Original Research Article

PGPR-INDUCED BIOCHEMICAL DEFENSE MECHANISMS FOR LEAFHOPPER (*AMRASCA DEVASTANS*) MANAGEMENT IN OKRA CULTIVATION

Abstract

Okra (*Abelmoschus esculentus* L. Moench) is a widely grown vegetable in Asia, valued for its nutritional properties and culinary uses. Nevertheless, it is significantly threatened by leafhoppers (Cicadellidae), which can transmit various plant viruses. To promote sustainable production and protect the crop from pest damage, a field study was conducted on a microplot in Kasilingapuram, Karungulam block, Thoothukudi district, during the Rabi season of 2020 and the Summer of 2021. Different Plant Growth-Promoting Rhizobacteria (PGPRs) were applied to the hybrid COBh 4 okra cultivars via soil, seed, and foliar treatments, and their effects on leafhopper populations were evaluated. The results indicated a notable reduction in leafhopper population in *Bacillus subtilis* Bbv57treated okra plants than untreated plants during both Rabi 2020 and Summer 2021 seasons. Likewise, the biochemical parameters were also observed in elevated levels in *Bacillus subtilis* Bbv57treated plants in both the seasons. These findings suggest that the use of PGPRs may be an effective approach for managing leafhopper populations in okra cultivation.

Keywords: PGPR, *Bacillus*, Rhizobacteria, Leafhopper, Biochemical, Defense, Resistance

1. Introduction

Okra (*Abelmoschus esculentus* L.), commonly known as Bhendi, is a significant dietary vegetable belonging to the Malvaceae family. It is cultivated extensively as both a vegetable and garden crop across tropical and subtropical regions worldwide [1]. Major producers of okra include India, Turkey, Japan, Iran, Bangladesh, Malaysia, Thailand, Ethiopia, and Pakistan [2]. Nutritionally rich, okra is an important component of the human diet, providing carbohydrates, proteins, fats, vitamins A and C, vitamin B6, folic acid, and various minerals including calcium,

magnesium, potassium, iron, zinc, and phosphorus, as well as dietary fiber [3, 4]. Despite its benefits, okra is vulnerable to numerous insect pests and diseases, requiring careful management to minimize losses. Approximately 72 species of insects have been documented as threats to okra crops [5], with significant damage occurring from both sucking and chewing pests. Notably, jassids (*Amrascadevastans* I.), thrips (*Thrips tabaci* L.), and whiteflies (*Bemisiatabaci* G.) are the primary sucking pests affecting the plant [6].

Among these, the leafhopper, *A. devastans* is particularly detrimental, residing on the undersides of leaves and feeding on sap, which leads to chlorophyll depletion and disrupts photosynthesis. Severe infestations result in symptoms such as downward cupping, yellowing, bronzing, and a burnt appearance known as hopper burn, significantly hindering crop growth, especially during the vegetative stage [7]. Losses attributed to leafhoppers are estimated to range from 50.00 to 63.41% [8]. Implementing alternative pest control strategies is crucial for effectively managing pest damage and minimizing the adverse impacts of synthetic pesticides, including food contamination, biodiversity loss, and disruptions to ecosystems.The widespread use of synthetic pesticides poses risks to environmental and public health [9, 10]. Consequently, sustainable practices, including the application of microorganisms for disease control, represent an environmentally friendly approach to pest management [11].

Plant Growth-Promoting Rhizobacteria (PGPR), constituting about 2–5% of rhizobacteria, can beneficially impact plant growth when reintroduced through inoculation into soils containing competitive microflora [12]. One major indirect mechanism by which PGPR promote growth is by serving as biocontrol agents [13]. Some PGPR enhance the plant's resistance to pathogens and pests by inducing systemic resistance through the formation of physical and chemical barriers [14]. They regulate plant signaling pathways and result in the production of secondary metabolites which confer resistance against insect pests [15]. Additionally, PGPR can directly support plant growth by improving nitrogen uptake, synthesizing phytohormones, solubilizing minerals, and chelating iron [16]. Certain PGPR may also suppress soil-borne pathogens by producing siderophores, antimicrobial compounds, or by competing for nutrients and niches [17].

2. Materials and Methods

2.1. Field study

A study was conducted to assess the impact of different Plant Growth-Promoting Rhizobacteria (PGPR) strains on the incidence of the leafhopper, *Amrascadevastans* (Ishida), in okra under field conditions. The experiment took place in microplots located at farmer's fields in Kasilingapuram, Karungulam block, Thoothukudi district during the Rabi 2020 and Summer 2021 seasons. The okra hybrid COBh 4 was planted in microplots measuring 5x3 m with a spacing of 45x30 cm, following seed treatment with different PGPR strains including *Bacillus subtilis* Bbv57, *Bacillus amyloliquefaciens*, *Rhizobium pusense*, *Ensifer* sp., and *Siphanobacter* sp. Talc formulations (containing 1x10□ $cfug^{-1}$) of each strain were applied at a rate of 10 g/kg of seed, as per the practices recommended by the Tamil Nadu Agricultural University (TNAU).

The talc formulations of *B. subtilis* Bbv57 and *B. amyloliquefaciens* were sourced from the Department of Plant Pathology, TNAU, Coimbatore, while those of *Rhizobium pusense*, *Ensifer* sp., and *Siphanobacter* sp. were obtained from the Microbiology Unit, Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Killikulam. Imidacloprid 48FS was applied as a seed treatment at a dose of 7 gkg^{-1} to serve as a chemical check.The treatments comprised *Bacillus subtilis* Bbv57 (T1), administered as a seed treatment (ST) at 10 g/kg of seed, soil application (SA) at 2.5 $kgha^{-1}$, and foliar spray (FS) at 5 g/liter of water. Similar applications (ST-SA-FS) were followed for *Bacillus amyloliquefaciens* (T2), *Rhizobium pusense* (T3), *Ensifer* sp. (T4), and *Siphanobacter* sp. (T5). In addition, Imidacloprid 48FS (T6) was used as a standalone seed treatment, and an untreated control group (T7) was maintained for comparison.Before sowing, soil applications of each PGPR talc formulation (1x10□ $cfug^{-1}$) at 2.5 kg/ha, along with 50 kg of vermicompost, were carried out. Foliar applications of the PGPR formulations (1x10□ $cfug^{-1}$) at 5 $gliter^{-1}$ of water were administered 30 days after emergence (DAE). The leafhopper incidence was monitored weekly, starting from 7 DAE until the end of the season. Leafhopper populations were assessed on three leaves per plant (representing the top, middle, and bottom portions) on ten randomly selected plants per replication and were recorded as the number of hoppers per plant.

2.2. Laboratory study

The estimation of biochemicals and defense-related secondary metabolites involved measuring total phenols, tannins, and defense enzyme activities such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL). Plant samples were

collected from five randomly selected plants at 30 days after emergence (DAE) for these estimations. Additionally, the induction of biochemical compounds and enzyme activities was assessed 72 hours after the foliar application of PGPR formulations.

2.2.1. Estimation of biochemicals

Total phenol

The total phenol content was determined using the protocol by Malik, Singh [18]. Leaf samples were collected from each plot and five hundred mg was weighed from each leaf sample and ground in a pestle and mortar. 10 ml of 80 per cent alcohol was used for homogenizing the samples. The sample extracts were centrifuged at 10000 rpm for 20 minutes, then the residue was discarded and the supernatant was collected and allowed to evaporate. After that 6 ml of distilled water was added to each sample and 0.5 ml sample was pipetted into test tubes and then volume was made to 3 ml by adding distilled water. The Folin–Ciocalteau Reagent (0.5 mL) was then added. 2 mL of a 20% sodium carbonate solution was added after three minutes, mixed and placed in a boiling water bath for one minute, then cooled. The absorbance was measured at 650 nm using the UV-VIS spectrophotometer (Agilent Cary Win®). The phenol content was measured and expressed in mg $g^{-1}$of fresh weight.

Tannin

Tannin content was estimated following a modified AOAC [19] method, using Folin-Denis reagent and saturated sodium carbonate, and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g on dry weight basis.

2.2.2.Estimation of defense enzymes

Plants samples were collected from each treatment and brought to the laboratory with chiller box. Enzyme extraction involved homogenisation of one gram of leaf sample in 5 ml of 0.1M sodium phosphate buffer (pH 7.0). The extract was centrifuged at 10000 rpm for 20 minutes at 4° C. Enzyme extracted was used to determine the activity of defence enzymes *viz*., Peroxidase (PO), Phenylalanine ammonia lyase (PAL) and Polyphenol oxidase (PPO).

Peroxidase (PO)

Consequently, peroxidase activity was determined according to Pütter [20]The reaction mixture was prepared with 3.5 ml of sodium phosphate buffer (pH 6.5), 200 µl enzyme extract and 0.1 ml freshly prepared O-dianisidine solution. 0.2 ml of 0.2 M hydrogen peroxide was

added to start the reaction. The changes in absorbance were noted at 430 nm and were recorded at every 30 seconds interval for three minutes by using the UV-VIS spectrophotometer (Agilent Cary Win®). The enzyme activity was expressed as changes in absorbance at 430 nm $min^{-1}g^{-1}$ of tissue.

Phenyl alanine ammonia lyase (PAL)

The PAL activity was estimated by the method given by Brueske [21]. The reaction mixture used for determining PAL activity was prepared with 0.5 ml buffer, 0.2 ml enzyme solution and 1.3 ml water in a test tube. The reaction was initiated with the addition of one ml of L- phenylalanine and incubated for 30min at 32°C. The reaction was stopped by adding 0.5 ml of 1 M trichloroacetic acid to the mixture. The absorbance at 290 nm was measured using UV-VIS spectrophotometer (Agilent Cary Win®). The enzyme activity was expressed as μ mol^{-1} min^{-1} $gram^{-1}$ of tissue.

Poly phenol oxidase (PPO)

Lastly, PPO activity was evaluated following the method of Augustin *et al.* [22]. 1 ml of 0.05 M catechol and 4.5 ml of 0.2 M phosphate buffer (pH 6.8 at 30°C) were used to prepare the reaction mixture. 0.5 mL enzyme extract was used to initiate the reaction. The rate of change in absorbance was determined at 410 nm every 30 seconds for three minutes using UV-VIS spectrophotometer (Agilent Cary Win®) against the blank. The enzyme activity was expressed as unit min^{-1} g^{-1} of tissue.

STATISTICAL ANALYSIS

The data on the aphid population and biochemical parameters was transformed using a square root method, and R software was employed for the analysis. The treatment mean values were compared for significant differences using the least significant difference (LSD) test at a 5% probability level.

3. RESULTS

Effect of PGPR on leafhopper incidence in okra

Observations on the leafhopper population during Rabi 2020 and Summer 2021 showed that PGPR significantly influenced the incidence on okra (Table 1 and 2). Among the PGPR treatments, *B. subtilis* Bbv57recorded weekly population of *A. devastans*as 0.23, 1.35, 1.66, 1.98, 2.02, 2.76, 3.48 and 5.07 leafhoppers per plant from 7 DAE to 56 DAE during Rabi 2020.

Similarly, *B. amyloliquefaciens*recorded less number of hoppers during various time of observations (0.38, 1.53, 1.96, 2.46, 2.59, 3.06, 3.91 and 7.85 hoppersplant^{-1}) when compared with untreated plants. Imidacloprid 48 FS treatment also showed a similar trend in leafhopper population up to 28 DAE, however, leafhopper population increased gradually and recorded as 5.56, 7.21, 9.12, 9.88 hoppers per plant from 35 DAE to 56 DAE, respectively at weekly intervals. The leafhopper population was high in untreated control from 7 DAE to 56 DAE (2.33, 4.11, 4.65, 7.70, 8.92, 9.06, 9.54 and 10.05 hoppersplant^{-1}) (Table 1).

During Summer 2021, the leafhopper incidence was low in *B. subtilis* Bbv57treatment with a population of 0.65, 2.76, 3.44, 3.78, 4.20, 5.88, 6.11 and 7.12 hoppers per plant from 7 DAE to 56 DAE, respectively followed by 0.98, 3.18, 3.84, 4.74, 4.87, 7.95, 9.11 and 10.66 hoppersplant^{-1} in *B. amyloliquefaciens*treated plants. The incidence of leafhoppers was low in the imidacloprid treated plants for the first three weeks (0.33, 0.78 and 2.88 hoppersplant^{-1} respectively). However, more number of leafhoppers was recorded in imidacloprid treatment from 28 DAE to 56 DAE with 3.96, 9.06, 11.74, 12.55 and 14.98 hoppers plant^{-1} respectively on weekly intervals. The leafhopper infestation was more in the untreated plants throughout the cropping period with a population of 2.49, 5.06, 7.59, 9.59, 10.25, 12.22, 13.36 and 18.32 hoppersplant^{-1} from 7 DAE to 56 DAE, respectively(Table 2).The population of leafhoppers was observed to be lowin *B. subtilis* Bbv57 treated okra plants than untreated plants during Rabi 2020 and Summer 2021.

Effect of PGPR on biochemical activity in okra

During the Rabi season, phenol and tannin content, along with the activity of defense enzymes, significantly increased in plants treated with PGPR. Among all treatments, *Bacillus subtilis* demonstrated the highest phenol content, reaching 1.96 mgg^{-1}at 72 hours after foliar application, outperforming other PGPR treatments and imidacloprid-treated plants (1.09 mgg^{-1}) (Fig. 1). Plants inoculated with *B. subtilis* also showed the highest tannin content (1.98mgg^{-1}), followed by those treated with *Bacillus amyloliquefaciens* (1.49mgg^{-1}). In contrast, untreated plants exhibited notably lower levels of phenol (0.54mgg^{-1}) and tannin (0.99mgg^{-1}).The activity of defense enzymes, including peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, significantly increased across all PGPR treatments at 72 hours after foliar application compared to untreated plants(Table 3). Among these treatments, *B. subtilis* showed the most

significant increase in enzyme activities, with peroxidase at 14.72 $min^{-1} g^{-1}$, polyphenol oxidase at 16.01 $min^{-1} g^{-1}$, and PAL at 113.64 µM $min^{-1} g^{-1}$, surpassing the activities recorded in imidacloprid-treated plants (3.88 $min^{-1} g^{-1}$, 6.92 $min^{-1} g^{-1}$, and 60.31 µM $min^{-1} g^{-1}$, respectively). Untreated plants showed even lower enzyme activities, with peroxidase at 2.09 $min^{-1} g^{-1}$, polyphenol oxidase at 3.44 $min^{-1} g^{-1}$, and PAL at 50.39 µM $min^{-1} g^{-1}$.

During the Summer, biochemical analysis revealed a further increase in phenol and tannin content in plants treated with *B. subtilis*. Phenol levels in these plants reached 2.54mgg^{-1}, followed by those treated with *B. amyloliquefaciens* (1.71mgg^{-1}) (Fig. 2). The tannin content also significantly increased after *B. subtilis* application, reaching 2.37mgg^{-1}, compared to untreated plants (1.18mgg^{-1}) and imidacloprid-treated plants (1.28mgg^{-1}). The activity of defense enzymes again increased notably in PGPR-treated plants(Table 4). At 72 hours after treatment, *B. subtilis*-treated plants recorded peroxidase activity of 16.82 $min^{-1} g^{-1}$, polyphenol oxidase activity of 18.16 $min^{-1} g^{-1}$, and PAL activity of 117.79 µM $min^{-1} g^{-1}$. In comparison, the activities in imidacloprid-treated plants were lower (4.22 $min^{-1} g^{-1}$, 11.31 $min^{-1} g^{-1}$, and 85.64 µM $min^{-1} g^{-1}$, respectively), and in untreated control plants, even lower still (2.17 $min^{-1} g^{-1}$, 8.71 $min^{-1} g^{-1}$, and 54.08 µM $min^{-1} g^{-1}$, respectively).

4. Discussion

The application of PGPR on okra through seed treatment, soil application and foliar spray significantly reduced the incidence of leafhoppers in okra during both Rabi 2020 and Summer 2021 seasons. The incidence of leafhopper,*Amrascadevastans* was less up to 14 DAE in *Bacillus subtilis* Bbv57 and imidacloprid 48 FS treated plants. However, the pest population increased subsequently over the growing period during Rabi 2020 and Summer 2021. The observations recorded on 35 DAE showed that the foliar application of *B. subtilis* on 30 DAE was able to maintain the population level of leafhoppers up to 42 DAE followed by *B. amyloliquefaciens* treatment compared to imidacloprid treated plants in both the seasons. These findings are consistent with the observations of Stout *et al.* [23], who reported that *Bacillus* treatment delayed population growth and resulted in a lower abundance of *A. gossypii* on cucumbers. Similarly, Murugan *et al.* [24]found that the application of *Pseudomonas fluorescens* significantly reduced leafhopper populations in okra. According to Murphy *et al.* [25], treating tomato plants with various *Bacillus* PGPR species as seed treatments reduced whitefly nymph densities by 40–43%.

Similarly, the application of *Bacillus subtilis* resulted in higher levels of biochemical compounds, such as phenols and tannins, as well as increased activities of defense enzymes like peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, compared to imidacloprid-treated and untreated plants. The reduced incidence of leafhoppers in PGPR-treated plants can likely be attributed to this enhanced defense enzyme activity and accumulation of biochemical compounds. Plant growth-promoting substances indirectly help suppress insect populations and enhance pest resistance by activating defense enzymes, including phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase, as well as proteinase inhibitors, thus protecting plants [26]. Additionally, the observed reduction in leafhopper infestations in PGPR-treated okra may be linked to the multiple biological processes triggered by PGPR colonization below ground, which can impact interactions with above-ground herbivores like sap-sucking and chewing insects. These processes influence plant growth, nutritional quality, and host defenses [27, 28].

5. LIMITATIONS

While the use of Plant Growth-Promoting Rhizobacteria (PGPR) showed potential in reducing leafhopper populations in okra, several limitations need consideration. The effectiveness of PGPR treatments can be influenced by environmental factors like soil type, moisture, and temperature, which may affect microbial colonization and activity. Furthermore, this study focused on a specific okra variety and was limited to a particular geographical region, potentially limiting the applicability of the findings to other crops or areas. Variability in field conditions, such as soil fertility and pest pressures, may also impact the consistency of the results. Additionally, while PGPR treatments successfully reduced leafhopper populations, their long-term impact on non-target organisms and the surrounding ecosystem requires further investigation.

6. FUTURE SCOPE

For future research, it is essential to validate the effectiveness of PGPR strains across various okra varieties and agro-climatic zones to ensure broader applicability. Exploring the synergistic effects of combining PGPR with other biological control agents or organic amendments could lead to more effective and integrated pest management strategies. Long-term studies should also assess the sustainability of PGPR applications, their impact on soil health, and their influence on non-target organisms. Further research into the molecular mechanisms

behind PGPR-induced resistance and alterations in pest behavior could provide valuable insights for optimizing their use. Additionally, developing stable and user-friendly PGPR formulations with improved shelf life and field efficacy could promote wider adoption among farmers, fostering sustainable and eco-friendly pest control practices in okra cultivation.

7. CONCLUSION

In conclusion, the use of Plant Growth-Promoting Rhizobacteria (PGPR) proved to be effective in reducing leafhopper infestations in okra. Among the PGPR strains studied, *Bacillus subtilis*as a PGPR played a crucial role in triggering enhanced levels of biochemical defenses, such as increased activity of defense-related enzymes viz., peroxidase, phenylalanine ammonia-lyase and polyphenol oxidase and higher production of secondary metabolites which contributed to stronger resistance against leafhoppers. Also, it was considered as the most successful treatment, significantly lowering pest populations byinducing systemic resistance, and creating an unfavorable environment for leafhoppers and improving plant health. Other strains like *Bacillus amyloliquefaciens*, *Rhizobium pusense*, *Ensifer sp*., and *Siphanobacter sp*. also demonstrated effectiveness in reducing leafhopper numbers when applied through a combination of seed treatment, soil application, and foliar spray. These findings underscore the importance of incorporating PGPR, especially *Bacillus subtilis*, into integrated pest management (IPM) practices as a sustainable and eco-friendly approach, reducing reliance on chemical pesticides, enhancing crop resilience, and ensuring environmental sustainability.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Table 1. Effect of PGPR on the incidence of leafhopper, *A. devastans*in okra during Rabi 2020

S.No.	Treatments	Number of leafhoppers per plant*							
		7 DAE	14 DAE	21 DAE	28 DAE	35 DAE	42 DAE	49 DAE	56 DAE
T_1	*Bacillus subtilis* Bbv57 (ST-SA-FS)	0.23 (0.85)^{a}	1.35 (1.36)^{ab}	1.66 (1.47)^{a}	1.98 (1.57)^{a}	2.02 (1.59)^{a}	2.76 (1.81)^{a}	3.48 (1.99)^{a}	5.07 (2.36)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	0.38 (0.94)^{a}	1.53 (1.42)^{ab}	1.96 (1.57)^{a}	2.46 (1.72)^{a}	2.59 (1.76)^{ab}	3.06 (1.89)^{a}	3.91 (2.10)^{a}	7.85 (2.89)^{abc}
T_3	*Rhizobium pusense*(ST-SA-FS)	0.92 (1.19)^{b}	1.70 (1.48)^{ab}	2.10 (1.61)^{a}	2.57 (1.75)^{a}	3.05 (1.88)^{ab}	3.57 (2.02)^{a}	5.12 (2.37)^{ab}	6.02 (2.55)^{ab}
T_4	*Ensifer*sp. (ST-SA-FS)	0.66 (1.08)^{ab}	1.86 (1.54)^{ab}	2.01 (1.58)^{a}	2.91 (1.85)^{a}	3.44 (1.98)^{ab}	5.29 (2.41)^{ab}	6.88 (2.72)^{ab}	7.66 (2.86)^{abc}
T_5	*Siphanobacter*sp. (ST-SA-FS)	1.03 (1.24)^{b}	1.98 (1.57)^{b}	2.51 (1.73)^{a}	3.48 (1.99)^{a}	4.25 (2.18)^{ab}	5.64 (2.48)^{ab}	7.02 (2.74)^{ab}	8.74 (3.04)^{bc}
T_6	Imidacloprid 48FS (ST alone)	0.25 (0.87)^{a}	0.96 (1.21)^{a}	1.65 (1.47)^{a}	2.27 (1.66)^{a}	5.56 (2.46)^{b}	7.21 (2.78)^{b}	9.12 (3.10)^{b}	9.88 (3.22)^{c}
T_7	Untreated control	2.33 (1.66)^{c}	4.11 (2.11)^{b}	4.65 (2.23)^{b}	7.70 (2.79)^{b}	8.92 (2.94)^{c}	9.06 (2.96)^{b}	9.54 (3.03)^{b}	10.05 (3.18)^{c}
CD (P = 0.05)		0.24**	0.33**	0.36**	0.52**	0.75**	0.73**	0.77**	0.57**

DAE – Days after emergence ST- Seed treatment SA- Soil application FS- Foliar spray

*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 leafhopper, *A. devastans*in okra during Summer 2021

S.No.	Treatments	Number of leafhoppers per plant*							
		7 DAE	**14 DAE**	**21 DAE**	**28 DAE**	**35 DAE**	**42 DAE**	**49 DAE**	**56 DAE**
T_1	*Bacillus subtilis* Bbv57 (ST-SA-FS)	0.65 (1.07)^{ab}	2.76 (1.81)^{b}	3.44 (1.98)^{a}	3.78 (2.07)^{a}	4.20 (2.17)^{a}	5.88 (2.53)^{a}	6.11 (2.57)^{a}	7.12 (2.76)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	0.98 (1.22)^{b}	3.18 (1.92)^{bc}	3.84 (2.08)^{a}	4.74 (2.29)^{bc}	4.87 (2.32)^{ab}	7.95 (2.91)^{ab}	9.11 (3.10)^{abc}	10.66 (3.34)^{ab}
T_3	*Rhizobium pusense*(ST-SA-FS)	1.22 (1.31)^{b}	3.84 (2.08)^{bc}	4.02 (2.13)^{a}	6.88 (2.72)^{bc}	5.48 (2.45)^{abc}	6.03 (2.56)^{a}	6.88 (2.72)^{ab}	11.85 (3.51)^{bc}
T_4	*Ensifer*sp. (ST-SA-FS)	1.14 (1.28)^{b}	3.36 (1.96)^{bc}	3.99 (2.12)^{a}	4.80 (2.3)^{bc}	7.99 (2.91)^{bcd}	8.58 (3.01)^{ab}	9.02 (3.09)^{abc}	10.33 (3.29)^{ab}
T_5	*Siphanobacter*sp. (ST-SA-FS)	1.26 (1.33)^{b}	4.02 (2.13)^{bc}	4.11 (2.15)^{a}	5.01 (2.35)^{bc}	5.63 (2.48)^{abc}	10.22 (3.27)^{c}	10.62 (3.33)^{bc}	11.22 (3.42)^{ab}
T_6	Imidacloprid 48FS (ST alone)	0.33 (0.91)^{a}	0.78 (1.13)^{a}	2.88 (1.84)^{a}	3.96 (2.11)^{ab}	9.06 (3.09)^{cd}	11.74 (3.50)^{c}	12.55 (3.61)^{c}	14.98 (3.93)^{bc}
T_7	Untreated control	2.49 (1.69)^{c}	5.06 (2.30)^{c}	7.59 (2.78)^{b}	9.59 (3.11)^{c}	10.25 (3.18)^{d}	12.22 (3.49)^{c}	13.36 (3.64)^{c}	18.32 (4.25)^{c}
CD (P = 0.05)		0.28**	0.43**	0.52**	0.53**	0.66**	0.60**	0.62**	0.74**

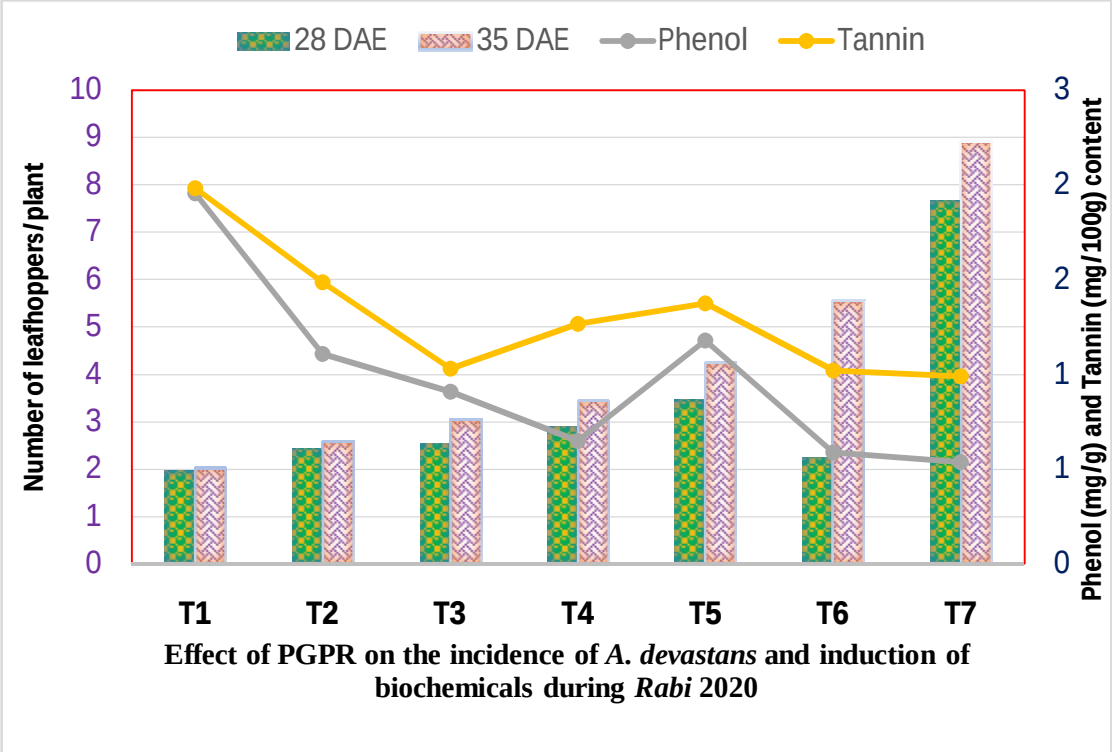
DAE – Days after emergence ST- Seed treatment SA- Soil application FS- Foliar spray

*Mean of three replications

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

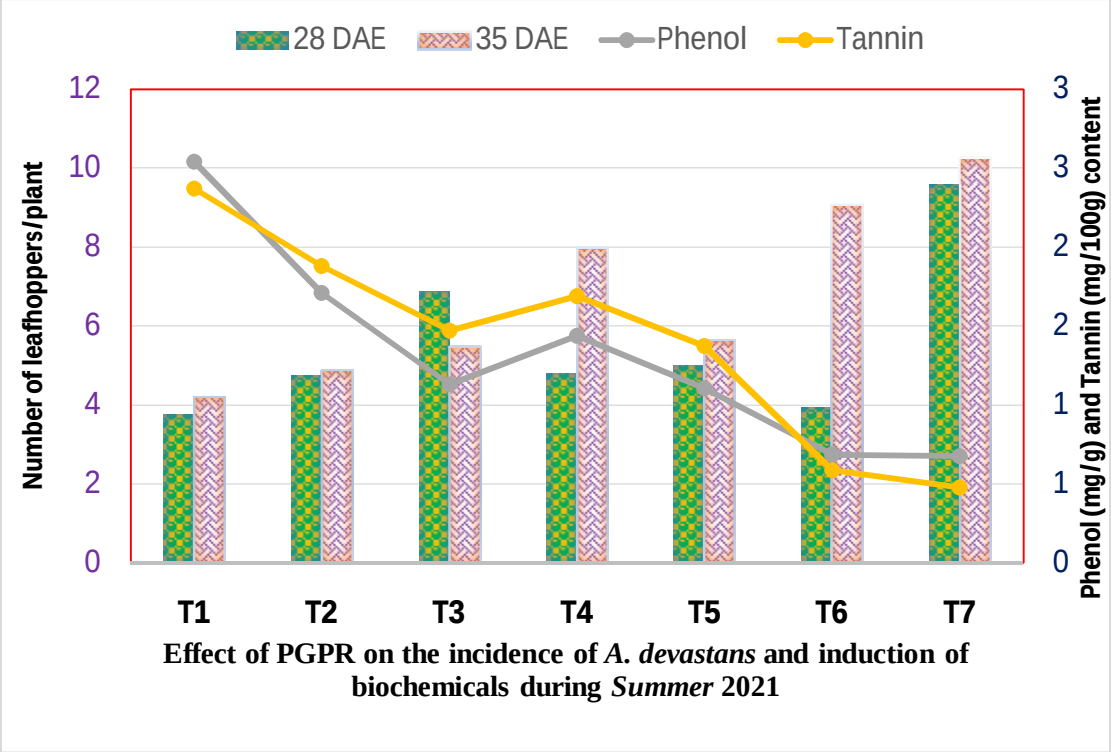
In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Fig 1. Effect of PGPR treatments on the incidence of *A. devastans*and induction of biochemicals in okraduring Rabi 2020



T_1- *B. subtilis* **T_2**- *B. amyloliquefaciens* **T_3**- *R. pusense*
T_4 -*Ensifer sp.* **T_5** - *Siphanobactersp.* **T_6**- Imidacloprid 48FS
T_7- Untreated check

Fig 2. Effect of PGPR treatments on the incidence of *A. devastans*and induction of biochemicals in okra during Summer 2021



T_1- *B. subtilis* **T_2**- *B. amyloliquefaciens* **T_3**- *R. pusense*
T_4 -*Ensifer sp.* **T_5** - *Siphanobacter sp.* **T_6**- Imidacloprid 48FS
T_7- Untreated check

Table 3. Effect of PGPR on activity of defence enzymes in okra during Rabi 2020

S.No.	Treatments	Peroxidase activity* min^{-1} g^{-1}		Polyphenol Oxidase* activity min^{-1} g^{-1}		PAL activity* µM min^{-1}g^{-1}	
		30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT
T_1	*Bacillus subtilis* Bbv57(ST-SA-FS)	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}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	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}
T_3	*Rhizobium pusense*(ST-SA-FS)	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}
T_4	*Ensifer*sp. (ST-SA-FS)	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}
T_5	*Siphanobacter*sp. (ST-SA-FS)	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}
T_6	Imidacloprid 48FS (ST alone)	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}
T_7	Untreated control	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)		0.04**	0.05**	0.04**	0.09**	0.14**	0.17**

DAE – Days after emergence HAT – Hours after treatment ST- Seed treatment SA- Soil application FS- Foliar spray

*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 activity of defence enzymes in okra during Summer 2021

S.No.	Treatments	Peroxidase activity* min^{-1} g^{-1}		Polyphenol Oxidase* activity min^{-1} g^{-1}		PAL activity* µM min^{-1}g^{-1}	
		30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT
T_1	*Bacillus subtilis* Bbv57(ST-SA-FS)	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 (10.88)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	2.36 (1.69)^{de}	13.21 (3.70)^{b}	10.94 (3.38)^{b}	12.89 (3.66)^{b}	97.25 (9.89)a^{b}	101.65 (10.11)^{ab}
T_3	*Rhizobium pusense*(ST-SA-FS)	4.28 (2.19)^{b}	11.47 (3.46)^{bc}	9.34 (3.14)^{bc}	10.59 (3.33)^{bc}	58.37 (7.67)^{cd}	61.05 (7.84)^{cde}
T_4	*Ensifer*sp. (ST-SA-FS)	3.54 (2.01)^{bc}	13.01 (3.68)^{b}	11.06 (3.40)^{b}	12.51 (3.61)^{b}	76.18 (8.76)^{bc}	79.14 (8.92)^{bc}
T_5	*Siphanobacter*sp. (ST-SA-FS)	2.96 (1.86)^{cd}	10.22 (3.27)^{c}	9.07 (3.09)^{bc}	10.98 (3.39)^{bc}	72.09 (8.52)^{c}	74.30 (8.65)^{cd}
T_6	Imidacloprid 48FS (ST alone)	2.02 (1.59)^{ef}	3.11 (1.90)^{d}	7.12 (2.76)^{cd}	8.01 (2.92)^{c}	57.16 (7.59)^{cd}	58.06 (7.65)^{de}
T_7	Untreated control	1.64 (1.44)^{f}	2.17 (1.61)^{e}	6.08 (2.52)^{d}	8.71 (2.97)^{c}	53.33 (7.17)^{d}	54.08 (7.22)^{e}
CD (P=0.05)		0.20**	0.24**	0.421**	0.50**	1.30**	1.27**

DAE – Days after emergence HAT – Hours after treatment ST- Seed treatment SA- Soil application FS- Foliar spray

*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)