International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(6): 1947-1950

Received: 12-08-2020
Accepted: 19-09-2020

S Adithya
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, Karnataka, India

MK Shivaprakash
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, Karnataka, India

K Harish Kumar
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, Karnataka, India

Corresponding Author:
S Adithya
Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore, Karnataka, India

In vitro evaluation of biocontrol potential of symbiotic bacteria of entomopathogenic nematodes against shoot and fruit borer (*Helicoverpa armigera*) in Solanaceous vegetable crops grown in seedling trays under greenhouse condition

S Adithya, MK Shivaprakash and K Harish Kumar

DOI: https://doi.org/10.22271/chemi.2020.v8.i6ab.11049

Abstract
The present study was carried out to test the biocontrol potential of symbiotic bacteria, *Photorhabdus* and *Xenorhabdus* against the shoot and fruit borer, *Helicoverpa armigera* in solanaceous vegetable crops grown under greenhouse conditions. A total of ten treatments were designed with symbiotic bacteria of entomopathogenic nematodes for testing their biocontrol potential by using *Bacillus thuringiensis* as a reference strain. The results had shown significantly higher biocontrol efficiency in the treatment T_9 *(H. armigera* + Consortia) with 85-90% biocontrol efficiency in all the vegetable crops followed by the treatment T_7 received *Bacillus thuringiensis.* Among the treatments received individual strains of entomopathogenic bacteria, T_3 and T_2 which received EPB3 and EPB1 with 75-80% biocontrol potential and are at par with each other. Other treatment received individual strains had shown more than 50% of biocontrol efficiency in vegetable crops grown in seedling trays under greenhouse conditions. Further these entomopathogenic bacteria can be tested for their effectivity under field trails and can be developed as bio-pesticide formulations either in the form of individual application or in integrated pest management for plant protection practices.

Keywords: Symbiotic bacteria, *Helicoverpa armigera*, biocontrol and bio-pesticide

Introduction
Photorhabdus and *Xenorhabdus* are fascinating bacteria live in mutualistic relationship with the genus *Heterorhabditis* spp. and *Steinernema* spp., respectively producing an array of protein toxins and toxic secondary metabolites with insecticidal potential and pathogenic activity against plant pathogens (Nielsen-LeRoux *et al.*, 2012; Stock, 2015) [1, 2]. Over the past two decades, significant progress was made in the mining of *Photorhabdus* genomes. Comparison across different species and strains has revealed the presence of several conserved biosynthesis gene clusters that are involved in the biosynthesis of diverse natural products. Furthermore, these small bioactive molecules have been shown to play key roles in the pathogenic and mutualistic phases of this bacterium. Despite this progress, there are yet many critical steps that must be pursued before their consideration of *Photorhabdus* secondary metabolites as agricultural pesticides. Most of what is known regarding the bioactivity of this bacterial secondary metabolites are based on structure comparisons and/or similarities with molecules of known insecticidal, antimicrobial or cytotoxic activity (Tobias *et al.*, 2017) [3].
The shoot and fruit borer, *Helicoverpa armigera* is one of the most important polyphagous noctuid pests in vegetable crops over 200 plant species worldwide. It cause damage both the vegetative parts and reproductive parts of the plats including leaves, stems, buds, inflorescence, pods and fruits. In India shoot and fruit borer, *H. armigera* is one of the most important pests in vegetable crops limiting the production and market value of vegetable crops. The fruit borer, *H. armigera* (Hubner) is the most destructive pest is commonly known as gram pod borer, American bollworm and tomato fruit borer and causes 40-50 percent damage to the solanaceous vegetable crops (Jat and Ameta, 2013) [4].

The earlier larval instars feed voraciously on foliage, flower buds and flowers, while the later instars of these insects bore into fruit and render them unmarketable. Due to wider host range, multiple generations, migratory behaviour, high fecundity and existing insecticide resistance this insect has become a difficult pest to tackle (Meena and Raju, 2014) [5].

All over the world, this pest alone causes annual loss of approximately 5 billion US dollars. Whereas the pest greatly hampers the production and productivity of the solanaceous crop by causing a yield loss in the range of 20 to 60% (Gadhiya *et al*., 2014; Talekar *et al*., 2006) [6, 7]. It has been reported on 181 cultivated and wild plant species belonging to 45 families in India. A thorough knowledge about the biology of insect and its status as a pest provides important basis for developing efficient pest management strategies (Herald and Tayde, 2018) [8]. Controlling the insect pests with insecticides causes serious side effects, including development of insecticide resistance in the insects, pest resurgence, environmental pollution, and health hazards. With this background, the present study was aimed to study biocontrol potential of symbiotic bacteria for the biocontrol of shoot and fruit borer in vegetable crops grown in seedling trays under greenhouse conditions.

Materials and Methods

Bacteria strains and Insect cultures

The entomopathogenic bacteria were isolated from two agro-climatic zones of Karnataka i.e., zone 5 and zone 6. A total of five entomopathogenic bacteria were isolated of which four isolates were *Photorhabdus luminescence* (EPB1, EPB4, EPB8 and EPB9) and one isolate was *Xenorhabdus* sp. (EPB3) (Adithya *et al*., 2020) [9]. *Bacillus thuringiensis* (*Bt*) strain was obtained from Biofertilizer lab, Department of Agricultural Microbiology, GKVK, Bangalore. The *Helicoverpa armigera* egg cards were obtained from National Bureau of Agriculturally Insect Resources (NBAIR), Hebbala, Bangalore.

Seedling tray experiment

The seedling tray experiment was conducted in the greenhouse facilities located in Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore. Completely randomized design was used in seedling tray experiment to test the biocontrol potential of symbiotic bacterial isolates of EPN in solanaceous vegetable crops namely Tomato (Var: Arka Vikas), Brinjal (Var: Arka Keshav), Chillies (Var: Arka Lohit) Capsicum (Var: Arka Mohini). The treatments imposed were are as follows:

Treatments	Treatment details
T_1 -	Negative control (*Helicoverpa armigera* alone)
T_2 -	*H. armigera* + EPB1
T_3 -	*H. armigera* + EPB3
T_4 -	*H. armigera* + EPB4
T_5 -	*H. armigera* + EPB8
T_6 -	*H. armigera* + EPB9
T_7 -	*H. armigera* + *Bt*
T_8 -	*H. armigera* + EPB1+ EPB3
T_9 -	*H. armigera* + Consortia
T_{10} -	Control (Sterilized soil)

Observations recorded

Biological control efficacy against the insect pest was calculated using the following formula given by Guo *et al.* (2004) [10].

$$\mathrm{BCE} = \frac{(\mathrm{PIC-PIT})}{\mathrm{PIC}} \text{ X } 100$$

Where, BCE – Biological control efficiency
PIPC- Pest incidence in control
PIT- Pest incidence in treatment group

Statistical analysis

All the experimental data were subjected to statistical analysis using analysis of variance (ANOVA) (Raudonius, 2017) [11] at *p*<0.05 level, further the treatment means were statically differentiated by performing Duncan's Multiple Range Test (DMRT) at *p*<0.05 level. Statistically differentiated means were denoted by different alphabets. For all the above analysis, the software, DSAASTAT developed by Dr. A. Onofri, DSAA, Italy (Onofri *et al*., 2010) [12].

Results and Discussion

Biocontrol potential of symbiotic bacteria of EPN on Helicoverpa armigera in Solanaceous vegetable crops in seedling trays under greenhouse condition

The data related to biocontrol efficiency of symbiotic bacteria of EPN against the shoot and fruit borer, Helicoverpa armigera in solanaceous vegetable crops grown in seedling trays were presented in the Table 1 and Figure 1.

Tomato

The biocontrol efficiency (BCE) was recorded for all the treatments supplemented with the symbiotic bacterial isolates and significantly highest BCE was recorded in the treatment T_9 (85.90%) followed by T_8 (78.00%), T_3 (75.16%), T_2 (74.94%) and T_7 (73.69%), and are promisingly different from each other as well as from other treatment groups. Significant variation of BCE was exhibited by the symbiotic isolates treated in the sapling trays and least BCE was recorded in control (T_1- *H. armigera*) with 0.01%.

Brinjal

Significant variation of BCE was exhibited by the symbiotic isolates treated in the seedling trays. The BCE was recorded highest in the treatments T_9 with 90.19% followed by T_8 (81.90%), T_2 (78.69%), T_3 (78.62%) and T_4 (77.37%), are substantially different from each other. The treatment uninoculated control (T_1- *H. armigera*) did not show any biocontrol efficiency, may be due to lack of symbiotic bacterial isolates in the treatments.

Chilli

The BCE of symbiotic bacterial isolates against the insect pest *H. armigera* was recorded and highest percent biocontrol efficiency was noticed in the treatment T_9 supplemented with *H. armigera* and consortia of symbiotic bacterial isolates with 89.70% followed by T_8, T_3, T_2 and T_7 with 86.00%, 82.86%, 82.62% and 81.24%, respectively and are at par with each other. The treatment uninoculated control (T_1) had recorded null biocontrol efficiency due to lack of treatment with symbiotic bacterial isolates.

Capsicum

The biocontrol efficiency (BCE) was recorded for all the treatments supplemented with the symbiotic bacterial isolates and highest BCE was recorded in the treatment T_9 (85.22%) significantly from the other treatments followed by T8 (81.70%), T3 (78.72%), T2 (78.49) and T_7 (77.18%). Significant variation of BCE was exhibited by the symbiotic

isolates treated in the seedling trays and uninoculated control (T_1 - *H. armigera*) did not shown any biocontrol efficiency.
Similarly, Nandini *et al.* (2019) [13] in one of the experiment tested the efficacy of EPNs under field application and the results revealed that there is a significant reduction in the infectivity of damage caused by the *H. armigera* of 50% after 7 days after foliar spray during the first application and ~75% reduction in the infectivity during the second foliar application in chickpea. Biochemical analysis of haemolymph of *H. armigera* larvae infected with EPN had significantly higher amount of triglycerides, and proteins than the uninfected larvae. This is due to the symbiotic bacteria present in the EPN i.e., *Photorhabdus* sp. that produce a medley of phospholipases and proteases which enable bacteria to utilize host contents there by compromising the growth of the larvae and finally to the death (Duchaud *et al.*, 2003) [14].
In one of the study the expression of *X. nematophila* gene encoding the XnGroEL protein in transgenic tobacco plants for conferring resistance against *H. armigera* larvae was tested. And demonstrated the protein's insecticide activity in tobacco transgenic plants. The transgenic lines showed healthy growth and were phenotypically normal. Insect bioassays revealed significant mortality of larvae (100%) that fed on leaves of transgenic plants compared to vector and untransformed lines (Kumari *et al.*, 2015) [15].
The wide range of insecticidal activity exhibited by *Photorhabdus* and *Xenorhabdus* may be conferred due the selective evolutionary pressure driving the distinctive lifestyle as mutualists of entomopathogenic nematodes. During the evolution, although the bacteria are unable to control the host insect environment provided by the nematodes, they can kill the insect prey to maintain the development of nematode and protect the carcass of insect against other food competitors in the soil, such as fungi, nematodes, protozoa and probably several other organisms (Reimer *et al.*, 2013) [16].

Table 1: Biocontrol efficiency of Symbiotic bacteria on *Helicoverpa armigera* in Solanaceous vegetables grown in seedling trays under greenhouse condition

Treatments	BCE (per cent)			
	Tomato	Brinjal	Chilli	Capsicum
T_1 *(Helicoverpa armigera)*	0.01 (0.50 ± 0.01)f	0.01 (0.50 ± 0.01)f	0.01 (0.52 ± 0.02)d	0.00 (0.00 ± 0.00)e
T_2 (*H. armigera* + EPB1)	74.94 (59.96 ± 1.73)bc	78.69 (62.51 ± 1.80)b	82.62 (65.36 ± 1.89)a	78.49 (62.37 ± 1.80)a
T_3 (*H. armigera* + EPB3)	75.16 (60.11 ± 1.74)bc	78.62 (62.67 ± 1.81)b	82.86 (65.55 ± 1.89)a	78.72 (62.53 ± 1.81)a
T_4 (*H. armigera* + EPB4)	59.06 (50.22 ± 1.45)de	62.01 (51.95 ± 1.50)de	65.11 (53.79 ± 1.55)b	61.85 (51.86 ± 1.50)cd
T_5 (*H. armigera* + EPB8)	64.42 (53.38 ± 1.54)cd	67.65 (55.33 ± 1.60)cd	71.03 (57.43 ± 1.66)b	67.48 (55.23 ± 1.59)bc
T_6 (*H. armigera* + EPB9)	48.32 (44.04 ± 1.27)e	50.74 (45.42 ± 1.31)e	53.27 (46.88 ± 1.35)c	50.61 (45.35 ± 1.31)d
T_7 (*H. armigera* + *Bt*)	73.69 (59.14 ± 1.71)bc	77.37 (61.60 ± 1.78)bc	81.24 (64.33 ± 1.86)a	77.18 (61.46 ± 1.77)ab
T_8 (*H. armigera* + EPB1 + EPB3)	78.00 (62.03 ± 1.79)ab	81.90 (64.82 ± 1.87)b	86.00 (68.02 ± 1.96)a	81.70 (64.67 ± 1.87)a
T_9 (*H. armigera* + Consortia)	85.90 (67.94 ± 1.96)a	90.19 (71.75 ± 2.07)a	89.70 (71.28 ± 2.06)a	85.22 (67.39 ± 1.95)a
T_{10} (Control)	-	-	-	-

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)
EDI – Emergence Disease Incidence; BCE – Biocontrol Efficiency; *Bt-Bacillus thuringiensis*
*Figures in parenthesis indicate the $\sqrt{x} + 0.5$ transformed values

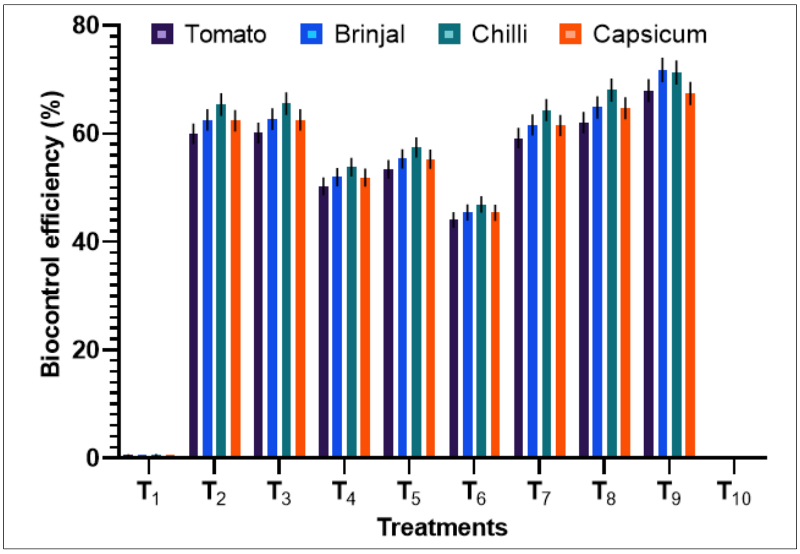

Fig 1: Biocontrol efficiency of different treatments of symbiotic bacteria of EPN on *Helicoverpa armigera* in Solanaceous vegetable crops grown in seedling trays under greenhouse conditions

Conclusion

In conclusion, the study showed the potential biocontrol ability of the symbiotic bacteria of entomopathogenic nematodes which are more effective than the commercially available bacillus thuringiensis. And the study has revealed that *Xenorhabdus* sp. were comparatively more virulent than the *Photorhabdus* sp. In future, these entomopathogenic bacteria are wide tested for their potential biocontrol abilities against the different insect pests and can be developed as a potential biocontrol agent in agriculturally important crops as a part of integrated pest management.

Acknowledgement

Authors are thankful to The Director, NBAIR, Hebbala, Bangalore- 560 065 for providing *Helicoverpa armigera* egg cards.

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