Pakistan J. Zool., vol. 48(3), pp. 887-890, 2016.

Short Communication

Biological Management of *Meloidogyne incognita* Using Entomopathogenic Bacterial Cell Suspensions with Other Bioproducts in Egg Plant

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ABSTRACT

In vitro assessment was performed with bacterial cell suspensions of *Xenorhabdus* spp. and *Photorhabdus* spp. with bio-products (abamectin, emamectin and azadirachtin) against *Meloidogyne incognita* Kofoid & White (Chitwood) on egg plants (*Solanum melongena* L.). Bacteria were isolated from *Steinernema asiaticum* and *Heterohabditis bacteriophora*, respectively. Maximum control (63.6%) for number of females was observed in combined treatment of *Xenorhabdus* spp. + abamectin, 60.7% in the case of *Photorhabdus* spp. + abamectin treatment, and 57% in abamectin treatment. The minimum control (45%) was observed in azadirechtin treatment as compared to control. In case of reproduction factor, *Photorhabdus* spp. + abamectin treatment proved the most effective against *M. incognita*, whereas azadirachtin and emmamectin treatments were the least effective. The results clearly showed synergistic effect of bacterial cell suspensions and abamectin in controlling *M. incognita* population.

Article information
Received 28 August 2015
Revised 15 October 2015
Accepted 31 October 2015
Available online 1 May 2016

Authors' Contributions:
NJ designed and supervised the project. HMA executed experimental work. MIU helped in rearing and maintaining EPNs culture. SA and SPL statistically analyzed the data. IM, YI and MA helped in preparation of manuscript.

Key words:
***Xenorhabdus* spp., *Photorhabdus* spp., *Steinernema asiaticum*, *Heterohabditis bacteriophora*, *Meloidogyne incognita*, bio-products.**

Eggplant, also known as brinjal or aubergine (*Solanum melongena* L.), is an important summer vegetable (Anonymous, 2011). In Punjab (Pakistan), this crop is cultivated on an area of 4.7 ha with an annual production of 59.2 tons and average yield of 12.6 tons.ha^{-1} (GOP, 2013). Pakistan stands at 18th position in the world ranking (FAO, 2012). In Pakistan, root-knot nematodes *Meloidogyne* (*Goeldi* spp.) are recognized as important pests of vegetable crops (Maqbool, 1992; Zaki, 2000, Khan *et al*., 2010).

Entomopathogenic nematodes (EPNs) and their associated bacteria have been marketed for the control of certain plant parasitic nematodes *i.e.*, root knot nematodes (RKNs); however variation has been observed in their efficacy against RKNs (Javed *et al*., 2012, Aatif *et al*., 2012, 2014; Khan *et al*., 2010a; Lewis and Grewal, 2005). Chaubey and Sharma *et al*. (2004) reported that EPNs (*Steinernema* spp. and *Heterorhabditis* spp.) are not only harmful to insects but inimical to plant parasitic nematodes (*Meloidogyne* spp.). EPNs belonging to the families Steinernematidae (Chitwood and Chitwood, 1937) and Heterorhabditidae (Poinar, 1976) infect and kill insects with the aid of symbiotic bacteria present in their intestines *i.e.*, *Steinernema* spp. carry *Xenorhabdus* spp. While *Heterorhabditis* spp., carry *Photorhabdus* spp. *Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* spp. infected juveniles (IJs) (Bird and Akhurst, 1983), while *Photorhabdus* spp. is distributed in the foregut and midgut of *Heterorhabditis* IJs (Boemare *et al*., 1996).

Alternative studies in the past shows positive and negative interactions with other soil pathogens indicating the potential of some EPNs species to suppress plant-parasitic nematodes (PPN). As an additional benefit, several researchers (Jagdale and Grewal, 2008; Molina *et al*., 2007) have demonstrated that EPNs can also be used as biological control agents against RKN infesting different crops in the field and green house. Other studies have shown that EPNs and symbiotic bacteria possibly may interfere with the reproduction and infection of some PPN (Grewal *et al*., 1999). EPNs and their associated bacteria inhibit egg hatching (Aatif *et al*., 2012; Samaliev *et al*., 2000) and cause mortality of J2s or repel J2s from the roots of the target crop (Sasnarukkit *et al*., 2002). EPNs have synergistic relationship with different

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0030-9923/2016/0003-0887 $ 8.00/0

nematicides. It has been seen that when nematicides like abamectin, nemaless and sincocin-A Gare applied with EPNs show more inhibition of life cycle of the RKNs than the alone nematicide chemicals (Turner and Schaeffer, 1989; El-Nagdi and Youssef, 2004).

Keeping in view the importance of the biological control, it was planned to investigate the biological control of RKNs through entomopathogenic bacteria. Experiment was carried out to assess compatibility of bacterial cell suspension with other liquid formulations of bio-products (abamectin, emamectin and azadirachtin) of Bayer-crop sciences on development of *M. incognita* in a host plant.

Materials and methods

Disposable pots (250 g) were filled with formalin sterilized soil. Bio-products *i.e.*, abamactin, emamectin and azadirachtin were thoroughly incorporated in the soil at the concentration of 0.1 % (v/w) and these doses were adjusted in 50 ml of water (Blackburn *et al.*, 1996). After 24 h, four weeks old brinjal seedlings at the 5-6 leaves stage were transplanted into these pots by making a hole in the center of each pot and were watered carefully. Over irrigation of the pots was avoided. A pure colony of *Xenorhabdus* and *Photorhabdus* species on nutrient agar bromothymol blue triphenyl tetrazolium chloride (NBTA) (Akhurst, 1980) was introduced into a nutrient broth for mass production of the bacteria. After four days of transplanting when the plants established their root system, inoculation of plants was done with *Xenorhabdus* and *Photorhabdus* spp. at the concentration of $4x10^7$ cell/ml. In these inoculated pots 1000±25 freshly hatched juveniles (24 h old) of *M. incognita* present in 15 ml water were added per plant by making holes (Campos and Campos, 2005). These pots were then filled with soil to prevent drying and kept for overnight in completely randomized design in the glass house. Treatments used were; t_1 = *Photorhabdus* spp. + abamectin, t_2 = *Photorhabdus* spp. + azadirechtin, t_3 = *Photorhabdus* spp. + emamectin, t_4 = *Xenorhabdus* spp. + abamectin, t_5 = *Xenorhabdus* spp. + azadirectin, t_6 = *Xenorhabdus* spp. + emamectin, t_7 = abamectin, t_8 = azadirechtin, t_9 = emamectin, t_{10} = RKN, t_{11} = *Photorhabdus* spp., t_{12} = *Xenorhabdus* spp.)**.** Experiment was repeated three times.

After 35 days, plants were removed from pots and the root balls were shaken until most of the soil was dislodged from the root. Number of females and nematode reproduction factor [Pf/Pi] was recorded, where Pf is final nematode population at harvest and Pi is initial inoculums.

SAS package was used for data analysis. Analysis of variance (ANOVA) was used to determine the effect of different treatments. Duncan Multiple range *i.e.* DMR test was used for the comparison of means.

Results

Analysis of variance (ANOVA) showed that cell suspension had significant effect over no. of females and reproductive potential of RKN (Table I). It was observed that all the treatments differed significantly in reducing the no. of females of *M. incognita* as compared to control treatment. Maximum control (63.6%) for no. of females of *M. incognita* on brinjal roots was observed in *Xenorhabdus* spp. + abamectin treatment followed by *Photorhabdus* spp. + abamectin treatment (60.7%) and abamectin treatment (57%). Minimum control was observed in azadirechtin alone (45%) as compared to control treatment where only RKNs were applied. Highest no. of females was in the control *i.e.* 341 whereas the least no. of females was in the treatment of *Xenorhabdus* spp. + abamectin *i.e.*134. It was observed that *Xenorhabdus* spp. alone application as well as in combination with bio-products was proved significantly more effective in reducing no. of females as compared to *Photorhabdus* spp. alone application as well as in combination.

Results showed that *Photorhabdus* spp. + abamectin proved the most effective for controlling the reproduction factor of *M. incognita* followed by *Xenorhabdus* spp. + abamectin, *Photorhabdus* spp. + emamectin, abamectin, *Xenorhabdus* spp. and *Photorhabdus* spp. application. Whereas azadirachtin and emmamectin treatments were the least effective.

Discussion

Compatibility of *Photorhabdus* spp. and *Xenorhbous* spp. and their associated toxins with bio-products *i.e.*, abamectin, emamectin and azadirechtin tested against RKNs shows that combine effect of Abamectin and *Xenorhabdous* spp. and their toxins is more effective against RKNs as compared to individual application. This proves that there was a synergistic effect among bio-products and bacteria against RKNs (Garabedian and Van Gundy, 1983).

During current study, both *Photorhabdus* spp. and *Xenorhabdus* spp. reduced J2s penetration into roots, and suppressed egg production in brinjal as compared to control treatment. Mechanism behind this effectiveness against RKN may be due to toxicity, biostasis and repellency of these bacterial species (Sasnarukkit *et al.*, 2002). When J2 of RKN was treated with different concentrations of pesticides to check the uptake of O_2, J2 of *M. incognita* became paralyzed after 24 hours of treatment of abamectin. This was in fact because of impeding of O_2 to RKN juveniles. Further, it was suggested that lack of oxygen would have created

Table I.- Effect of bacterial cell suspension with other bio-product on number of Females and Reproductive Potential of RKN.

Treatments	No. of Females	Reproductive Potential
T1 (*Photorhabdus* spp. ($4X10^7$ cells/mL) + abamectin (1%))	403	4.95±0.04 E
T2 ((*Photorhabdus* spp. ($4X10^7$ cells/mL) + azadirectin (1%)	516	5.36±0.18 CD
T3 (*Photorhabdus* spp. ($4X10^7$ cells/mL) + emamectin (1%))	506	5.30±0.06 DE
T4 (*Xenorhabdus* spp. ($4X10^7$ cells/mL) + abamectin (1%)	373	5.03±0.09 DE
T5 (*Xenorhabdus* spp.($4X10^7$ cells/mL) + azadirectin (1%)	490	5.33±0.03 CD
T6 (*Xenorhabdus* spp. ($4X10^7$ cells/mL) + emamectin(1%)	495	5.68±0.10 BC
T7 (1% abamectin)	438	5.12±0.12 DE
T8 (1% azadirectin)	561	5.86±0.04 B
T9 (1% emamectin)	545	5.94±0.104 B
T10 (RKN alone)	1025	20.28±0.29 A
T11 (*Photorhabdus* spp. $4X10^7$ cells/mL)	422	5.19±0.03 DE
T12 (*Xenorhabdus* spp. $4X10^7$ cells/mL)	395	5.07±0.04 DE

additive effect which produced toxicity of bio products resulted in death of RKN There are still chances that depletion of oxygen may be due to some other microorganism like the bacteria *Photorhabdus* spp. and *Xenorhabdus* spp. (in combine treatment) or already present in the soil which utilized it (Nordmeyer and Dickson, 1989). So, it is inferred from above discussion that bio-products prevent the motility of RKN.

Nemastatic mode of action of neem compounds is similar to those of carbamate and oxamyl pesticides (Elskamp *et al.,* 1974). These pesticides block the production of acetylcholinesterase enzyme which is involved in transferring the signal to neurosystem resulting in avoiding the breakdown of acetylcholine, which is a signaling substance to the muscular system (Elskamp *et al*., 1974). When breakdown of acetylcholine stopped it result the accumulation of acetylcholine causing convulsion, paralysis and ultimately death of nematodes. In general, it may be concluded that bio-products halt the nematodes and reduce invasion of nematodes. Bio-products higher doses especially of abamectin and emamectin exhibited good results by monitoring the attack of nematodes into brinjal roots. Azadirachtin do not cause the immobility and mortality of RKN (Javed *et al.,* 2008b) which means having no toxic effect on the J2. This proposes that azadirachtin works as protective bio-product within brinjal roots and avoid nematodes development. Furthermore, it was seen in our experiments that combine effect of bio-chemicals with symbiotic bacteria gave better results than bio-chemicals alone. These results are in accordance with many research workers (Barker, 1978; Garabedian and Van Gundy, 1983).

In Pakistan, different practices are in fashion to control insect-pest management. Integrated pest management could ameliorate pest management under field conditions. The *Photorhabdus* spp and *Xenorhabdus* spp. in present study could be made part of an IPM program to manage RKN. Further, improvement may be brought by using resistant genotypes, organic amendments, cultural practices and other biological agents, to frame a workable management of RKN.

Acknowledgements

The authors gratefully acknowledge the financial assistance through research funding received for Indigenous 5000 PhD. Fellowship Program Batch-IV by Higher Education Commission, Pakistan and Prof. Dr. Nazir Javed, Plant Pathology Department, University of Agriculture, Faisalabad Punjab, Pakistan for necessary correction and going through the manuscript.

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