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Evaluation of biocontrol efficiency of symbiotic bacteria of entomopathogenic nematodes against *Plutella xylostella*, Diamondback Moth in cruciferous vegetable crops in seedling trays under greenhouse conditions

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Abstract
Photorhabdus and *Xenorhabdus* bacteria share a mutualistic relationship with *Steinernematidae* and *Heterorhabditidae* nematode families, respectively. The present study was undertaken to determine the biocontrol efficiency of the symbiotic bacteria of entomopathogenic nematodes for diamondback moth, *Plutella xylostella* in cruciferous vegetable crops. Biocontrol efficiency of symbiotic bacteria was determined in seedling trays with different treatment combinations of individual as well as combination of bacteria along with *Bacillus thuringiensis* as reference strain and the results had shown that the treatments imposed with the consortia of all the five bacterial isolates were able to show significantly higher biocontrol efficiency compared to the other treatment groups imposed with the individual symbiotic bacterial isolates. Among the treatments with individual bacterial isolates the treatments imposed with EPB3 (*Xenorhabdus* sp.) had significantly higher biocontrol efficiency compared to the *Photorhabdus* strains. This study shows that the symbiotic bacteria of entomopathogenic nematodes has greater potential to be exploited in the management of diamondback moth further in the field studies.

Keywords: *Photorhabdus*, *Xenorhabdus*, biocontrol efficiency, symbiotic bacteria and diamondback moth

Introduction
Diamondback moth (DBM), *Plutella xylostella* is one of the major pest in cruciferous vegetable crops including cabbage, cauliflower, turnip, Brussels, Chinese cabbage, radish and broccoli. Larvae of DBM cause complete defoliation of the foliage leaving the leaf veins intact and also it will disrupt the head formation. Repeated use of insecticides to control DBM has resulted in development of resistance particularly in sub-tropical and tropical countries, where farmers tend to apply mixtures of chemical insecticides sometimes more than twice a week (Sarfraz and Keddie, 2005) [1]. Consequently, alternative methods of insect control, including biocontrol agents, are being investigated (Mahar *et al.*, 2004) [2]. Microbial bio-pesticides are known to possess all these qualities and are now encouraged against several insect pests.

There is a presenting demand to find substitute for chemical pesticides due to the environmental and health risk issues associated with greater extent usage of chemicals and its residual accumulation in soil affecting soil health. To overcome this, currently the research is focused mainly on the *Bacillus thuringiensis* (Bt) toxins. There is also another potential field wherein the toxins produced by symbiotic bacteria (*Photorhabdus luminescens* and *Xenorhabdus nematophilus*) associated with the entomopathogenic nematodes (EPNs) can also be used to overcome this problem. The toxins are commonly termed as Tc toxins (toxin complex). *Photorhabdus luminescens* and *Xenorhabdus* spp. belong to gram negative bacterium form a symbiotic complex with Entamopathogenic nematodes *Heterorhabditis* spp. and *Steinernema* spp. respectively. These nematode-bacteria complexes are highly virulent to insect pests and are considered as one of the best alternative for non-chemical pest control. The symbiotic bacteria can be grow under *in-vitro* conditions after isolation from the nematode host. The toxins of bacteria are considered as highly potent biopesticides and the gene has the potency to be cloned into plants for development of insect resistant transgenic crops similar to *Bt* toxins (Nawaz *et al.*, 2016) [3].

This study set out to determine the biocontrol efficacy of symbiotic bacteria of EPNs in the management of DBM under greenhouse conditions.

We tested the bacterial isolates for their ability to infect and kill DBM larvae in laboratory experiments (Adithya *et al*., under publication). Based on the results of these experiments we then tested isolates in greenhouse experiment for their ability to control DBM and compared their efficacy with a commercially available biocontrol agent, *Bacillus thuringiensis* (*Bt*).

Materials and Methods: In the present study the biocontrol efficiency of symbiotic bacterial isolates isolated previously by Adithya *et al*. (2020) [4] against the *Plutella xylostella*, Diamondback moth (obtained from NBAIR, Hebbala) under greenhouse conditions in cruciferous vegetable crops namely Cabbage, Cauliflower, Knol-Khol and Broccoli. The bacterial biocontrol agents used were presented in the table below

Table 1: Symbiotic bacterial isolates used as biocontrol agents

S. No.	Symbiotic Bacterial isolate	Bacterial identity
1.	EPB1	*Photorhabdus luminescence* subsp. *thracensis*
2.	EPB3	*Xenorhabdus bovienii*
3.	EPB4	*P. luminescence* subsp. *Kayaii*
4.	EPB8	*P. caribbeanensis*
5.	EPB9	*P. luminescence* subsp. *Laumondi*
6.	*Bt*	*Bacillus thuringenesis**

*Obtained from Biofertilizers lab, Dept. of Agril. Microbiology

The experiment was carried out in the greenhouse located in Department of Agricultural Microbiology, UAS, GKVK, Bangalore. A total of ten treatments were imposed with three replications in completely randomized design to test the biocontrol efficiency of symbiotic bacterial isolates of EPN in cruciferous vegetable crops. The treatments imposed were are as follows: T_1 – Negative control (DBM alone), T_2 – DBM + EPB1, T_3 – DBM + EPB3, T_4 – DBM + EPB4, T_5 – DBM + EPB8, T_6 – DBM + EPB9, T_7 – DBM + *Bacillus thuringiensis*, T_8 – DBM + EPB1 + EPB3, T_9 - DBM + EPB1 + EPB3 + EPB4 + EPB8 + EPB9 and T_{10} – Absolute control (Sterile pot mixture).

Biological control efficacy was calculated using the following formula given by Guo *et al.* (2004).

$$BCE = \frac{(PIC-PIT)}{PIC} \times 100$$

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

Results and discussion

The results representing the biocontrol efficiency of symbiotic bacterial isolates against the insect pest diamondback moth, *Plutella xylostella* in cruciferous vegetable crops were presented in the Table 2 and Figure 1.

Cabbage

The effect of symbiotic bacterial isolates had shown prominent difference among the treatment groups for biocontrol efficiency against the insect pest DBM, *P. xylostella*. Highest biocontrol efficiency (BCE) was recorded in the treatment T_9 (DBM + EPB1 + EPB3 + EPB4 + EPB8 + EPB9) with 92.47% followed by T_8 (87.95%) and T_3 (84.21%), and are significantly different from the other treatments. Significant variation of BCE was exhibited by the symbiotic isolates treated in the seedling trays and uninoculated control (T_1 - DBM) had shown null biocontrol efficiency.

Cauliflower

The BCE was recorded highest in the treatment T_9 with 87.85% followed by the treatment T_8 (83.85%), T_3 (80.00%), and T_2 (74.70%), and are significantly different from the other treatment groups. The treatment uninoculated control (T_1) did not show any biocontrol efficiency, may be due to lack of symbiotic bacterial isolates in the treatments.

Knol-Khol

The BCE of symbiotic bacterial isolates against the diamondback moth in greenhouse conditions were recorded and highest percent biocontrol efficiency was observed in the treatment T_9 supplemented with *P. xylostella* and consortia of symbiotic bacterial isolates with 91.47% followed by T_8 and T_3 with 89.47% and 84.21%, respectively and are significantly different from each other. The treatment uninoculated control, T_1 did not show any biocontrol efficiency.

Broccoli

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 (96.05%) followed by T_8 (93.95%) and T_3 (88.42%), and are in parallel as well as considerably different from other treatments. Significant variation of BCE was exhibited by the symbiotic isolates treated in the seedling trays and uninoculated control (T_1 - DBM) had shown null biocontrol efficiency.

Similar results were witnessed by Schroer and Ehlers (2005) [5] for *S. carpocapsae* and combination of the nematode and *B. thuringiensis* with a polymer. Other researchers have reported on the efficacy of EPNs for control of *P. xylostella* with similar results, i.e., less than 50% of targeted populations were controlled with high concentrations of EPNs (Somvanshi *et al.*, 2006) [6]. Nyasani *et al.* (2008) [7] used three EPN isolates, *Steinernema* sp., *S. weiseri* and *H. indica* in a field experiment to test their ability to reduce DBM populations and damage to kale and the results had shown that all the three nematodes caused significant reductions in populations of DBM and DBM damage, with the population reductions being similar to those caused by application of *Bacillus thuringiensis* ssp. *kurstaki*.

NanGong *et al.* (2016) [8] reported that *Xenorhabdus nematophila* HB310 has high insecticidal activity when fed to DBM along with *B. thuringiensis* which indicates that no cross-resistance exists between *X. nematophila* and *B. thuringiensis*. Although clear evidence is not yet available for *X. nematophila*, that these two bacteria have different modes of action and that the Xn toxin toxins and Bt Cry proteins utilize different binding sites within those moths. Whereas *Bt*

mainly targets the gut epithelium, natural infections involving *Xenorhabdus* primarily begin from the haemocoel after release from the nematode hosts (Nielsen-Leroux *et al.*, 2012). Because of their non-overlapping and cooperative pathogenic pathways, *Bt*-resistant DBM remained susceptible to *X. nematophila* throughout the experimental period there by out coming the effect *Bt* resistant diamondback moth when treated with symbiotic bacteria of EPNs.

Table 2: Biocontrol efficiency of Symbiotic bacteria on *Plutella xylostella* in Cruciferous vegetables grown in seedling trays under greenhouse condition

Treatments	BCE* (per cent)			
	Cabbage	**Cauliflower**	**Knol-Khol**	**Broccoli**
T_1 (*Plutella xylostella* alone)	0.01 (0.51 ± 0.01)g	0.01 (0.50 ± 0.01)g	0.00 (0.00 ± 0.00)f	0.00 (0.00 ± 0.00)g
T_2 (*P. xylostella* + EPB1)	78.64 (62.47 ± 1.80)cd	74.70 (59.80 ± 1.73)bcd	78.42 (62.32 ± 1.80)bc	82.34 (65.15 ± 1.88)cd
T_3 (*P. xylostella* + EPB3)	84.21 (66.59 ± 1.92)bc	80.00 (63.43 ± 1.83)abc	84.21 (66.59 ± 1.92)ab	88.42 (70.11 ± 2.02)bc
T_4 (*P. xylostella* + EPB4)	73.69 (59.14 ± 1.71)d	70.00 (56.79 ± 1.64)cd	73.68 (59.14 ± 1.71)cd	77.37 (61.59 ± 1.78)de
T_5 (*P. xylostella* + EPB8)	69.16 (56.27 ± 1.62)de	65.70 (54.15 ± 1.56)de	63.16 (52.63 ± 1.52)de	66.32 (54.52 ± 1.57)ef
T_6 (*P. xylostella* + EPB9)	52.64 (49.97 ± 1.44)ef	55.70 (48.28 ± 1.39)ef	68.42 (55.81 ± 1.61)cde	71.84 (57.95 ± 1.67)def
T_7 (*P. xylostella* + *Bt*)	52.11 (46.21 ± 1.33)f	49.50 (44.72 ± 1.29)f	57.89 (49.54 ± 1.43)e	60.79 (51.23 ± 1.48)f
T_8 (*P. xylostella* + EPB1 + EPB3)	87.95 (69.69 ± 2.01)ab	83.55 (66.07 ± 1.91)ab	89.47 (71.07 ± 2.05)a	93.95 (75.76 ± 2.19)ab
T_9 (*P. xylostella* + Consortia)	92.47 (74.08 ± 2.14)a	87.85 (69.60 ± 2.01)a	91.47 (73.02 ± 2.11)a	96.05 (78.53 ± 2.27)a
T_{10} (Absolute 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

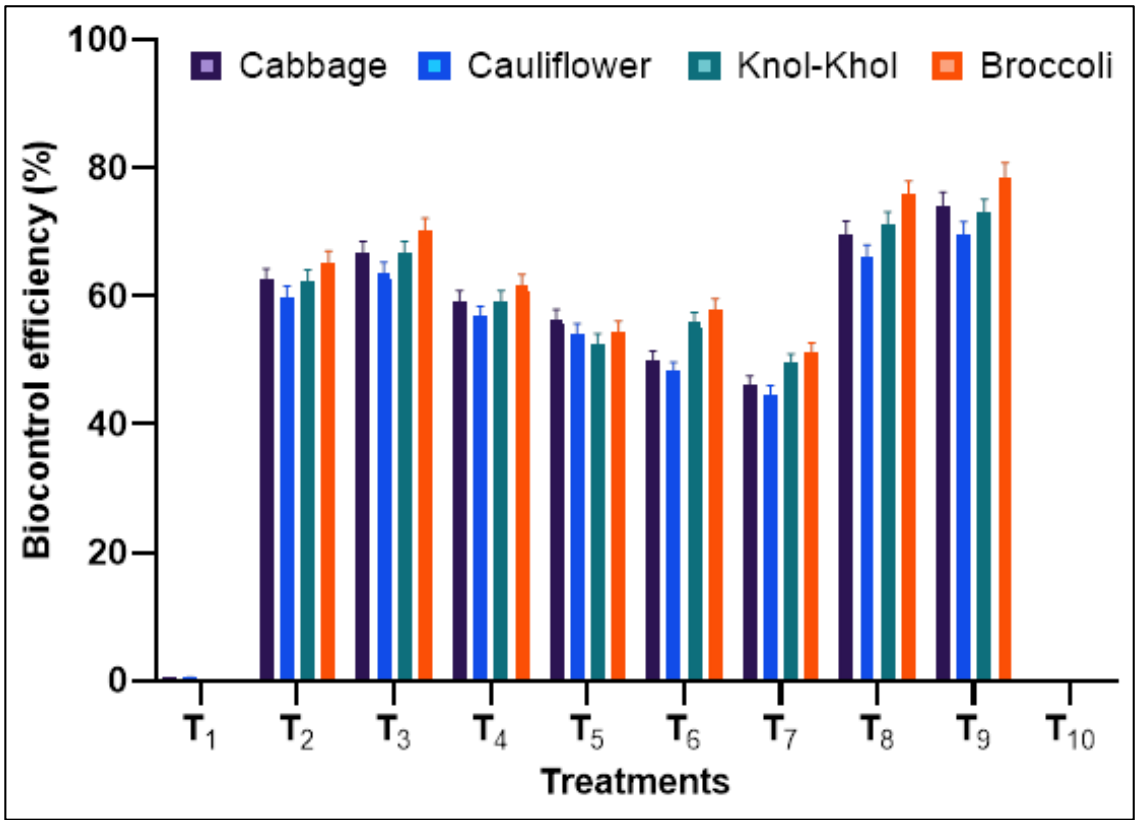

Fig 1: Biocontrol efficiency of different treatments of symbiotic bacterial isolates on *Plutella xylostella* in Cruciferous vegetable crops grown in seedling trays under greenhouse conditions.

Conclusion

In conclusion, our study clearly implies that symbiotic bacteria of entomopathogenic nematodes have the ability of reducing damage caused by DBM larvae and they can thus be used as biopesticides against this important pest. Among the symbiotic bacteria, *Xenorhabdus* had comparatively more potential than the *Photorhabdus* which has to be further confirmed with the field studies.

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