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Impact of Plant Growth-promoting Rhizobacteria on the Incidence of Aphids (*Aphis gossypii*) in Okra

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Okra (*Abelmoschus esculentus* L. Moench) is a widely cultivated vegetable in Asia, facing ongoing challenges from aphids (*Aphis gossypii* Glover). 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. Various plant growth-promoting rhizobacteria (PGPRs) were applied to the hybrid COBh 4 okra cultivars through soil, seed, and foliar treatments, and their impact on aphid populations was assessed. The findings revealed a significant reduction in aphid numbers and enhanced production of defensive compounds and enzymes in plants treated with *Bacillus subtilis*

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Bbv57 during both the seasons. These results suggest that incorporating PGPRs could be an effective strategy for managing aphid populations by boosting up the biochemical compounds in okra cultivation and can be incorporated in IPM practices.

Keywords: Aphid; PGPR; defense; biochemical; bacillus; okra; enzymes.

1. INTRODUCTION

Okra, also known as lady's finger (*Abelmoschus esculentus* L. Moench), is a significant vegetable crop from the Malvaceae family and is extensively cultivated in India. Globally, okra is grown over an area of 1.26 million hectares, with a production of 22.29 million tonnes and a productivity rate of 15.10 t/ha (Kumar and Singh, 2022). However, okra cultivation faces numerous challenges, with pest infestations being a primary concern (Kumawat et al., 2000; Bhatt et al., 2018). A total of 72 insect species have been identified as pests in okra crops, with key threats being the shoot and fruit borer (*Earias spp*.), aphids (*Aphis gossypii* Glover), leafhoppers (*Amrasca biguttula biguttula* Ishida), and whiteflies (*Bemisia tabaci* Gennadius), which are particularly harmful in southern Indian states (Rao and Rajendran, 2002; Anitha and Nandihalli, 2008; Deevaraja et al., 2020). Sucking pests like aphids and leafhoppers are especially destructive, causing a yield loss of 23-54% in okra crops (Rai et al., 2014). Aphids, notably *A. gossypii*, infest young plants, leading to stunted growth, wilting, and, in severe cases, plant death. Additionally, aphids excrete honeydew, which promotes the growth of sooty mold and disrupts photosynthesis. During the reproductive stage, these pests damage flower buds, flowers, and fruits, resulting in substantial economic losses (Kedar et al., 2014).

While chemical insecticides offer an affordable and immediate solution to pest control, their prolonged use poses environmental risks such as crop residues, pest resistance, and resurgence (Maurya et al., 2022). Therefore, developing an ecologically sound strategy to minimize chemical pesticide usage in okra cultivation is crucial. One promising approach is the utilization of plant growth-promoting rhizobacteria (PGPR), which are beneficial root-colonizing bacteria. These rhizobacteria positively influence plant growth by inducing physiological and biochemical changes (Kloepper et al., 1980). Besides growth promotion, PGPRs strengthen the physical structure of plant cell walls and trigger defense responses, leading to the production of defense chemicals that protect against pathogens and insect pests (Ramamoorthy et al., 2001). 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). Numerous PGPR strains from genera like *Bacillus*, *Pseudomonas* and *Serratia* are effective in colonizing crop roots and providing pest resistance (Zehnder et al., 1997). Therefore, incorporating PGPRs in okra cultivation could be a viable method to control aphids and other sucking pests, reducing dependency on chemical insecticides.

2. MATERIALS AND METHODS

The impact of various Plant Growth-Promoting Rhizobacteria (PGPR) strains on the incidence of aphid, *Aphis gossypii* (Glover) in okra was investigated under field conditions in microplots at farmer holdings in Kasilingapuram, Karungulam block, Thoothukudi district, during the Rabi season of 2020 and the Summer of 2021. The experiment followed a Randomized Block Design (RBD) with seven treatments and three replications, detailed as follows. The hybrid okra cultivar COBh 4 was sown in microplots measuring 5x3 meters, with a planting spacing of 45x30 cm, following seed treatments with different PGPR strains. The bacterial strains used included *Bacillus subtilis* Bbv57, *Bacillus amyloliquefaciens*, *Rhizobium pusense*, *Ensifer* sp., and *Siphanobacter* sp. Talc-based formulations of each strain ($1x10^8$ cfu/g) were applied at a rate of 10g per kilogram of seeds. The talc formulations of *B. subtilis* Bbv57 and *B. amyloliquefaciens* were sourced from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, while the other strains were obtained from the Microbiology Unit of the Department of Soil Science and Agricultural Chemistry at the Agricultural College and Research Institute, Killikulam. As a chemical control, seeds were treated with Imidacloprid 48 FS at a dosage of 7g per kg. Before sowing, a soil application of each PGPR talc formulation ($1x10^8$ cfu/g) was done at a rate of 2.5 kg per hectare along with 50 kg of

List 1. The treatment details are as follows

S.No.	Treatments	Dose
T_1	*Bacillus subtilis* Bbv57 (ST-SA-FS)	Seed treatment (ST) @ 10 g/kg of seed
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	Soil Application (SA) @ 2.5 kg/ha
T_3	*Rhizobium pusense* (ST-SA-FS)	Foliar application (FS) @ 5 g/lit of water
T_4	*Ensifer* sp. (ST-SA-FS)	(Each talc formulation containing $1x10^8$ cfu/g)
T_5	*Siphanobacter* sp. (ST-SA-FS)	
T_6	Imidacloprid 48FS (ST alone)	7 g/kg of seed
T_7	Untreated control	-

vermicompost was carried out. Additionally, a foliar spray of each PGPR formulation ($1x10^8$ cfu/g) was applied at a concentration of 5g per liter of water, 30 days after crop emergence (DAE). Aphid populations were monitored weekly, starting at 7 DAE, by counting both nymphs and adults on the top three fully expanded leaves of ten randomly selected plants per replication. The results were expressed as the number of aphids per plant, and the observations were recorded throughout the crop season to evaluate the effectiveness of the treatments.

The estimation of biochemicals and secondary metabolites involved measuring total phenol, tannin, and the activity of key defense enzymes like peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) using standard protocols. Plant samples were collected at 30 days after emergence (DAE) from five randomly selected plants to assess these biochemicals and enzymes. Similarly, biochemical induction and enzyme activity were measured 72 hours after foliar application of PGPR formulations. Total phenol content was estimated following the protocol by Malik, Singh(1980), where phenol was extracted from leaf samples using Folin-Ciocalteu reagent. The absorbance was then measured at 650 nm using a UV-VIS spectrophotometer (Agilent Cary Win®), and the phenol content was expressed in mg/g on a fresh weight basis. Additionally, tannin content in the leaves was determined using a modified AOAC (1931) method, involving Folin-Denis reagent and saturated sodium carbonate, and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g on a dry weight basis. Likewise, peroxidase activity was analyzed using the method by Pütter (1974), monitoring absorbance changes at 430 nm every 30 seconds for three minutes and expressing the results as changes in absorbance per minute per gram of tissue. Subsequently, PAL activity was measured at 290 nm following the method by Brueske (1980) and expressed as µmol per minute per gram of tissue. Finally, PPO activity was determined based on the procedure by Augustin *et al.* (1985), recording the rate of change in absorbance at 410 nm every 30 seconds for three minutes against a blank and expressed as units per minute per gram of tissue.

2.1 Statistical Analysis

The data on aphid population and biochemical parameters were transformed using square root transformation and analysis of data was done using R software. The significance of difference between the treatments mean values were compared by using least significance difference (LSD) at 5 per cent probability.

3. RESULTS

3.1 Effect of PGPR on the Incidence of Aphids in Okra

The effect of PGPR on the incidence of *A. gossypii* in okra during Rabi 2020 season is presented in Table 1. Among the different PGPR treatments, *Bacillus subtilis* Bbv57 had a low number of aphids (0.09/plant) than the untreated plants (6.77/plant) on 7 days after emergence (DAE). However, it is statistically on par with imidacloprid 48FS treatment (0.15/plant). The other PGPR treatments *viz., Bacillus amyloliquefaciens*, *Rhizobium pusense, Siphanobacto*r sp. and *Ensifer* sp. recorded mean aphid population as 1.51, 2.12, 2.98 and 3.22 per plant respectively and there was no significant difference between the treatments. On 14 DAE, an increase in the aphid population was observed. However, the same pattern of variation was observed among the treatments. Imidacloprid 48 FS recorded 0.35 aphids/plant followed by *B. subtilis* Bbv57 (0.21 aphids/plant), *B. amyloliquefaciens* (2.55 aphids/plant) and the

untreated plants recorded 10.47 aphids/plant. A similar trend was recorded up to 28 DAE, where the pest population increased up to 17.75, 27.21, 45.26 aphids per plant in *B. subtilis* Bbv57, *B. amyloliquefaciens* and untreated control respectively. The observations recorded on 35 DAE *i.e.*, five days after the foliar application of PGPR showed that PGPR applied plants were able to maintain the aphid population. On 35 DAE, the treatments *B. subtilis* Bbv57 and *B. amyloliquefaciens* had 23.41 and 42.92 aphids per plant, respectively and significantly different from imidacloprid 48 FS (50.77/plant) and untreated plants (88.54/plant).

The effect of PGPR on the incidence of *A. gossypii* in Okra during Summer 2021 is given in Table 2. The observations showed that the population of aphids was ranging from 0.21–2.88 aphids/plant on 7 DAE and imidacloprid 48 FS recorded 0.21 aphids/plant followed by *B. subtilis* Bbv57 (0.45 aphids/plant) compared to untreated plants (2.88 aphids/plant). The population of aphids increased significantly at different growth stages. However, *B. subtilis* Bbv57 treated plots had significantly lower population among the PGPR treatments. Observation on 35 DAE *i.e.,* five days after the foliar application of PGPR showed that the population of aphids was low in *B. subtilis* Bbv57 treated plants (64.02 aphids/plant) compared to the imidacloprid treatment (118.83 aphids/plant). There was an increasing trend in the aphid population from 42 DAE to 56 DAE in all treatments, however incidence of *A. gossypii* was less in *B. subtilis* Bbv57 (71.11, 102.23 and 148.33 aphids/plant) treated plants when compared to imidacloprid treated plants (148.33,195.15 and 210.66) and untreated plants (186.65, 201.85 and 235.84 aphids/plant) on 42 DAE, 49 DAE and 56 DAE respectively. As a result, the mean population of aphid was observed to be 31.72 and 59.04 in *B. subtilis* Bbv57 treated okra plants as against 71.62 and 120.24 in untreated plants during Rabi 2020 and summer 2021, respectively.

3.2 Effect of PGPR on the Biochemical Changes in Okra

During the Rabi season (2020), plants treated with Plant Growth-Promoting Rhizobacteria (PGPR) exhibited a significant increase in phenol and tannin content, along with enhanced defense enzyme activity. Among all treatments, *Bacillus subtilis* showed the highest phenol content, reaching 1.96 mg/g at 72 hours after foliar application, outperforming other PGPR treatments and imidacloprid-treated plants (1.09 mg/g) (Table 3). Additionally, plants inoculated with *B. subtilis* recorded the highest tannin content at 1.98 mg/g, followed by those treated with *Bacillus amyloliquefaciens* (1.49 mg/g), while untreated plants displayed lower levels of phenol (0.54 mg/g) and tannin (0.99 mg/g). The activity of key defense enzymes, such as peroxidase, polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), increased significantly across all PGPR treatments compared to untreated plants. *B. subtilis* stood out among these treatments, with the highest peroxidase activity (14.72 min^{-1} g^{-1}), PPO activity (16.01 min^{-1} g^{-1}), and PAL activity (113.64 µM min^{-1} g^{-1}), surpassing enzyme levels 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). In contrast, untreated plants showed even lower enzyme activities, with peroxidase at 2.09 min^{-1} g^{-1}, PPO at 3.44 min^{-1} g^{-1}, and PAL at 50.39 µM min^{-1} g^{-1}.

During the Summer (2021), biochemical analysis indicated an even greater increase in phenol and tannin content in plants treated with *B. subtilis*. Phenol levels in these plants reached 2.54 mg/g, followed by those treated with *B. amyloliquefaciens* (1.71 mg/g) (Table 4). Tannin content also rose significantly after *B. subtilis* application, reaching 2.37 mg/g, compared to untreated plants (1.18 mg/g) and imidacloprid-treated plants (1.28 mg/g). Furthermore, defense enzyme activity was notably higher in PGPR-treated plants. At 72 hours post-treatment, *B. subtilis*-treated plants recorded peroxidase activity of 16.82 min^{-1} g^{-1}, PPO activity of 18.16 min^{-1} g^{-1}, and PAL activity of 117.79 µM min^{-1} g^{-1}. In comparison, imidacloprid-treated plants displayed lower enzyme activities (4.22 min^{-1} g^{-1}, 11.31 min^{-1} g^{-1}, and 85.64 µM min^{-1} g^{-1}, respectively), while untreated control plants had the lowest levels (2.17 min^{-1} g^{-1}, 8.71 min^{-1} g^{-1}, and 54.08 µM min^{-1} g^{-1}, respectively).

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

S.No	Treatments	Number of aphids 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)	0.09 (0.77)^{a}	0.21 (0.84)^{a}	2.82 (1.82)^{a}	17.75 (4.27)^{a}	23.41 (4.89)^{a}	49.22 (7.05)^{a}	68.84 (8.33)^{a}	91.44 (9.59)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	1.51 (1.42)^{b}	2.55 (1.75)^{a}	9.09 (3.10)^{b}	27.21 (5.26)^{abc}	42.92 (6.59)^{b}	59.11 (7.72)^{ab}	75.53 (8.72)^{a}	102.58 (10.15)^{ab}
T_3	*Rhizobium pusense* (ST-SA-FS)	2.12 (1.62)^{bc}	4.85 (2.31)^{c}	15.33 (3.98)^{bc}	33.82 (5.86)^{cd}	60.02 (7.78)^{b}	72.19 (8.52)^{abc}	89.32 (9.48)^{ab}	119.66 (10.96)^{bcd}
T_4	*Ensifer* sp. (ST-SA-FS)	3.22 (1.93)^{c}	5.02 (2.35)^{c}	18.91 (4.41)^{cd}	29.34 (5.46)^{bcd}	39.11 (6.29)^{ab}	61.55 (7.88)^{ab}	92.21 (9.63)^{ab}	110.22 (10.52)^{bc}
T_5	*Siphanobacter* sp. (ST-SA-FS)	2.98 (1.87)^{c}	4.03 (2.13)^{bc}	23.41 (4.89)^{cd}	38.37 (6.23)^{cd}	57.21 (7.60)^{b}	77.42 (8.83)^{abc}	98.71 (9.96)^{ab}	126.66 (11.28)^{cde}
T_6	Imidacloprid 48FS (ST alone)	0.15 (0.81)^{a}	0.35 (0.92)^{b}	2.98 (1.87)^{a}	20.03 (4.53)^{ab}	50.77 (7.16)^{b}	81.74 (9.07)^{bc}	121.13 (11.03)^{b}	132.13 (11.51)^{de}
T_7	Untreated control	6.77 (2.64)^{d}	10.47 (3.24)^{d}	28.35 (5.25)^{d}	45.26 (6.61)^{d}	88.54 (9.22)^{c}	110.66 (10.31)^{c}	137.66 (11.49)^{b}	145.25 (12.03)^{e}
CD (P = 0.05)		0.44**	0.55**	0.93**	1.18**	1.63**	1.83**	1.99**	0.91**

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 aphid, *A. gossypii* in okra during Summer 2021

S.No.	Treatments	Number of aphids 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)	0.45 (0.97)^{ab}	2.82 (1.82)^{a}	20.98 (4.63)^{a}	62.42 (7.93)^{a}	64.02 (8.03)^{a}	71.11 (8.46)^{a}	102.23 (10.14)^{a}	148.33 (12.20)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	0.84 (1.16)^{bc}	3.86 (2.09)^{abc}	26.12 (5.16)^{a}	66.32 (8.17)^{ab}	90.02 (9.51)^{ab}	106.63 (10.35)^{ab}	115.44 (10.77)^{ab}	166.26 (12.91)^{ab}
T_3	*Rhizobium pusense* (ST-SA-FS)	1.11 (1.27)^{c}	5.08 (2.36)^{bcd}	30.47 (5.56)^{ab}	71.14 (8.46)^{ab}	121.36 (11.04)^{bc}	136.94 (11.72)^{bc}	164.41 (12.84)^{bc}	189.41 (13.78)^{bc}
T_4	*Ensifer* sp. (ST-SA-FS)	0.95 (1.20)^{bc}	5.24 (2.40)^{cd}	29.98 (5.52)^{a}	82.39 (9.10)^{ab}	110.21 (10.52)^{bc}	134.47 (11.62)^{bc}	156.31 (12.52)^{abc}	181.31 (13.48)^{bc}
T_5	*Siphanobacter* sp. (ST-SA-FS)	1.20 (1.30)^{c}	6.40 (2.63)^{de}	24.12 (4.96)^{ab}	98.44 (9.95)^{bc}	116.27 (10.81)^{bc}	141.93 (11.93)^{bc}	178.66 (13.38)^{c}	203.66 (14.29)^{cd}
T_6	Imidacloprid 48FS (ST alone)	0.21 (0.84)^{a}	2.98 (1.87)^{ab}	22.74 (4.82)^{a}	72.24 (8.53)^{ab}	118.83 (10.92)^{bc}	148.33 (12.20)^{bc}	195.15 (13.99)^{c}	210.66 (14.53)^{cd}
T_7	Untreated control	2.88 (1.81)^{d}	9.32 (3.07)^{e}	45.89 (6.66)^{b}	128.85 (11.12)^{c}	150.65 (12.02)^{c}	186.65 (13.37)^{c}	201.85 (13.90)^{c}	235.84 (15.32)^{d}
CD (P = 0.05)		0.27**	0.51**	1.19**	1.98**	2.14**	2.37**	2.57**	1.09**

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 3. Effect of PGPR on biochemicals and defence enzyme 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 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT
T_1	*Bacillus subtilis* Bbv57(ST-SA-FS)	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}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	1.01 (1.23)^{b}	1.11 (1.27)^{bc}	1.28 (1.33)^{bc}	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}
T_3	*Rhizobium pusense* (ST-SA-FS)	0.60 (1.05)^{de}	0.91 (1.19)^{c}	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}
T_4	*Ensifer* sp. (ST-SA-FS)	0.88 (1.17)^{bc}	0.65 (1.07)^{d}	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}
T_5	*Siphanobacter* sp. (ST-SA-FS)	0.78 (1.13)^{cd}	1.18 (1.3)^{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}
T_6	Imidacloprid 48FS (ST alone)	0.63 (1.06)^{de}	0.66 (1.08)^{d}	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}
T_7	Untreated control	0.50 (0.99)^{e}	0.53 (1.01)^{d}	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)		0.06**	0.09**	0.02**	0.03**	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 biochemicals and defence enzyme 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 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT	30 DAE	72 HAT
T_1	*Bacillus subtilis* Bbv57(ST-SA-FS)	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 (10.88)^{a}
T_2	*Bacillus amyloliquefaciens* (ST-SA-FS)	1.21 (1.31)^{a}	1.71 (1.49)^{b}	1.37 (1.37)^{ab}	1.88 (1.54)^{b}	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)	0.77 (1.13)^{bcd}	1.13 (1.28)^{c}	1.16 (1.29)^{b}	1.47 (1.4)^{c}	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)	0.95 (1.2)^{b}	1.44 (1.39)^{b}	1.34 (1.36)^{b}	1.69 (1.48)^{bc}	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)	0.82 (1.15)^{bc}	1.11 (1.27)^{c}	1.22 (1.31)^{b}	1.37 (1.37)^{c}	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)	0.65 (1.07)^{cd}	0.69 (1.09)^{d}	0.55 (1.02)^{c}	0.59 (1.04)^{d}	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	0.60 (1.04)^{d}	0.68 (1.08)^{d}	0.43 (0.96)^{c}	0.48 (0.98)^{d}	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)		0.10**	0.11**	0.11**	0.12**	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)

4. DISCUSSION

The application of PGPR to okra through seed treatment, soil application, and foliar spray significantly decreased aphid populations during both the Rabi 2020 and Summer 2021 seasons. The incidence of *Aphis gossypii* (Glover) remained low up to 14 days after emergence (DAE) in plants treated with *Bacillus subtilis* Bbv57 and imidacloprid 48 FS. However, aphid populations began to rise as the growing period progressed in both seasons. Observations recorded at 35 DAE indicated that foliar application of *B. subtilis* at 30 DAE successfully maintained lower aphid levels up to 42 DAE, followed closely by treatments with *B. amyloliquefaciens*, compared to imidacloprid-treated plants. These results align with the findings of Stout *et al.* (2002), who noted that *Bacillus* treatments delayed population growth, resulting in minimal *A. gossypii* numbers on cucumbers. Similarly, Murugan *et al.* (2005) reported that the application of *Pseudomonas fluorescens* significantly reduced the populations of aphids, leafhoppers, and whiteflies in okra. The reduction in pest populations is likely due to the induction of systemic resistance in plants, which affects aphid development and feeding behavior (Serteyn et al., 2020). Additionally, *Bacillus subtilis* was more effective at reducing aphid populations than other PGPR and insecticides, potentially due to its production of antimicrobial compounds, enhancement of plant defense mechanisms, improved nutrient availability, formation of protective biofilms, and minimal impact on beneficial insects.

5. LIMITATIONS

Despite the promising results of using PGPR strains in managing *Aphis gossypii* in okra, their effectiveness faces several limitations. Environmental factors like soil conditions, moisture, and temperature can significantly influence PGPR efficacy, leading to variable results across different regions. Additionally, the effects are strain-specific, requiring careful selection and validation. Unlike conventional insecticides, PGPR act gradually, enhancing plant growth and systemic resistance rather than providing immediate relief in severe infestations. Their indirect action, without direct toxicity to aphids, may be insufficient under high pest pressure. Proper agronomic practices, application timing, and soil health are critical to achieving desired results. Furthermore, producing and applying consistent, high-quality formulations can be costly and challenging for small-scale farmers. PGPR strains are also vulnerable to biotic and abiotic stresses, which could limit field efficacy. Thus, integrating PGPR with other pest management strategies is essential for consistent success.

6. FUTURE PERSPECTIVES

Utilizing PGPR for aphid management in okra and other crops involves several promising avenues. First, optimizing PGPR strains through genetic engineering can enhance their biocontrol capabilities and adaptability to environmental stresses. Additionally, improvements in formulation techniques, such as nanoformulations and encapsulation, can increase the stability and shelf life of PGPR in the field. Combining PGPR with other biocontrol agents, like beneficial fungi or natural predators, may yield synergistic effects for sustainable pest management. Gaining insights into how PGPR induce plant defense mechanisms will facilitate the development of more precise treatments. Standardizing application protocols tailored to specific regional conditions is essential for maximizing effectiveness. Furthermore, creating multi-functional PGPR strains that promote both pest resistance and overall plant health can provide added advantages to farmers. Incorporating precision agriculture technologies and digital tools can further enhance PGPR application, allowing for real-time monitoring and adjustments. Lastly, fostering policy support and sustainability initiatives, including incentives for sustainable practices and training programs for farmers, can encourage broader adoption of these strategies. Overall, these approaches aim to refine PGPR utilization, making them more effective, cost-efficient, and integral to sustainable agriculture for managing aphids and other pests.

7. CONCLUSION

The study conclusively demonstrated that applying various Plant Growth-Promoting Rhizobacteria (PGPR) strains significantly reduced infestations of *Aphis gossypii* in okra. Among the treatments, *Bacillus subtilis* Bbv57 was the most effective, leading to substantial decreases in aphid populations compared to untreated controls. This efficacy is attributed to the multifaceted actions of PGPR, which enhanced plant vigor and nutrient quality, induced systemic resistance, and improved biochemical defense responses. *B. subtilis*

excelled by producing antimicrobial compounds, enhancing defense enzymes such as peroxidase and polyphenol oxidase, increasing phenol and tannin levels. These mechanisms collectively strengthened plant health and resilience against aphid infestations. The integrated application approach, which combined soil, seed, and foliar treatments, effectively maintained low aphid populations throughout the cropping period. These findings highlight the significance of incorporating PGPR strains into standard agronomic practices as an eco-friendly alternative to chemical insecticides, promoting sustainable pest control, reducing environmental impact, and enhancing crop resilience. Utilizing PGPR in okra cultivation also sets the stage for broader applications in sustainable agriculture.

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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