The Bioscan 16(3): 183-187, 2021
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EVALUATION OF BIOCONTROL EFFICIENCY OF ENTOMOPATHOGENIC BACTERIA AGAINST TOBACCO CATERPILLAR (*Spodoptera litura* F.) IN SOLANACEOUS VEGETABLE CROPS GROWN IN SEEDLING TRAYS UNDER GREENHOUSE CONDITION

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KEYWORDS
Biocontrol
Entomopathogenic bacteria
Photorhabdus
Xenorhabdus

Received on :
30.12.2020

Accepted on :
10.07.2021

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ABSTRACT
An investigation was carried out to test the biocontrol efficiency of entomopathogenic bacteria in solanaceous vegetable crops grown in seedling trays under greenhouse conditions. *Spodoptera litura* is one of the serious pest in solanaceous vegetable crops namely tomato, brinjal, chilli and capsicum. Entomopathogenic bacteria, *Photorhabdus* and *Xenorhabdus* were tested for their biocontrol efficiency with individual as well as consortia of bacteria isolates with *Bacillus thuringiensis* as reference strain. Among the treatments, T_9 treatment received all the five entomopathogenic bacteria had shown 93 % of biocontrol efficiency in brinjal and 89% in tomato, chilli and capsicum. In case of treatments groups received individual organisms' treatment T_3 imposed with EPB3 (*Xenorhabdus* sp.) had shown significantly higher biocontrol efficiency of 75% followed by T_2 imposed with EPB1 (*Photorhabdus luminescence*) with 65% compared to the reference strain *B. thuringiensis* with ~50% biocontrol efficiency. Apart from the biocontrol efficiency, these entomopathogenic bacteria had shown significant effect in enhancing the seedling vigour of the vegetable crops. 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.

INTRODUCTION

Cultivation of vegetables is now becoming a viable commercial enterprise with the introduction of liberal trade policies throughout the year in all parts of the country. Solanaceous vegetables namely tomato, brinjal, chilli, potato and capsicum are grown in an area of 32.98 lakh ha with the production of 441.7 lakh tones. They constitutes ~ 47% in the total vegetable production of the country. Inadequate availability of pest and disease free high quality planting material as well as slow dissemination and adaptability of improved high seeds, inadequate facility for identification of nutrient deficiency, lack of pests and diseases outbreak forecast services *etc.* are the major limiting factors solanaceous vegetables productivity growth (Anonymous, 2015).

Spodoptera litura is a polyphagous pest in tropical countries causing huge damage to economically important cultivated field crops and also a significant pest of crops such as tomato, brinjal, capsicum, sweet peppers and chilli in glasshouse cultivation (Vashisth *et al.*, 2012). The larvae feeds voraciously on the leaves of the host plants resulting in skeletonization of leaves. Although they can be controlled by insecticides, there have been instance where they have developed resistance.

Repeated usage of insecticides against *S. litura* over a period of time has driven the development of insecticide resistance to many older conventional pesticides such as organophosphates and pyrethroids as well as to latest insecticides like spinosad, avermectins and imidacloprid (Abbas *et al.*, 2012, Armes *et al.*, 1997, Imran *et al.*, 2017). Variation in detoxification enzyme activity among S. litura from different origins can be matched to insecticide usage patterns (Karuppaiah *et al.*, 2017). Hence the researchers are directed towards the bio-control of tobacco caterpillar, *S. litura*.

Photorhabdus spp. and *Xenorhabdus* spp. are gram-negative motile bacteria lives in symbiotic relationship with genus *Heterorhabditis* spp.and *Steinernema* spp. of entomopathogenic nematodes (EPNs), respectively(Herbert and Goodrich-Blair, 2007, Waterfield *et al.*, 2009). In the nature when the EPNs enter the larvae they release the bacteria into the gut which causes septicemia and finally death of the larvae by the range of toxins produced by the symbiotic bacteria. Over the past few years, considerable progress has been made in cloning of toxin genes from *Photorhabdus* and *Xenorhabdus* (Ffrench-Constant, 2007).Because of the significant pathogenic capability against some important pests of commercial crops, *Photorhabdus* spp. and *Xenorhabdus* spp. are considered to be new biological insecticides similar to *Bacillus thuringiensis* (Bt). With this background, the present investigation was carried to evaluate the biocontrol efficiency of entomopathogenic bacteria against the insect pest *S. litura* in solanaceous 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). *Bacillus thuringiensis* (*Bt*) strain was obtained from Biofertilizer lab, Department of Agricultural Microbiology, GKVK, Bangalore.The *Spodoptera litura* 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. 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 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 (*Spodoptera litura* alone)
T_2 -	*S. litura* + EPB1
T_3 -	*S. litura* + EPB3
T_4 -	*S. litura* + EPB4
T_5 -	*S. litura* + EPB8
T_6 -	*S. litura* + EPB9
T_7 -	*S. litura* + *Bt*
T_8 -	*S. litura* + EPB1 + EPB3
T_9 -	*S. litura* + Consortia
T_{10} -	Control (Sterilized soil)

Observations recorded

During the experimental period, germination percentage, root length, shoot length seedling vigour index (SVI) and biocontrol efficiency (BCE) was recorded. Biological control efficacy against the insect pest was calculated using the formula given by Guo *et al.* (2004).

Statistical analysis

All the experimental data were subjected to statistical analysis using analysis of variance (ANOVA) (Gomez and Gomez, 1984) 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).

RESULTS AND DISCUSSION

Efficiency of symbiotic bacteria on wilt pathogens in enhancing seedling vigour of solanaceous vegetable crops

The data pertaining the efficiency of entomopathogenic bacteria in enhancing seedling vigour in solanaceous vegetable crops were presented in Table 1 and Table 2

Tomato

The maximum percent germination of seeds were recorded in treatment T_9 (*S. litura* + Consortium) with 94.44% followed by T_8 (*S. litura* + EPB1 + EPB3) with 91.67% germination. Least per cent germination was observed in the treatment T_1 (*S. litura*) of 44.44%. The data related to the efficiency of symbiotic bacterial in enhancing seedling vigour of cabbage is given in Table 61. The highest root length (7.56 cm) was observed in the treatment T_9 (*S. litura* + Consortia) followed by T_8 (7.21 cm), and are significantly different from the other treatments. The lowest root length of 4.12 cm was recorded in the negative control (T_1). The shoot length was recorded highest in the treatment T_1 (9.52 cm) followed T_8 (9.12 cm) and T_3 (8.80 cm), are considerably different from each other. There is a promising difference in the seedling vigour index (SVI) in the treatment groups and highest SVI was reported in the treatment T_9 (1613) followed by T_8 (1497) and T_3 (1359) and are considerably different from each other.

Brinjal

The highest percent germination was noted in the treatment T_9 (*S. litura* + Consortium) with 86.11% followed by T_8 (*S. litura* + EPB1 + EPB3) and T_3 (*S. litura* + EPB3) with 80.56% and 75%, are significantly different from each other. The percent germination was observed lowest in the treatment T_1 (*S. litura*) with 50%. The effect of symbiotic bacterial isolates on seedling vigour index in cauliflower crop is presented in the Table 1. The highest root length was observed in the treatment T_9 (6.38 cm) followed by T_8 (6.32 cm), T_6 (6.12 cm) and T_3 (5.92 cm) which are in parallel and considerably different from the other treatments. The shoot length was recorded highest in the treatment T_9 (5.48 cm) followed T_8 (5.36 cm) and T_3 (5.36 cm), are significantly different from each other. There is a promising difference among the treatments for the seedling vigour index treated with the symbiotic bacterial isolates. Highest seedling

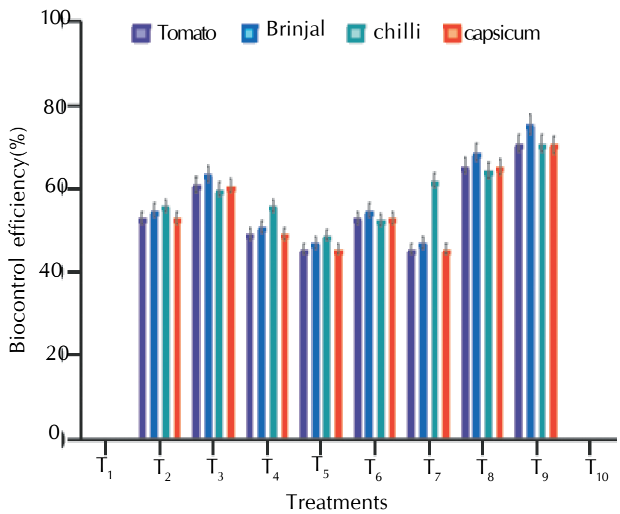

Figure 1. Biocontrol efficiency of different treatments of symbiotic bacterial isolates on *Spodoptera litura* in Solanaceous vegetable crops grown in seedling trays under greenhouse conditions.

Table 1: Effect of entomopathogenic bacteria in enhancing seedling vigour of tomato and brinjal grown in seedling trays under greenhouse condition

Treatments	Tomato				Brinjal			
	Percent Germination	Root length(cm)	Shoot length(cm)	SVI	Percent Germ ination	Root length (cm)	Shoot length (cm)	SVI
T_1 *(Spodoptera litura)*	44.44^{h}	4.12^{f}	6.50^{h}	472^{g}	50.00^{f}	4.16^{e}	3.60^{e}	388^{f}
T_2 (*S. litura* + EPB1)	75.00^{ef}	6.35^{cd}	8.50^{cd}	1114^{e}	69.44^{d}	5.76^{c}	4.89^{d}	858^{d}
T_3 (*S. litura* + EPB3)	86.11^{c}	6.98^{b}	8.80^{bc}	1359^{c}	75.00^{c}	5.92^{bc}	5.36^{ab}	909^{c}
T_4 (*S. litura* + EPB4)	80.56^{d}	6.45^{c}	8.10^{e}	1172^{d}	66.67^{d}	5.44^{d}	5.16^{bc}	824^{d}
T_5 (*S. litura* + EPB8)	77.78^{de}	6.42^{cd}	7.90^{e}	1114^{e}	66.67^{d}	5.42^{d}	4.88^{d}	744^{e}
T_6 (*S. litura* + EPB9)	72.22^{fg}	6.65^{c}	8.20^{de}	1073^{e}	69.44^{d}	5.92^{bc}	4.94^{cd}	754^{e}
T_7 (*S. litura* + *Bt*)	69.44^{g}	6.12^{d}	7.50^{f}	946^{f}	61.11^{e}	6.12^{ab}	5.15^{bc}	751^{e}
T_8 (*S. litura* + EPB1 + EPB3)	91.67^{b}	7.21^{b}	9.12^{b}	1497^{b}	80.56^{b}	6.32^{a}	5.36^{ab}	973^{b}
T_9 (*S. litura* + Consortia)	94.44^{ab}	7.56^{a}	9.52^{a}	1613^{a}	86.11^{a}	6.38^{a}	5.48^{a}	1054^{a}
T_{10} (Control)	97.22^{a}	5.45^{e}	7.10^{g}	1220^{d}	88.89^{a}	5.24^{d}	4.78^{d}	946^{bc}

Note: Means with same superscript, in a column do not differ significantly at P= <0.05 as per Duncan Multiple Range Test (DMRT).*Bt- Bacillus thuringiensis;* SVI- Seedling vigour index

Table 2: Effect of entomopathogenic bacteria in enhancing seedling vigour of Chilli and Capscicum grown in seedling trays under greenhouse condition

Treatments	Chilli				Capsicum			
	Percent Germination	Root length(cm)	Shoot length(cm)	SVI	Percent Germination	Root length(cm)	Shoot length(cm)	SVI
T_1 *(Spodoptera litura)*	44.44^{e}	4.66^{g}	4.05^{f}	387^{g}	50.00^{g}	5.60^{f}	7.18^{e}	639^{g}
T_2 (*S. litura* + EPB1)	77.78^{c}	8.23^{bc}	6.30^{cd}	1130^{d}	80.56^{cd}	7.28^{cd}	8.85^{bc}	1120^{d}
T_3 (*S. litura* + EPB3)	80.56^{bc}	8.52^{b}	6.42^{cd}	1204^{c}	80.56^{cd}	7.56^{c}	9.02^{b}	1244^{c}
T_4 (*S. litura* + EPB4)	77.78^{c}	7.92^{cd}	6.28^{cd}	1104de	77.78^{d}	7.32^{cd}	8.10^{d}	1028^{e}
T_5 (*S. litura* + EPB8)	69.44^{d}	7.53^{e}	6.58^{bc}	980^{f}	72.22^{e}	7.14^{d}	8.52^{cd}	1044^{e}
T_6 (*S. litura* + EPB9)	72.22^{d}	8.38^{b}	6.25^{d}	1057^{e}	69.44^{ef}	7.48^{cd}	8.46^{cd}	1107^{d}
T_7 (*S. litura* + *Bt*)	77.78^{c}	7.65^{de}	6.42^{cd}	1094de	66.67^{f}	7.36^{cd}	8.32^{d}	958^{f}
T_8 (*S. litura* + EPB1 + EPB3)	83.33^{b}	8.95^{a}	6.83^{ab}	1315^{b}	83.33^{c}	8.16^{b}	9.12^{b}	1392^{b}
T_9 (*S. litura* + Consortia)	88.89^{a}	9.05^{a}	7.05^{a}	1431^{a}	88.89^{b}	8.52^{a}	9.76^{a}	1574^{a}
T_{10} (Control)	91.67^{a}	6.57^{f}	5.65^{e}	1120^{d}	94.44^{a}	6.12^{e}	8.25^{d}	1277^{c}

Note: Means with same superscript, in a column do not differ significantly at P= <0.05 as per Duncan Multiple Range Test (DMRT).*Bt- Bacillus thuringiensis;* SVI- Seedling vigour index

vigour index was observed in the treatment T_9 (1054) followed by T_8 (973) and T_3 (909).

Chilli

Seed germination percent was observed highest in the treatment T_9 (*S. litura* +Consortium) with 88.89% followed by T_8 and T_3 with 83.33% and 80.56%, respectively and promisingly different from each other as well as from other treatments. The percent germination was recorded lowest in the treatment T_1 supplemented with only *S. litura* (44.44%). The data representing the efficiency of symbiotic bacterial in enhancing seedling vigour of Knol-Khol is presented in Table 2. Significantly highest root length was witnessed in the T_9 (9.05 cm) followed by the treatment T_8 (8.95 cm) and T_3 (8.52 cm), and are significantly different from each other. Similar trend was there with respect to the shoot length with highest shoot length in T_9 (7.05 cm) followed by T_8 (6.83 cm). There is a prominent difference in the treatment groups related to seedling vigour index (SVI) was noticed and highest SVI of 1431 was reported in T_9 followed by T_8 (1315) and T_3 (1204), and are significantly different from each other.

Capsicum

The germination percent was recorded highest in the treatment T_9 (*S. litura* +Consortium) with 88.89% followed by T_8 (*S. litura* +EPB1+EPB3) and T_3 (*S. litura* +EPB3) with 83.33% and 80.56% of germination, respectively and are considerably different from each other. The lowest percent germination was observed in the treatment T_1 (*S. litura*) of 50%. Significantly highest root length was recorded in the T_9 (8.52 cm) followed by the treatment T_8 (8.16 cm) and T_3 (7.56 cm), are in parallel with each other and significantly different from other treatments. The highest root length of 9.76 cm was observed in the treatment T_9 followed by T_8 (9.12 cm) and T_3 (9.02 cm). There is a significant difference between the treatments pertaining to seedling vigour index treated with the symbiotic bacterial isolates. Highest seedling vigour index was observed in the treatment T_9 (1574) followed by T_8 (1392) and T_3 (1244). The lowest SVI was observed in the uninoculated negative control treatment T_1 (639).

Evaluation of biocontrol efficiency of entomopathogenic bacteria against the insect pest *Spodoptera litura*

The results pertaining to the biocontrol efficiency of entomopathogenic bacteria against *Spodoptera litura* in solanaceous vegetable crops were presented in the Table 3 and Figure 1.

Tomato

The effect of entomopathogenic bacterial isolates had shown prominent difference among the treatment groups for biocontrol efficiency against the insect pest *S. litura*. The highest biocontrol efficiency (BCE) was recorded in the treatment T_9 (*S. litura* + Consortia) with 89.25% followed by T_8 (82.88%)

Table 3:Biocontrol efficiency of entomopathogenic bacteria on *Spodoptera litura* in Solanaceous vegetable crops grown in seedling trays under greenhouse condition

Treatments	BCE (per cent)			
	Tomato	Brinjal	Chilli	Capsicum
T_1 *(Spodoptera litura)*	0 (0.00 ± 0.00)^{e}	0 (0.00 ± 0.00)^{e}	0 (0.00 ± 0.00)^{g}	0 (0.00 ± 0.00)^{e}
T_2 *(S. litura* + EPB1)	63.75 (52.98 ± 1.53)^{c}	66.94 (54.90 ± 1.58)^{c}	68.82 (56.06 ± 1.62)^{cde}	63.59 (52.89 ± 1.53)^{c}
T_3 *(S. litura* + EPB3)	76.5 (61.00 ± 1.76)^{b}	80.33 (63.67 ± 1.84)^{b}	74.71 (59.81 ± 1.73)^{bc}	76.31 (60.87 ± 1.76)^{b}
T_4 (*S. litura* + EPB4)	57.38 (49.24 ± 1.42)^{cd}	60.24 (50.91 ± 1.47)^{cd}	68.82 (56.06 ± 1.62)^{cd}	57.23 (49.16 ± 1.42)^{cd}
T_5 (*S. litura* + EPB8)	51 (45.57 ± 1.32)^{d}	53.55 (47.04 ± 1.36)^{d}	57.06 (49.06 ± 1.42)$^{d\ f}$	50.87 (45.50 ± 1.31)^{d}
T_6 (*S. litura* + EPB9)	63.75 (52.98 ± 1.53)^{c}	66.94 (54.90 ± 1.58)^{c}	62.94 (52.50 ± 1.52)^{def}	63.59 (52.89 ± 1.53)^{c}
T_7 (*S. litura* + *Bt*)	51 (45.57 ± 1.32)^{d}	53.55 (47.04 ± 1.36)^{d}	78.06 (62.07 ± 1.79)^{bc}	50.87 (45.50 ± 1.31)^{d}
T_8 (*S. litura* + EPB1 + EPB3)	82.88 (65.55 ± 1.89)^{ab}	87.02 (68.88 ± 1.99)^{b}	81.47 (64.50 ± 1.86)^{ab}	82.67 (65.40 ± 1.89)^{ab}
T_9 (*S. litura* + Consortia)	89.25 (70.86 ± 2.05)^{a}	93.71 (75.48 ± 2.18)^{a}	89.35 (70.96 ± 2.05)^{a}	89.03 (70.65 ± 2.05)^{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).BCE – Biocontrol Efficiency; *Bt-Bacillus thuringiensis;**Figures in parenthesis indicate the "5ØeÜ + 0.5 transformed values.

and T_3 (76.50%), 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 - *S. litura*) had shown null biocontrol efficiency.

Brinjal

The BCE of symbiotic bacterial isolates against the insect pest *S. litura* in greenhouse conditions were recorded and highest percent biocontrol efficiency was observed in the treatment T_9 supplemented with *S. litura* and consortia of symbiotic bacterial isolates with 93.71% followed by T_8 and T_3 with 87.02% and 80.33%, respectively and differs significantly from each other. The treatment uninoculated control, T_1 hasn't shown any biocontrol efficiency.

Chilli

The BCE was recorded highest in the treatment, T_9 (*S. litura* + Consortia) with 89.35% followed by the treatment T_8 (*S. litura* + EPB1 + EPB3) and T_7 (*S. litura* + *Bt*) with 81.47% and 78.06%, respectively 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.

Capsicum

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 (89.03%) followed by T_8 (82.67%) and T_3 (76.31%), and are at par which each other 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 - *S. litura*) had shown null biocontrol efficiency.

Benfarhat Touzri *et al*. (2014) demonstrated improvement in toxicity when *P. luminescens* and *B. thuringiensis* were used in combination against *Spodoptera littoralis*, leading to a strategy allowing the use of *P. luminescens* without its nematode host. The use of mixture of wild-type insecticidal strains would be cost-effective than constructing recombinants or pure toxins for the development of formulations as final bio-product. These findings paves a way for dealing the resistance problem of *B. thuringiensis* in insect pests in field conditions. Similarly, Rezaei *et al*. (2015) tested the pathogenecity of entomopathogenic nematodes *S. feltiae* and *H. bacteriophora* against the greenhouse whitefly, *Trialeurodes vaporariorum* and the results indicated that highest mortality of larval instrars was observed when the EPNs were applied in combinations. The study reported herein establishes the basis for further applied studies to search for elaborate, new environmentally compatible strategies that allow the enhancement of pest control and crop health in greenhouse productions.

The entomopathogeinc nematodes, *Heterorhabditis amazonensis* along with the combination of different percentages of insecticides were used to control the tomato leaf minor (*Tuta absoluta*) in tomato crop under greenhouse conditions and the results showm control of leaf minor significantly compared to other control methods and suggested that the preventive soil application of EPNs will be a beter stategy to contorl tomato leaf minor as well as helps in the reduction of inapprapriate use of insecticides (Sabino et al., 2019) .

The effciency of EPN *Steinernema* sp. and its bacterial partner *Xenorhabdus nematophila* were evaluated for biological control effeciency against rootknot nematode in tomato and results shown in the reduction of gall index in the treatments imposed with biocontrol agents and a significant increase in the yield of tomato compared to control group (Kepenekci *et al*., 2018).

CONCLUSION

In the present study, entomopathogenic bacteria had shown significantly higher biocontrol efficiency in the solanaceous vegetable crops compared to the control. The efficency of these entomopathogenic bacteria has to be further tested in the field conditions and can be tested their compatibility with the different concentrations of insecticides so as to include them as a part of the integrated pest management.

Conflict of interest: The authors declare no conflict of interest

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