68	3.81	0.23	0.32	NS	NS

Note: T_1: Emamectin benzoate @71.3g a.i./ha, T_2: Emamectin benzoate @95.0g a.i./ha, T_3: Emamectin benzoate @142.5g a.i./ha, T_4: Emamectin benzoate @190.0g a.i./ha, T_5: Emamectin benzoate @285.0g a.i./ha, T_6: Untreated control; Initial nematode population (*Meloidogyne* spp.) was 428.3/200cc of soil in seed bed and 411.1/200cc of soil in main field Gall index: 1-5 Scale:1 = no galls and/or egg masses, 2 = 1-10, 3 = 11-30, 4 = 31-100 and 5 > 100 galls and/or egg masses per plant root system; *Figures in the parentheses indicate "per cent decrease over control".

Table 3: Effect of emamectin benzoate 1.9%WP on the *Meloidogyne* spp., gall indexing and free living nematodes population applied as pre sowing soil drenching (15 DBS) in chilli nursery bed

Treatments	Population of *Meloidogyne* spp.(j_2)/200 cc of soil			Number of females / g of root		Root gall index		Free living nematodes /200 cc of soil	
	35 DAS (Seedling)	90 DAS (Flowering)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)	35 DAS (Seedling)	180 DAS (Fruiting)
T_1	295.0 (33.0)*	373.7(28.3)	471.1(20.4)	17.3(21.4)	26.3(17.3)	2.8	3.7	675.8	679.6
T_2	261.5 (40.5)	308.8(40.8)	436.3(26.3)	14.3(35.0)	22.0(30.9)	2.5	3.3	761.3	621.4
T_3	210.2(52.2)	286.4(45.0)	371.0(37.4)	12.3(44.0)	20.8(34.6)	2.3	3.0	655.5	687.4
T_4	144.5 (67.1)	246.9(52.6)	295.3(50.1)	11.0(50.0)	17.3(45.6)	2.0	2.8	654.3	762.0
T_5	135.0 (69.3)	209.6(56.8)	279.5(52.8)	10.3(54.5)	16.5(48.1)	2.0	2.5	639.0	690.0
T_6	439.8	521.2	592.2	22.0	31.8	3.5	4.2	609.3	738.8
SEm ±	0.93	2.01	1.96	0.84	1.33	0.16	0.13	104.6	50.6
CD (0.05)	2.80	6.06	5.89	2.54	4.01	0.48	0.39	NS	NS

Note: T_1: Emamectin benzoate @71.3g a.i./ha, T_2: Emamectin benzoate @95.0g a.i./ha, T_3: Emamectin benzoate @142.5g a.i./ha, T_4: Emamectin benzoate @190.0g a.i./ha, T_5: Emamectin benzoate @285.0g a.i./ha, T_6: Untreated control; Initial nematode population (*Meloidogyne* spp.) was 406.0/200cc of soil in seed bed and 425.1/200cc of soil in main field; Gall index: 1-5 scale: 1 = no galls and/or egg masses, 2 = 1-10, 3 = 11-30, 4 = 31-100 and 5 > 100 galls and/or egg masses per plant root system; *Figures in the parentheses indicate "per cent decrease over control".

chilli plants. The reduction in number of nematodes in soil by various treatments compared to control ranged from 64.2 to 75.0 per cent, the greatest being 75.0% for emamectin benzoate 1.9% WP @ 285.0g a.i./ha. Data related to nematode population at flowering stage (90 DAS) and at fruiting stage (180 DAS), collected from the chilli experimental plots having differentially treated plants at nursery bed, have been presented in table-1. Here also in both cases nematode populations were recorded as lowest for emamectin benzoate 1.9% WP @ 285.0g a.i./ha, reduction being 57.6% and 66.6% respectively. The female root-knot nematode populations on the root were recorded at transplanting (35 DAS) and at fruiting stage (180 DAS) and were revealed differentially for different treatments. The data relating to the observation have been presented in table-1. It has been observed that with the increase in doses of emamectin benzoate a greater reduction in the nematode population was achieved. The result showed the highest reduction of female root-knot nematode population over control at transplant (35 DAS) as well as at 180 DAS for the treatment consisted of emamectin benzoate 1.9% WP @ 285.0g a.i./ha, being 51.3% and 51.5% respectively. This result was reflected in the gall index too (35 DAS- 3.0, 180 DAS- 4.3). None of the treatments significantly differed in their effects on populations of free-living nematodes at 35 DAS and at 180 DAS.

Experiment-II

The results clearly indicated that all the treatments applied as soil drenching at 7 days before sowing (DBS) had significantly reduced the nematode population in soil in nursery bed when compared with control (Table- 2). Among them, T_5, emamectin benzoate 1.9%WP @ 285.0g a.i./ha recorded the greatest reduction of root knot nematode soil population in the nursery bed which was followed by T_4 , T_3, T_2 and T_1 being 73.4, 72.9, 57.3, 47.7 and 38.3 per cent respectively (Table 2). The same trend in the performance of the mentioned treatment was also noted in the soil population at 90 DAS and at 180 DAS in the main field.

Reduction in female nematode population in chilli over untreated plant at transplanting (35 DAS) indicated an ascending trend in performance of the treatments: T_1, T_2, T_3, T_4 and T_5: commensurable to the dosages of emamectin benzoate. Reduction in population was to the tune of 52% for T_5, emamectin benzoate 1.9% WP @ 285.0g a.i./ha followed by 50.5, 40.7, 32.7 and 31.3 per cent for T_4, T_3,T_2 and T_1 respectively (Table 2).

Female nematode population in root at fruiting stage was recorded minimum for the treatment T_5 (13.5) which were at par with the treatment T_4 (13.8) (Table 2). Both the treatments were significantly superior over others. Consequent to these the gall index recorded against the treatments was commensurable to their performance in reducing the population. Here also the population of free-living nematode was not affected by any of the treatments in 35 DAS and 180 DAS.

Experiment-III

The experimental results revealed that application of different doses of emamectin benzoate applied as soil drenching at 15 DBS was quite effective to control root-knot nematode over the untreated control. Among the dosages applied, emamectin benzoate 1.9% WP @ 285.0g a.i./ha (T_5) was found the best to reduce the soli population in chilli nursery bed (Table 3). Reduction of soil population of nematode was 69.3% for this treatment followed by 67.1, 52.2, 40.5 and 33 per cent for T_4, T_3, T_2 and T_1 respectively. Now with regard to the data of soil nematode population at 90 DAS and at 180 DAS in the main field, population reduction over the untreated plants was recorded with a gradually descending trend for the treatments T_5, T_4, T_3, T_2 and T_1 being 56.8%, 52.6%, 45.0%, 40.8% and 28.3% at 90 DAS and 52.8, 50.1, 37.4, 26.3, and 20.4 per cent at fruiting stage respectively.

All the treatments performed better over the untreated plants in reducing the female nematode population in chilli both at transplanting and at fruiting stage. In both the cases per cent reduction of root population increased with the increase of the dosages. The treatment T_5 performed the best reducing the root population by 54.5% and 48.1% over that of the untreated plants at transplanting and at fruiting respectively (Table 3). The same treatment (T_5) also showed lesser root galling due to *Meloidogyne* spp. per 200cc of soil at 35 DAS and 180 DAS comparing others (Table 3). The population of free-living nematode was not significantly differed with different doses of emamectin benzoate both at transplanting and at fruiting stage.

DISCUSSION

During the experiment, emamectin benzoate 1.9%WP proved to be an excellent nematicide against root-knot nematodes when applied in the nursey bed of chilli. It has been observed that with the increase in doses of emamectin benzoate a greater reduction in the nematode populations were achieved and the highest reduction of juvenile root-knot nematodes (*Meloidogyne* spp.) in soil were achieved by emamectin benzoate 1.9%WP @ 285.0g a.i./ha in all three experiments. The present findings are in parity with the results of Blackburn *et al*. (1996) observed that avermectin B1 at 0.2 and 0.4 kg a.i/ha significantly reduced the population of *Hoplolaimus galeatus* and *Tylenchorynchus dubius* compared to untreated control. Similarly, Jayakumar *et al*., (2005) observed reduction of nematodes/200 g soil (140.0), untreated control (523.3), and number of female nematodes/ g root (6.5), untreated control (23.30) by the application of crude avermectin 100% as seed treatment in bhendi against reniform nematode, *Rotylenchulus reniformis*.

Regarding female root-knot populations and gall indexing, our experiment showed that different concentrations of emamectin benzoate 1.9%WP were effectively reduced the root-knot nematodes population per g of root by soli drenching though highest reduction was achieved by the highest dose of emamectin benzoate *i.e.* 285.0g a.i./ha, in every occasions in the nursery or in the main field. In This study, reduction of nematode populations in roots caused by the emamectin benzoate may be attributed to the affect of nematode physiology because after a soil treatment with highest dose of avermectin B1 (0.6 mg/dm^3 of soil), the few *Meloidogyne arenaria* which entered the tomato roots were unable to develop and moult (Cayrol *et al*., 1993). Or the reason perhaps lie in the fact that Avermectin, which is a streptomycete-derived macrocyclic lactone biological nematicide that blocks the transmittance of electrical activity in nerves and muscle cells by stimulating the release and binding of gamma-aminobutyric acid at nerve endings (Putter *et al*., 1981), which causes paralysis of the neuromuscular systems that adversely affect nematode hatching and movement in soil, subsequently reducing the extent of root invasion (Tobin *et al*., 2008; Huang *et al*., 2014). It may also depends on the fact that, in a soil rhizosphere, abamectin, a member of avermectin family, as like emamectin, is bind tightly to the soil particles, and has a low water solubility, resulting in the pore movement of product through the soil profile (Bull *et al*., 1985; Mrozik, 1994; Chukwedebe *et al*., 1996) resulting its extremely low leaching potential through many types of soil (Khalil, 2013). While avermectin B1 and B2 shown equally high activities in *in vitro* tests against *M. incognita*, on tobacco grown in the micro plots where granulated formulations of these entities were incorporated and avermectin B2 was about 10-40 times more potent than several organophosphorus and carbamate nematicides (Putter *et al*., 1981; sasser *et al*., 1982; Nordmeyer and Dickson, 1985). Our findings are also in agreement with Nwadinobi *et al*. (1989), who reported the reduction of number of galls in root dipping of 14 days old tomato seedlings in 1mg/L of avermectin B1. The treatment delayed nematode invasion and development up to 20 days. These results correlate with other workers. Author like, Sasser and Kirkpatrick (1982), who had reported that application of avermectin at the rates ranging from 0.05 to 0.50 Kg a.i/ha suppressed root galling per plant by 21-86%.

Literatures on the effect of emamectin benzoate to free-living nematodes are not available for comparison. However, Abamectin has shown low toxicity to non target beneficial arthropods, which was considered a motivation to use it in IPM programs (Khalil, 2013). Wright *et al*. (1984) had reported that in addition to *M. incognita,* a triphasic locomotor response to avermectins has been observed in the plant parasitic nematode *Heterodera schachtii* but was not found in the free-living species *Caenorhabditis elegans* which is advantageous as because free-living nematodes are involved in soil nutrient cycling and help to create a healthier soil environment (Wang *et al*., 2004; Wang and McSorley, 2005).

In summary, our investigations demonstrated that using emamectin benzoate 1.9%WP as soil drenching in nursery bed is an effective way of reducing early root-knot nematodes penetration in the roots of chilli seedlings as well as in the main field till harvest without any visual signs of phytotoxic effect on chilli crop. In general, all these justifications for the suppression of female nematode population in roots and juvenile population in soil due to application of emamectin benzoate 1.9%WP could be pointed to low fecundity and hatching as encouraged by nematicidal principles present in the emamectin benzoate 1.9%WP which is a novel semi-synthetic derivative of natural product avermectin.

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