The Pharma Innovation Journal 2021; SP-10(11): 148-152

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(11): 148-152
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www.thepharmajournal.com
Received: 10-09-2021
Accepted: 12-10-2021

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Effect of PGPR on the incidence of shoot and fruit borer, *Earias vittella* (Fabricius) in okra

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Abstract
Okra, *Abelmoschus esculentus* L. (Moench) is an important vegetable crop grown throughout the world and its economic production is hampered by shoot and fruit borers. Plant growth promoting rhizobacteria (PGPR) are well known to induce biochemicals and defence enzymes against the insect pests of crop plants. Okra plants inoculated with Bacillus subtilis had low incidence of *Earias vittella* (Fabricius) and recorded higher amount of biochemicals and defence enzymes than the untreated plants in both Rabi 2020 and Summer 2021 seasons. Bacillus subtilis could be used as a tool to induce systemic resistance against the shoot and fruit borer of okra.

Keywords: PGPR, okra, *Earias vittella, Bacillus subtilis*, induced resistance

Introduction
Okra, *Abelmoschus esculentus* L. (Moench) (F: Malvaceae) is an economically important vegetable crop and a significant contributor to vegetable production in India. Okra is a rich source of Vitamin A, Vitamin B and minerals (Habib *et al.*, 2016) [6]. India ranks first in okra production accounting for 73 per cent of total global production. In India, okra is being cultivated in an area of 5.26 lakh hectares with an average productivity of 12.28 tonnes per hectare (Panwar *et al*., 2019) [10]. However, infestation of insect pests is the major concern in okra cultivation and more than 72 species of insect pests are known to infest the crop and cause significant yield loss (Srinivasan and Krishnakumar 1983) [13].
The shoot and fruit borer, *Earias vittella* F. is one of the major destructive pests of okra and causes damage by boring into the growing shoots, flower buds, flowers and young fruits. Infestation results in drooping of shoots, shedding of the fruiting bodies and distorted fruits plugged with excreta. The shoot and fruit borers could cause 22 to 54 per cent yield loss in okra (Suryawanshi *et al*., 2000) [14].
Plant growth promoting rhizobacteria (PGPR) regulate plant signaling pathways and result in the production of secondary metabolites which confer resistance against insect pests (Kloepper 1978) [7]. PGPR induced systemic resistance (ISR) showed that the host plants are able to withstand herbivore attack through increased production of secondary metabolites at equal amounts when the plants are damaged by herbivores (Choudhary *et al.*, 2007) [4]. This study evaluated the performance of the PGPR strains *viz*., *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Rhizobium puscense*, *Ensifer* spp. and *Siphanobactor* spp. against the shoot and fruit borer in okra.

Materials and Methods
The effect of PGPR strains on the incidence of shoot and fruit borer was evaluated under field conditions at Agricultural College and Research Institute, Killikulam farm during Rabi 2020 and Summer 2021 seasons. Okra F_1 hybrid COBh 4 seeds were treated with talc formulations @ 10g/kg of seed containing $1x10^8$ cfu/g of *B. subtilis* Bbv57, *B. amyloliquefaciens*, *Rhizobium puscense*, *Ensifer* spp. and *Siphanobactor* spp. The experiments were conducted in completely randomized block design with three replications. Soil application of each PGPR @ 2.5 kg/ha was done before sowing and foliar spray of PGPR formulations @ 5g per litre of water was done on 30 DAS. Seed treatment with Imidacloprid 48 FS @ 7g/kg of seed served as chemical check. The treated seeds were sown in 5x3m plots with a spacing of 45x30 cm and raised as per the package of practices recommended by TamilNadu Agricultural University.
Shoot infestation by *E. vittella* was recorded on 25 randomly selected plants per replication at 35 days after sowing (DAS) and 45 DAS. Total number of healthy and infested shoots were

counted and per cent shoot damage was worked out. Fruit infestation was recorded in ten randomly selected plants. Total number of fruits and *E. vittella* infested fruits were counted from first harvest to last harvest and mean per cent fruit damage was worked out.

Estimation of biochemicals

Total phenol, tannin and defence enzyme activity *viz*., peroxidase, polyphenol oxidase and PAL activity were estimated as per the standard protocols during both Rabi 2020 and Summer 2021. Plant samples were collected at 30 DAS from randomly selected five plants for the estimation of biochemicals and defence enzymes. Similarly, the induction of biochemicals and defence enzyme activity was estimated at 72 hours after the foliar application of PGPR formulations.

Total phenol

The total phenol was estimated by as per the protocol given by Maliak and Singh (1980) [9]. Phenol was extracted from the collected leaf samples using Folin – Ciocalteau reagent and absorbance was measured at 650 nm using UV-VIS spectrophotometer (Agilent Cary Win®). The phenol content was expressed as mg/g on fresh weight basis.

Tannin

Tannin content of leaf sample was determined using AOAC (1980) [1] method with some modifications using Folin-Denis reagent and saturated sodium carbonate and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g on dry weight basis.

Defense enzymes activity

Peroxidase activity (PoX) (Putter, 1974) was studied by measuring the changes in absorbance read at 430 nm and recorded at every 30 seconds interval for three minutes and expressed as changes in absorbance at 430 nm min^{-1} g^{-1} of tissue. The Phenyl alanine ammonia lyase (PAL) activity (Brueske 1980) [3] was measured at 290 nm expressed as µ mol^{-1} min^{-1} g^{-1} of tissue. Polyphenol oxidase (PPO) activity was determined as per the procedure given by Augustin *et al* (1985) [2]. The rate of change in absorbance was determined at 410 nm every 30 seconds for three minutes against the blank and expressed as unit min^{-1} g^{-1} of tissue.

Results

Effect of PGPR on the incidence of fruit and shoot borer *E. vittella* in okra

The field experiments revealed that the shoot damage was less in the PGPR treated plants over the untreated plants during Rabi 2020 and Summer 2021 (Table 1 and 2). Incidence of *E. vittella* was less during the vegetative stage and increased during the reproductive stage. During Rabi 2020, shoot infestation by *E. vittella* was low in *B. subtilis* treated plants (1.33% and 12.15%) compared to 12.81 per cent and 22.34 per cent infestation in imidacloprid treated okra plants on 35 DAS and 45 DAS respectively. The shoot infestation in untreated plants was higher than all other PGPR treatments at 35 DAS (16.66%) and 45 DAS (25.33%). Similarly, during Summer 2021 season the shoot damage caused by *E. vittella* was low in *B. subtilis* treated plants on 35 DAS (1.33%) and 45 DAS (14.30%) when compared with infestation on the untreated plants at 35 DAS (18.67%) and 45 DAS (31.33%).

The fruit damage by *E. vittella* was less in *B. subtilis* treated plants (8.64%) during Rabi 2020 followed by *B. amyloliquefaciens* treated plants (9.88%). The imidacloprid treated plants and untreated plants recorded fruit damage as 17.25 and 19.25 per cent respecively. During Summer 2021, *B. subtilis* recorded 10.65% fruit damage followed by *B. amyloliquefaciens* (13.33%). However, the imidacloprid treated plants and untreated plants had 19.12 and 24.40 per cent fruit damage respectively.

Effect of PGPR on biochemical activity in okra

Phenol, tannin content and defense enzyme activity were increased significantly in PGPR applied plants. *B. subtilis* treated plants recorded the phenol content as 1.96 mg g^{-1} at 72 hours after the foliar which was higher than all other PGPR treatments and imidacloprid treated plants (1.09 mg g^{-1}). Plants inoculated with *B. subtilis* had increased tannin content in plant sample (1.98 mg g^{-1}) followed by *B. amyloliquefaciens* (1.49 mg g^{-1}) treatment. Both Phenol (0.54 mg g^{-1}) and tannin content (0.99 mg g^{-1}) were low in the untreated plants. The activity of defense enzymes like Peroxidase, Polyphenol oxidase and Phenyl alanine ammonia lyase showed a significant increase in all treatments at 72 hours after foliar application of PGPR strains compared to untreated check. Among the PGPR, *B. subtilis* treated plants showed significantly increased activity of defense enzymes *viz*., Peroxidase, Polyphenol oxidase and Phenyl alanine ammonia lyase (14.72 min^{-1} g^{-1}, 16.01 min^{-1} g^{-1} and 113.64 µM $min^{-1}g^{-1}$ respectively) at 72 hours after treatment (HAT) compared to 3.88 $min^{-1}g^{-1}$, 6.92 $min^{-1}g^{-1}$ and 60.31 µM min^{-1} g^{-1} respectively in imidacloprid treatment. Whereas, the untreated control plants showed low activity of Peroxidase (2.09 min^{-1} g^{-1}), Polyphenol oxidase (3.44 min^{-1} g^{-1}) and Phenyl alanine ammonia lyase **(**50.39 µM min^{-1} g^{-1}**)**.

Biochemical analysis of plants during Summer 2021 season showed that the phenol and tannin content of the plants increased significantly in *B. subtilis* treated plants (2.54 mg/g) followed by *B. amyloliquefaciens* (1.71 mg/g) treated plants. The tannin content of plants after application of *B. subtilis* was significantly increased (2.37 mg g^{-1}) over the untreated plants (1.18 mg g^{-1}) and imidacloprid treated plants (1.28 mg g^{-1}). Similarly, the activity of defense enzymes increased significantly after foliar application of PGPR formulations compared to the untreated control. At 72 HAT, the defense enzymes Peroxidase, Polyphenol oxidase and Phenyl alanine ammonia lyase had the enhanced activity of 16.82 min^{-1} g^{-1}, 18.16 $min^{-1}g^{-1}$ and 117.79 µM min^{-1} g^{-1} respectively in the *B. subtilis* treated plants. The activity of Peroxidase, Polyphenol oxidase and Phenylalanine ammonia lyase and activity was less (4.22 min^{-1} g^{-1}, 11.31 min^{-1} g^{-1} and 85.64 µM $min^{-1}g^{-1}$ respectively) in imidacloprid and untreated control plants (2.17 min^{-1} g^{-1}, 8.71 min^{-1} g^{-1} and 54.08 µM $min^{-1}g^{-1}$ respectively) (Table 3 and 4).

Table 1: Effect of PGPR on the incidence of fruit borer *E. vittella* in okra during Rabi 2020

S. No	Treatments	Per cent shoot damage*		Per cent fruit damage*
		35 DAS	45 DAS	
T1	*Bacillus subtilis* Bbv57(ST-SA-FS)	1.33 (6.62)[a]	12.15 (20.40)[a]	8.64 (1.35)[a]
T2	*Bacillus amyloliquefaciens* (ST-SA-FS)	4.00 (11.54)[b]	14.33 (22.24)[a]	9.88 (2.12)[ab]
T3	*Rhizobium puscense* (ST-SA-FS)	8.00 (16.43)[c]	16.00 (23.58)[ab]	15.66 (2.92)[cd]
T4	*Ensifer* sp. (ST-SA-FS)	9.33 (17.79)[cd]	17.12 (24.44)[abc]	11.56 (3.14)[abc]
T5	*Siphanobactor* sp. (ST-SA-FS)	10.67 (19.06)[cd]	14.67 (22.52)[ab]	14.88 (3.34)[bcd]
T6	Imidacloprid 48FS (ST alone)	12.81 (20.97)[de]	22.34 (28.21)[cd]	17.25 (3.65)[cd]
T7	Untreated check	16.67 (23.63)[e]	25.33 (29.71)[d]	19.25 (3.81)[d]
CD (P = 0.05)		4.45**	5.74**	4.83**

DAS – Days after sowing
*Mean of three replications
Figures in parentheses are $\sqrt{x+0.5}$ transformed values.
In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Table 2: Effect of PGPR on the incidence of fruit borer *E. vittella* in okra during Summer 2021

S. No	Treatments	Per cent shoot damage*		Per cent fruit damage*
		35 DAS	45 DAS	
T1	*Bacillus subtilis* Bbv57(ST-SA-FS)	1.33 (6.63)[a]	14.30 (22.22)[a]	10.65 (1.35)[a]
T2	*Bacillus amyloliquefaciens* (ST-SA-FS)	9.33 (17.79)[bc]	18.33 (25.35)[ab]	13.33 (2.12)[ab]
T3	*Rhizobium puscense* (ST-SA-FS)	6.67 (14.96)[b]	22.67 (28.43)[abc]	18.01 (2.92)[bc]
T4	*Ensifer* sp. (ST-SA-FS)	8.00 (16.43)[b]	21.33 (27.51)[abc]	15.05 (3.14)[ab]
T5	*Siphanobactor* sp. (ST-SA-FS)	13.33 (21.42)[cd]	24.00 (29.33)[bc]	17.65 (3.34)[bc]
T6	Imidacloprid 48FS (ST alone)	14.66 (22.51)[d]	28.00 (31.95)[bc]	19.12 (3.65)[bc]
T7	Untreated check	18.67 (25.11)[d]	31.33 (33.54)[c]	24.4 (3.81)[c]
CD (P = 0.05)		4.72**	6.81**	5.68**

DAS – Days after sowing
*Mean of three replications
Figures in parentheses are $\sqrt{x+0.5}$ transformed values.
In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Table 3: Effect of PGPR on biochemicals and defense enzymes activity in okra during Rabi 2020

S. No.	Treatments	Phenol content mg g^{-1} fresh weight*		Tannin content mg $100g^{-1}$ dry weight*		Peroxidase activity* $min^{-1} g^{-1}$		Polyphenol Oxidase activity* $min^{-1} g^{-1}$		PAL activity* µM $Min^{-1}g^{-1}$	
		30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT
T1	*Bacillus subtilis*	1.36 (1.36)[a]	1.96 (1.57)[a]	1.39 (1.37)[a]	1.98 (1.58)[a]	4.68 (2.28)[a]	14.72 (3.90)[a]	10.35 (3.29)[a]	16.01 (4.06)[a]	110.37 (10.53)[a]	113.64 (10.68)[a]
T2	*Bacillus amyloliquefaciens*	0.56 (1.03)[d]	1.01 (1.23)[d]	1.28 (1.33)[b]	1.49 (1.41)[b]	3.77 (2.07)[b]	11.63 (3.48)[b]	9.64 (3.18)[b]	12.67 (3.63)[c]	94.49 (9.75)[b]	96.84 (9.86)[b]
T3	*Rhizobium puscense*	0.76 (1.12)[c]	1.13 (1.28)[bc]	0.91 (1.19)[f]	1.03 (1.24)[e]	2.95 (1.86)[c]	9.01 (3.08)[e]	8.04 (2.92)[c]	12.21 (3.56)[bc]	55.86 (7.51)[e]	58.80 (7.70)[e]
T4	*Ensifer* sp.	0.52 (1.01)[e]	0.65 (1.07)[e]	1.20 (1.30)[c]	1.27 (1.33)[d]	2.65 (1.77)[d]	11.19 (3.42)[c]	10.18 (3.27)[a]	10.14 (3.26)[d]	72.27 (8.53)[c]	75.93 (8.74)[c]
T5	*Siphanobactor* sp.	0.48 (0.99)[f]	1.18 (1.30)[b]	1.12 (1.27)[d]	1.37 (1.37)[c]	4.55 (2.25)[a]	10.17 (3.27)[d]	7.79 (2.88)[c]	13.32 (3.72)[b]	69.85 (8.39)[c]	71.16 (8.46)[d]
T6	Imidacloprid 48 FS	0.98 (1.22)[b]	1.09 (1.26)[c]	0.98 (1.22)[e]	1.05 (1.24)[e]	2.88 (1.84)[c]	3.88 (2.09)[f]	6.74 (2.69)[d]	6.92 (2.72)[e]	60.22 (7.79)[d]	60.31 (7.80)[e]
T7	Untreated check	0.50 (1.00)[ef]	0.54 (1.02)[f]	0.80 (1.14)[g]	0.99 (1.22)[e]	2.01 (1.58)[e]	2.09 (1.61)[g]	3.11 (1.90)[e]	3.44 (1.98)[f]	50.15 (7.12)[f]	50.39 (7.13)[f]
CD (P=0.05)		0.017**	0.021**	0.021**	0.023**	0.035**	0.035**	0.041**	0.090**	0.144**	0.168**

DAS – Days after sowing, HAT – Hours after treatment
*Mean of three replications
Figures in parentheses are $\sqrt{x+0.5}$ transformed values.
In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Table 4: Effect of PGPR on biochemicals and defense enzymes activity in okra during Summer 2021

S. No.	Treatments	Phenol content mg g^{-1} fresh weight*		Tannin content mg $100g^{-1}$ dry weight*		Peroxidase activity* $min^{-1} g^{-1}$		Polyphenol Oxidase activity* $min^{-1} g^{-1}$		PAL activity* µM $Min^{-1}g^{-1}$	
		30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT	30 DAS	72 HAT
T1	*Bacillus subtilis*	1.48 (1.41)^{a}	2.54 (1.74)^{a}	1.66 (1.47)^{a}	2.37 (1.69)^{a}	5.81 (2.51)^{a}	16.82 (4.16)^{a}	15.27 (3.97)^{a}	18.16 (4.32)^{a}	113.28 (10.67)^{a}	117.79 (10.87)^{a}
T2	*Bacillus amyloliquefaciens*	1.21 (1.31)^{b}	1.71 (1.49)^{b}	1.37 (1.37)^{b}	1.88 (1.54)^{b}	2.36 (1.69)^{b}	13.21 (3.70)^{b}	10.94 (3.38)^{d}	12.89 (3.66)^{b}	97.25 (9.89)^{b}	101.65 (10.11)^{b}
T3	*Rhizobium puscense*	0.77 (1.13)^{e}	1.13 (1.28)^{d}	1.16 (1.29)^{d}	1.47 (1.40)^{d}	4.28 (2.19)^{d}	11.47 (3.46)^{c}	9.34 (3.14)^{e}	10.59 (3.33)^{d}	58.37 (7.67)^{f}	61.05 (7.84)^{e}
T4	*Ensifer* sp.	0.95 (1.20)^{c}	1.44 (1.39)^{c}	1.34 (1.36)^{b}	1.69 (1.48)^{c}	3.54 (2.01)^{c}	13.01 (3.68)^{b}	11.06 (3.40)^{b}	12.51 (3.61)^{b}	76.18 (8.76)^{d}	79.14 (8.92)^{d}
T5	*Siphanobactor* sp.	0.82 (1.15)^{d}	1.11 (1.27)^{e}	1.22 (1.31)^{c}	1.37 (1.37)^{e}	2.96 (1.86)^{e}	10.22 (3.27)^{d}	9.07 (3.09)^{f}	10.98 (3.39)^{cd}	72.09 (8.52)^{e}	74.30 (8.65)^{d}
T6	Imidacloprid 48 FS	0.72 (1.10)^{f}	0.82 (1.15)^{f}	1.25 (1.32)^{c}	1.28 (1.33)^{f}	3.02 (1.88)^{f}	4.22 (2.17)^{e}	10.99 (3.39)^{c}	11.31 (3.44)^{c}	82.16 (9.09)^{c}	85.64 (9.28)^{c}
T7	Untreated check	0.59 (1.04)^{g}	0.68 (1.09)^{g}	0.91 (1.19)^{e}	1.18 (1.30)^{g}	1.64 (1.46)^{g}	2.17 (1.63)^{f}	6.08 (2.56)^{g}	8.71 (3.03)^{e}	53.33 (7.34)^{g}	54.08 (7.39)^{f}
CD (P=0.05)		0.017**	0.017**	0.021**	0.020**	0.030**	0.031**	0.070**	0.071**	0.078**	0.158**

DAS – Days after sowing, HAT – Hours after treatment

*Mean of three replications

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values.

In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Discussion

The incidence of *E. vittella* on okra F_1 hybrid CoBh4 during Rabi 2020 and Summer 2021 seasons was significantly low in *B. subtilis* treated plants when compared with imidacloprid treated plants and untreated plants. Similarly, the induction of biochemicals phenols and tannins and the activity of defense enzymes *viz.*, Peroxidase, Polyphenol oxidase and Phenylalanine ammonia lyase were high in the *B. subtilis* treated plants than in the imidacloprid treated plants and untreated Plants. The possible reasons for the low incidence of shoot and fruit borer in PGPR treated plants might be due to the increased activity of defense enzymes and biochemicals. Thiruveni *et al* (2012) [15] showed that PGPR strain of *Pseudomonas fluorescens* was effective in reducing the damage caused by *E. vittella*. In the present investigation, the incidence of *E. vittella* had a negative trend with increase in the levels of biochemicals and activity of defense enzymes in the okra plants which is in accordance with findings of Lukefahr *et al* (1974) [8] who stated the antibiotic effects of tannins, reduced nutritional quality and non-availability of nutrients for the development of spotted boll worm, *E. vittella* in cotton. Plant growth promoting substances, indirectly contribute to the suppression of insect population and induce resistance to crop pests through induction of defense enzymes such as phenylalanine ammonia lyase, peroxidase and polyphenol oxidase as well as certain proteinase inhibitors and protect plants (Prasad *et al.*, 2015) [11]. Plants with high levels of phenolics are generally less preferred by herbivores as they become less palatable and polyphenolics such as tannins are important antifeedants (Fahey Jr and Jung 1989) [5].

Conclusion

The research findings suggested that PGPR *viz.*, *B. subtilis* and *B. amyloliquefaciens* could be employed to induce resistance against *E. vittella* in okra.

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