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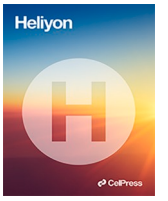

Research article

Impact of bacterial volatiles on the plant growth attributes and defense mechanism of rice seedling

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ABSTRACT

Rice is a major dietary element for about two billion people worldwide and it faces numerous biotic and abiotic stress for its cultivation. Rice blast disease caused by *Magnaporthe oryzae* reduce up to 30 % rice yield. Overuse of synthetic chemicals raises concerns about health and environment; so, there is an urgent need to explore innovative sustainable strategies for crop productivity. The main aim of this study is to explore the impact of bacterial volatiles (BVCs) on seedling growth and defense mechanisms of rice under *in-vitro* condition. On the basis of plant growth promoting properties, six bacterial strains were selected out of ninety-one isolated strains for this study; *Pantoea dispersa* BHUJPVR01, *Enterobacter cloacae* BHUJPVR02, *Enterobacter* sp. BHUJPVR12, *Priestia aryabhattai* BHUJPVR13, *Pseudomonas* sp. BHUJPVWRO5 and *Staphylococcus* sp. BHUJPVWLE7. Through the emission of bacterial volatiles compounds (BVCs), *Enterobacter* sp., *P. dispersa* and *P. aryabhattai* significantly reduces the growth of rice blast fungus *Magnaporthe oryzae* by 69.20 %, 66.15 % and 62.31 % respectively. Treatment of rice seedlings with BVCs exhibited significant enhancement in defence enzyme levels, including guaiacol peroxidase, polyphenol oxidase, total polyphenols, and total flavonoids by a maximum of up to 24 %, 48 %, 116 % and 80 %, respectively. Furthermore, BVCs effectively promote shoot height, root height, and root counts of rice. All BVCs treated plant showed a significant increase in shoot height. *P. dispersa* treated plants showed the highest increase of 60 % shoot and 110 % root length, respectively. Root counts increased up to 30% in plants treated with *E. cloacae* and *Staphylococcus* sp. The BVCs can be used as a sustainable approach for enhancing plant growth attributes, productivity and defence mechanism of rice plant under biotic and abiotic stresses.

1. Introduction

Exponential increase in the human population over the decades elevates the demand for agricultural products globally. Rice (*Oryza sativa* L.) is a major dietary element for approximately two billion people worldwide. Consequently, over 100 nations cultivate rice across the world, however the top ten rice producers accounts for about 85 % of global production [1]. Among this India and China accounts approximately 50 % of total rice production globally [2]. In addition, the current food demands rise 20 % in rice production

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for avoiding food crisis. To meet the global food demand, the synthetic chemicals and biofertilizers have been extensively used [3]. However, as health and environmental concerns are increasing, there is a shift towards the application of biofertilizers, including endophytic bacteria and their byproducts.

Innumerable microbial species interact with plants in both mutualistic and parasitic way. Out of these, plant endophytes are residents of the inside plant parts in mutualistic way and emits a complex mixture of volatiles, secondary metabolites, plant and microbial hormone [4]. This relationship helps to host plant for taking up essential nutrients and generate tolerance against various biotic and abiotic stresses [5]. From seed germination onwards, plants are in contact with bacterial volatiles compounds (BVCs). Microbial volatiles promote improve seed germination and plant biomass, and to protect plants from abiotic and biotic stress BVCs plays a pivotal role for inducing plant systemic tolerance.

Global rice production is severely impacted by rice blast disease, which is caused by a filamentous fungus namely *Magnaporthe oryzae* (synonym of *Pyricularia oryzae)* [6]. Up to 30 % of yield losses of rice possibly due to the rice blast disease [7]. Multiple strategies have been employed to reduce the severity of this condition, among them application of endophytes and their byproducts in different bioformulation having inhibitory effects on the blast fungus and plant growth promotion as well. From literature approximately 2000 VOCs have been identified which were emitted by a bacteria and fungi and were discovered on a limited number of microorganisms. BVCs are categorized in alcohols, alkenes, ketones, tepenoids, and other chemical classes [8–10].

BVCs possess the ability to stimulate plant defense mechanisms by elevating the activity of defense related enzymes such as polyphenol oxidase, guaiacol peroxidase, total flavonoids, and total polyphenols. To fufill the gaps on BVCs and their impact on rice plant growth and elevating the defense response mechanisms, this study is (a) to identify the rice endophytes having properties of reducing blast severity through their volatiles (b) to promote plant growth by the action of volatiles and (c) to enhance synthesis of defense related enzymes in plant by the action of BVCs. We hypothesised that BVCs have ability to show positive impact on plant growth, development and biocontrol under biotic and abiotic stresses by elevating plant defense systems.

2. Material and methods

2.1. Isolation of endophytic strains from rice plant

Isolation of endophytic bacteria from rice were done by serial dilution methods. Different plants part were washed under the tap water and deionized water to remove dirt particles. Different parts (seeds, leaf, root and, stem) cut into sections 2–3 cm and surface sterilised by using 0.1 % mercury chloride ($HgCl_2$) and 70 % ethanol [11]. The bacterial endophytes were then isolated from the sterilised rice plant parts via crushing and serial dilution in agar and broth containing nutrient agar (NA) and tryptone soya agar or trypto-casein soy agar (TSA). Then the agar plates and broth inoculated with rice plant parts were incubated at 28 ± 2 °C for 2–5 days, and the broth was incubated in an incubator with shaking at 130 rpm. Following incubation, many microbial colonies were found on the particular plates. Based on the colony morphology, shapes, sizes, colours, we picked the microbial colony and prepared slant and glycerol stock and stored in −20 °C and −80 °C.

2.2. Biochemical characterization of isolated bacterial strains

Gram staining and biochemical properties were used to characterize isolated endophytic isolates [12,13].The plant growth promoting biochemical properties of isolated rice plant endophytes were explored by investigating the indole-3-acetic acid (IAA) [14], NH_3 (ammonia) production [15], phosphate solubilization [16,17], siderophore production [18], zinc solubilization [19], Silicate solubilization [20,21], Potassium solubilization [22], and antagonistic effect of BVCs were observed against the plant pathogen *Magnaporthe oryzae* [23,24].

2.3. Molecular characterisation of isolates

DNA of endophytes were isolated by using CTAB (cetyltrimethylammonium bromide) methods [25], and the identification of isolates were done by PCR, which were conducted by using the universal 16s rRNA gene (Forward primer 27F 5′-AGAGTTTGATCCTGGCTCAG-3′, and Reverse primer 939R 5′-TACGGTTACCTTGTTACGACTT-3′) [26]. The PCR products were resolved in 1.2 % (w/v) agarose gel, alongside a 100 bp DNA ladder (Thermo Scientific, Lithuania). Predominantly 1000 bp PCR products were observed. For the sequencing of amplified 16s rRNA genes, purification of the PCR products was carried out by PCR purification kit (Invitrogen, PCR purification kit, USA).

2.4. Effects of the endophytic volatile components on rice seedling growth

Rice seeds surface sterilisation was done by using of 0.1 % mercury chloride and 70 % ethanol [11]. We took two opened small (4 & 8 cm) petri plates containing microbial inoculum and other contain sterilised rice seed and placed both petri plate inside a bigger plate (15 cm). After that bigger petri plate covered with lid and sealed properly to evaluate the effect of BVCs on seed germination of rice and defense elevation. Then the whole plate placed in the plant growth chamber (12 h and 12 h duration of light and dark respectively, moisture was ranging from 75 to 80 %, and the temp. was ranged from 28 to 30 °C). The whole plant growth promoting experiments was measured for 15 days. The height of root, shoot and numbers of root and leaves were recorded.

2.5. Plant biochemical assay

After the experiments, plants were collected and measured the activity of different plant defence enzymes including; guaiacol peroxidase [27–29], polyphenol Oxidase [30–32], total phenols [28,33] and flavonoids content [34].

2.6. Statistical analysis

This study was conducted with seven treatments having three replicates. The research data were represented as mean ± SD. Data analysis was done by use of ANOVA with Duncan comparison tests using SPSS software. The significant values were taken at $p \leq 0.05$.

3. Results

3.1. Isolation and culture collection

A total of 91 morphologically diverse endophytes were isolated from different parts of the rice plant samples. On the basis of their plant growth promoting biochemical properties (IAA production, phosphate solubilization, potassium solubilization, ammonium production, siderophore production, Silicate solubilization, Zinc solubilization) only 4 endophytes were selected for the study of their volatiles on plant growth promotion and defense activation. Another 2 microorganisms were taken from lab that were previously characterized in Chouhan et al. (2023) [35] namely BHUJPVWRO5 (*Pseudomonas* sp.), and BHUJPVWLE7 (*Staphylococcus* sp.).

3.2. Characterization of plant growth promoting properties of rice endophytes

Biochemical characteristics (IAA production, phosphate solubilization, potassium solubilization, ammonium production, siderophore production, silicate solubilization, zinc solubilization) were used to characterize the isolates. Upon 48h of incubation, out of 91 only 47 isolated strains from rice plants and rhizosphere soil produced IAA where *Enterobacter* sp. strain BHUJPVR12 (56.54 μg/ml), *Staphylococcus* sp. strain BHUJPVWLE7 (56.07 μg/ml) *Pseudomonas* sp. strain BHUJPVWRO5 (49.62 μg/ml) and *Enterobacter cloacae* strain BHUJPVR02 (42.29 μg/ml) produced highest amount of IAA. 27 isolated endophytes showing positive result for ammonia production in the range from 9.16 to 21.30 μg/ml and the highest activity was showing in *Priestia aryabhattai* strain BHUJPVR13 (21.30 μg/ml). All endophytes solubilized phosphate, but the most significant strain was *Enterobacter cloacae* strain BHUJPVR02 (943.51 μg/ml) and *Pantoea dispersa* strain BHUJPVR01 (823.51 μg/ml). *Enterobacter* sp. strain BHUJPVR12 (38.6 μg/ml) and *Pseudomonas* sp. strain BHUJPVWRO5 (14.45 μg/ml) significantly produced siderophore in comparison to other isolates. All selected strains solubilize zinc, but *Pantoea dispersa* strain BHUJPVR01 and *Pseudomonas* sp. strain BHUJPVWRO5 being the most effective solubilizers. *Pseudomonas* sp. strain BHUJPVWRO5, *Staphylococcus* sp. strain BHUJPVWLE7, and *Pantoea dispersa* strain BHUJPVR01 solubilize more silicate than the others. *Pseudomonas* sp. strain BHUJPVWRO5 having the highest potassium solubilizing activity, followed by *Pantoea dispersa* strain BHUJPVR01, *Enterobacter cloacae* strain BHUJPVR02, *Enterobacter* sp. strain BHUJPVR12, and *Staphylococcus* sp. strain BHUJPVWLE7 (Table 1).

3.3. Identification of culturable rice endophytes by 16s rDNA sequencing

On the basis on their PGP properties 4 isolates were selected for 16s rDNA sequencing. From the analysis of their sequence, we found out that BHUJPVR01 is *Pantoea dispersa*, BHUJPVR02 is *Enterobacter cloacae,* BHUJPVR12 is *Enterobacter* sp. and BHUJPVR13 is *Priestia aryabhattai* (Table 2, Supplementary Figs. 1A and B).

Table 1
Plant growth promoting properties of selected endophytic strains.

Isolate	IAA production (ug/ml) (with tryptophan)	Ammonia production	PSB	Siderophore production	Zinc	Silica	Potassium
BHUJPVR01	14.90 ± 1.02^{a}	11.01 ± 1.26^{ab}	823.51 ± 8.48^{c}	6.62 ± 0.81^{ab}	+++	++	+
BHUJPVR02	42.29 ± 0.11^{c}	11.46 ± 1.55^{ab}	943.51 ± 14.12^{d}	4.69 ± 0.79^{a}	++	+	+
BHUJPVR12	56.54 ± 2.21^{d}	9.16 ± 1.47^{a}	741.94 ± 11.12^{b}	38.6 ± 5.76^{e}	+	+	+
BHUJPVR13	25.62 ± 0.43^{b}	21.30 ± 1.64^{c}	578.81 ± 34.82^{a}	3.02 ± 1.14^{a}	++	–	–
BHUJPVWRO5	49.62 ± 0.11^{c}	18.51 ± 1.64^{bc}	607.82 ± 34.82^{ab}	14.45 ± 1.55^{c}	+++	+++	+++
BHUJPVWLE7	56.07 ± 2.21^{d}	14.45 ± 1.55^{b}	609.00 ± 34.82^{ab}	21.30 ± 1.64^{d}	+	+++	+

Note: Here BHUJPVR01= *Pantoea dispersa*; BHUJPVR02 = *Enterobacter cloacae*; BHUJPVR12 = *Enterobacter* sp.; BHUJPVR13= *Priestia aryabhattai*; BHUJPVWRO5= *Pseudomonas* sp.; BHUJPVWLE7 = *Staphylococcus* sp. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

3.4. Effect of bacterial volatile compounds (BVCs) on the growth of rice blast fungus Magnaporthe oryzae

Bacterial volatile compounds from selected microorganisms were evaluated for the potential of antifungal activity on rice pathogenic fungi *M. oryzae.* All the microorganisms significantly reduce the mycelial growth of fungi but T4 (*Enterobacter* sp. BHUJPVR12), T2 (*P. dispersa* BHUJPVR01) and T5 (*P. aryabhattai* BHUJPVR13) up to 69.20 %, 66.15 % and 62.31 %, respectively (Table 3, Fig. 1A and B).

3.5. Effects of bacterial volatile compounds (BVCs) on rice plant growth promotion

After 15 days of BVCs treatment shoot length, root length, leaf number and root number were recorded, and a significant difference were recorded when compared to the control (T1). All BVCs treated plants showed a significant increase in height, with T2 (*P. dispersa* BHUJPVR01) and T5 (*P. aryabhattai* BHUJPVR13) treated plants have longer shoot length of 14.17 and 12.03 cm, respectively, as compared to control T1 (8.83 cm). Apart from T3 (*E. cloacae* BHUJPVR02), all BVCs treated plants showed higher root length of up to 10.70 cm when compared to control (T1) 5.10 cm. In treatments T3 (*E. cloacae* BHUJPVR02), T4 (*Enterobacter* sp. BHUJPVR12) and T7 (*Staphylococcus* sp. BHUJPVWLE7) root counts were also increased to 14.33, 14.00 and 14.33 than control (T1), respectively (Fig. 2 & Table 4).

3.6. Effect of BVCs on the defense related enzymes

BVCs treated 15 days old rice seedlings were assayed for their defense enzymes such as guaiacol peroxidase and polyphenol oxidase. All treatments showed enhanced activity of POX enzymes, with T4 (*Enterobacter* sp.) and T6 (*Pseudomonas* sp.) having the maximum activity of 0.59 and 0.57 Unit/L, respectively, as compared to the T1 control 0.47 unit/L. PPO activity was also enhanced in all treatments where T3 (*E. cloacae*) and T6 (*Pseudomonas* sp.) showing the maximum activity up to 0.37 Unit/L. Total polyphenols and total flavonoids were found more in BVCs treated rice seedlings (Fig. 3 & Table 5).

4. Discussion

In this study, we explored the impact of bacterial volatiles on the growth of rice plant and their impact on defense related enzymes. Based on the PGP properties (IAA production, phosphate solubilization, potassium solubilization, ammonium production, siderophore production, silicate solubilization and zinc solubilization), only 6 strains were selected for the study. In here, we demonstrated the effect of bacterial volatiles on plant growth promotion, elevation of defense related enzymes and inhibition of rice blast fungus *M. oryzae* in plate assay.

In our study, we were targeting fungal pathogen *M. oryzae,* the causative agent of rice blast disease. Blast disease can cause significant yield loss of up to 30 % and in some instance up to 100 % yield loss was observed [7,36]. It was previously reported, bacterial volatiles can inhibit the growth of plant pathogen of up to 69 % [37]. For instance, several microbial cultures are known to produce volatile compounds making plant more resistant to phytopathogen attack by elevating their systemic resistance [38–40]. In this study, we demonstrated that bacterial volatiles reduce the mycelial growth of rice blast fungus *M. oryzae* up to 69.20 % in plate assay. All selected strains effectively reduce the growth of plant pathogenic fungus by the action of their volatiles, with *Enterobacter* sp. strain BHUJPVR12 reduces maximum growth followed by *P. dispersa* strain BHUJPVR01 and *P. aryabhattai* BHUJPVR13.

Ryu et al. (2003) [41], first reported the positive impact caused by bacterial volatiles promoting the growth of Arabidopsis, since then very few research was conducted on the growth promotion of rice by the action of bacterial volatiles [10]. In our experimental setup, six isolates were used to see if their volatiles could promote rice growth when grown on two petri plates with only airborne signals being transferred between the bacteria and the plants. Our findings showed that bacterial volatiles promote rice plant growth in the form of shoot height, root height, root numbers and leaf numbers. When rice seedlings were exposed to bacterial volatiles for 15 days, all treated plants showed a significant increase in shoot height, with *P. dispersa* BHUJPVR01 treated plants showed the highest increase of 60.48 % as compared to control. Similarly, more than 85 % increment was observed in root length of plants treated with bacterial volatiles of *P. dispersa* BHUJPVR01, *Enterobacter* sp. BHUJPVR12, *P. aryabhattai* BHUJPVR13, *Pseudomonas* sp. BHUJPVWRO5 and *Staphylococcus* sp. BHUJPVWLE7. Leaf counts were practically identical to control, whereas root counts increased a maximum of up to 30 % in plants treated with *E. cloacae* BHUJPVR02 and *Staphylococcus* sp. BHUJPVWLE7.Additional study was conducted for estimation of elevated defense response in plants treated with bacterial volatiles by assaying defense related enzymes such as guaiacol peroxidase, polyphenol oxidase, total polyphenols, and total flavonoids. Bacterial volatiles treated plants exhibited enhanced activity of guaiacol peroxidases, polyphenol oxidases, and increased levels of total phenols and flavonoids. Guaiacol

Table 2
Molecular identification of isolates through 16srDNA sequencing.

Strain	Bacterial Name	Accession number	Percent identity match	Reference organism	Reference organism Accession no.
BHUJPVR01	*Pantoea dispersa*	OQ892233	100 %	*Pantoea dispersa* strain AA9	MT275631
BHUJPVR02	*Enterobacter cloacae*	OQ892234	100 %	*Enterobacter cloacae* strain MBB8	MT138639
BHUJPVR12	*Enterobacter* sp.	OR603106	97.83 %	*Enterobacter* sp. strain HSTU-ASn40	MN559048
BHUJPVR13	*Priestia aryabhattai*	OR603107	100 %	*Priestia aryabhattai* strain AYG1023	OQ569480

Table 3
Impact of bacterial volatiles compounds (bVOCs) for growth inhibition of plant pathogenic fungus.

Treatment	Average diameter (cm)	Percent growth inhibition (PGI)
T1	4.33 ± 0.05^{a}	–
T2	1.47 ± 0.05^{e}	66.15
T3	1.73 ± 0.05^{d}	59.99
T4	1.33 ± 0.11^{e}	69.20
T5	1.63 ± 0.11^{d}	62.31
T6	1.93 ± 0.11^{c}	55.35
T7	2.10 ± 0.10^{b}	51.53

Note: Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

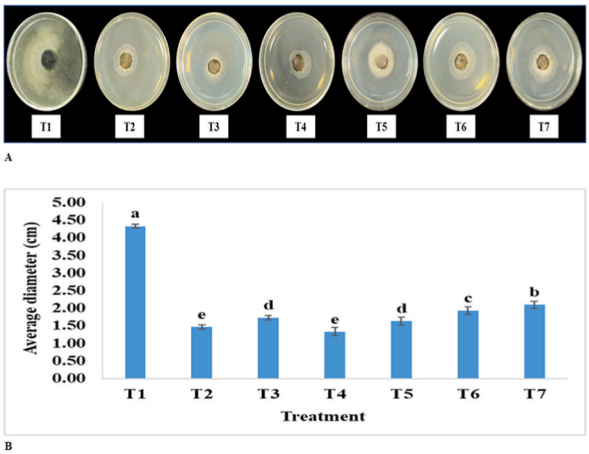

Fig. 1. A: Antifungal activity of bacterial volatiles against *Magnaporthe oryzae* for showing growth inhibition as compare to control; B: Diameter of fungal growth inhibition by bacterial volatiles compunds. Note: Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

peroxidases activity was observed highest in *Enterobacter* sp. strain BHUJPVR12 and *Pseudomonas* sp. strain BHUJPVWRO5 treated rice plants. Polyphenol oxidase activity was found highest in *E. cloacae* strain BHUJPVR02 and *Pseudomonas* sp. strain BHUJPVWRO5 treated rice plants. According to a recent study, tomato leaves exposed to bacterial volatiles exhibited increased level of phenolics, which improves plant resistance to *F. oxysporum* f.sp. *lycopersici* and effectively promoted plant growth as compared to untreated pathogen challenged control [42]. Increased phenol content in plant was due to the exposure of bacterial volatiles and were responsible for induced systemic resistance (ISR) and systemic acquired resistance [43]. Few studies reported that, 3-pentanol and 6-pentyl-α-pyron volatiles produced by *Bacillus amyloliquefaciens* strain IN937a and *Trichoderma,* respectively, induce systemic resistance via modulation of salicylic acid and jasmonic acid pathways resulting in reduction of phytopathogenic attack in pepper and *Arabidopsis thaliana* [44,45]. Many phenols such as gallic acid were reported to protect plant from fungal pathogen by degrading fugal chitin by enhancing the activities of chitinases and peroxidases [46,47]. From aforementioned reports, it was clear that our finding of increased level of phenols in volatiles treated plants was indication of elevated defense response against fungal pathogens. Furthermore, Tang et al. (2023) [48] reported that flavonoids treatment can contribute to plant resistance to whitefly *(Bemisia tabaci)* by the callose accumulation and jasmonic acid expression. In corelation with above results, our findings of increased level of flavonoids in volatiles treated rice plants may show more resilience towards insect pest attack. Our findings align with several studies demonstrating that increased levels of defence enzymes in plants leads to the breakdown of reactive oxygen species (ROS), effectively protecting plants from pathogenic attack [49–52]. Considering the outcomes of the above study, we may hypothesize that bacterial volatiles exposure could promote plant growth and defense response in rice plants from a variety of biotic and abiotic stresses.

5. Conclusion

Bacterial volatile compounds (BVC) from plant growth promoting bacterial strains could be an ideal strategy to increase plant growth by improving plant physiological property and suppressing the disease. The volatiles compounds of rice endophytes

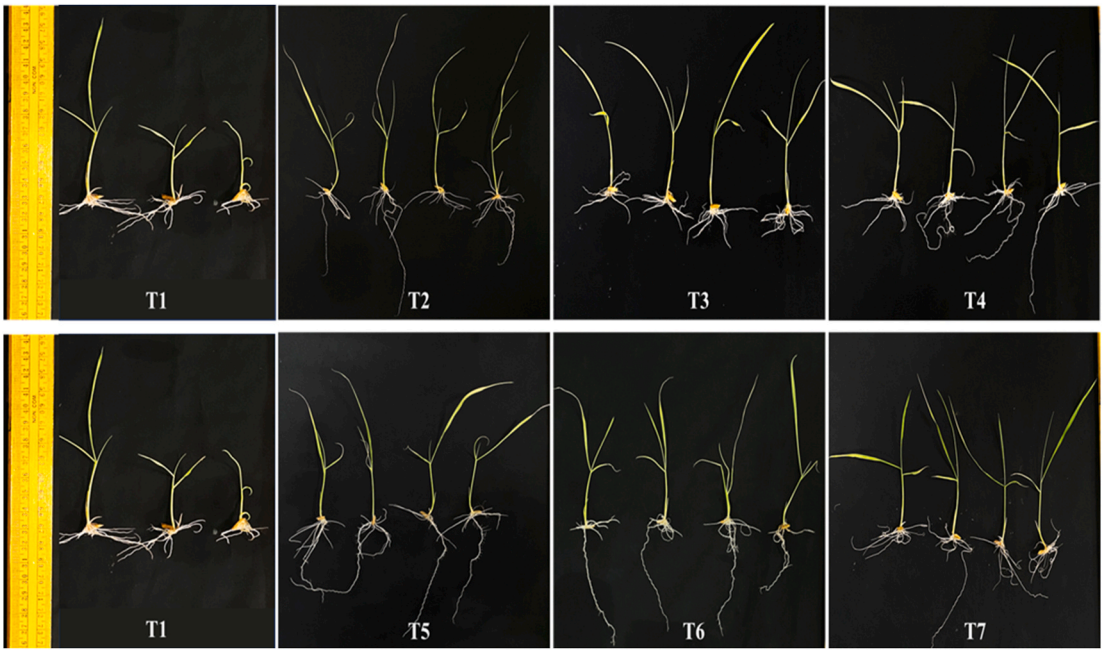

Fig. 2. Figure represents the effect of bacterial volatiles on growth of rice seedlings, Note: Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7.

Table 4
Bacterial volatiles compounds mediated plant growth promotion of rice seedlings at 15 days.

Treatment	Shoot length	Root length	Leaf numbers	Root numbers
T1	08.83 ± 2.63^{a}	05.10 ± 1.15^{a}	2.67 ± 0.57^{a}	11.00 ± 1.00^{a}
T2	14.17 ± 1.25^{c}	10.70 ± 2.98^{b}	3.00 ± 0.00^{a}	11.67 ± 0.57^{a}
T3	11.13 ± 1.06^{ab}	05.27 ± 0.66^{a}	3.00 ± 0.00^{a}	14.33 ± 0.57^{b}
T4	10.90 ± 0.17^{ab}	10.00 ± 1.48^{b}	3.00 ± 0.00^{a}	14.00 ± 1.00^{b}
T5	12.03 ± 0.84^{bc}	10.00 ± 0.79^{b}	3.00 ± 0.00^{a}	11.33 ± 2.51^{a}
T6	11.10 ± 0.87^{ab}	10.00 ± 1.00^{b}	3.00 ± 0.00^{a}	12.67 ± 1.15^{ab}
T7	11.37 ± 0.72^{b}	09.53 ± 2.66^{b}	3.00 ± 0.00^{a}	14.33 ± 0.57^{b}

Note: Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

Enterobacter sp. BHUJPVR12, *Pantoea dispersa* BHUJPVR01 and *Priestia aryabhattai* BHUJPVR13 directly reduce growth of the rice pathogens *Magnaporthe oryzae* by 69.20 %, 66.15 %, and 62.31 %, respectively under *in vitro* condition experiments. A significant increase of plant growth attributes is observed in the rice plants treated with volatiles of *Pantoea dispersa* BHUJPVR01, *Enterobacter cloacae* BHUJPVR02 and *Staphylococcus* sp. BHUJPVWLE7 as compared to untreated plants. Beside the plant growth BVCs also elevated the defense activity of host plants by activation of key defense related enzymes such as guaiacol peroxidase, polyphenol oxidase, total phenol and total flavonoids content which are able to improve plant sustainability under biotic and abiotic stresses. The bacterial volatiles compounds will be an effective technology for biocontrol against different phytopathogen. This technology will be environment friendly, cost effective and socially acceptable for agricultural production.

CRediT authorship contribution statement

Tushar Goyal: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Arpan Mukherjee:** Writing – review & editing, Methodology, Formal analysis. **Gowardhan Kumar Chouhan:** Software, Formal analysis, Conceptualization. **Anand Kumar Gaurav:** Software, Methodology, Formal analysis, Data curation. **Deepak Kumar:** Software, Methodology, Formal analysis, Data curation. **Saman Abeysinghe:** Writing – review & editing, Project administration. **Jay Prakash Verma:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

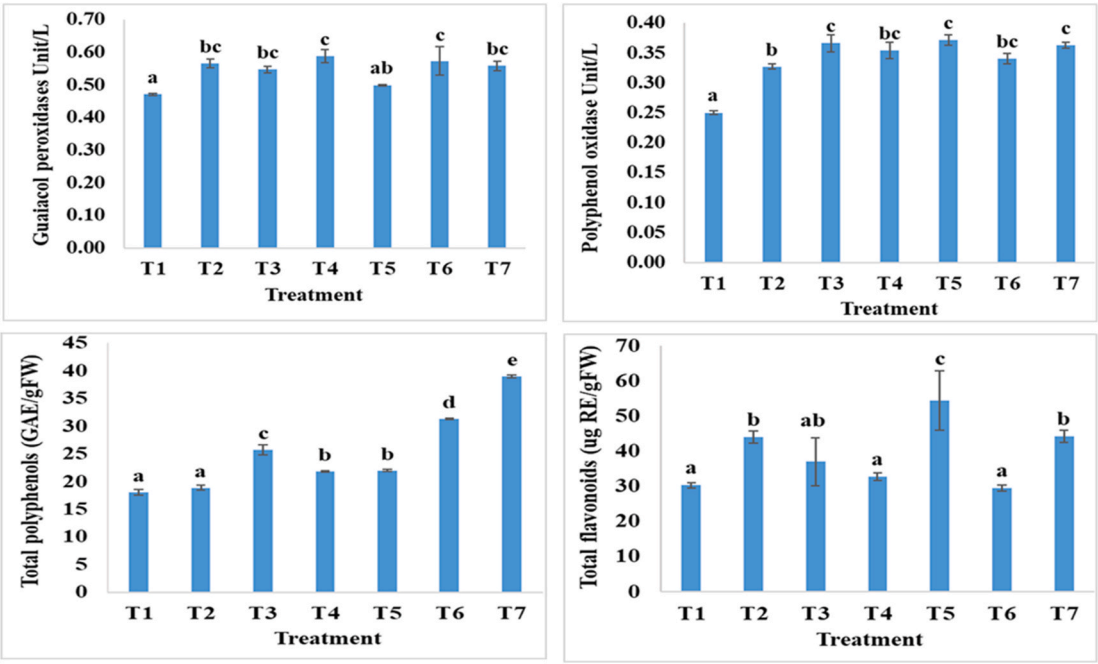

Fig. 3. Defense elevation of bacterial volatiles treated rice plant. Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

Table 5
Enzymatic activity of rice plants treated with bVOCs.

Treatment	Guaiacol peroxidase (Unit/L)	Polyphenol oxidase (Unit/L)	Total polyphenols (GAE/gFW)	Total flavonoids (μg RE/gFW)
T1	0.47 ± 0.02^{a}	0.25 ± 0.03^{a}	18.02 ± 0.49^{a}	30.30 ± 0.42^{a}
T2	0.56 ± 0.01^{bc}	0.33 ± 0.04^{b}	18.84 ± 0.45^{a}	44.03 ± 0.94^{b}
T3	0.54 ± 0.09^{bc}	0.37 ± 0.01^{c}	25.76 ± 0.92^{c}	36.98 ± 3.94^{ab}
T4	0.59 ± 0.01^{c}	0.35 ± 0.01^{bc}	21.79 ± 0.10^{b}	32.73 ± 0.64^{a}
T5	0.50 ± 0.02^{ab}	0.37 ± 0.05^{c}	21.99 ± 0.20^{b}	54.48 ± 4.84^{c}
T6	0.57 ± 0.04^{c}	0.34 ± 0.04^{bc}	31.27 ± 0.12^{d}	29.45 ± 0.52^{a}
T7	0.56 ± 0.01^{bc}	0.36 ± 0.05^{c}	38.97 ± 0.24^{e}	44.27 ± 0.99^{b}

Note: GAE-Gallic acid; FW- Fresh weight & RE-Rutin equivalent. Treatment T1 = Control; T2 = *Pantoea dispersa* BHUJPVR01; T3 = *Enterobacter cloacae* BHUJPVR02; T4 = *Enterobacter* sp. BHUJPVR12; T5 = *Priestia aryabhattai* BHUJPVR13; T6 = *Pseudomonas* sp. BHUJPVWRO5; T7 = *Staphylococcus* sp. BHUJPVWLE7. Data are shown as mean ± SD (n = 3), and in each column the superscript letters are used to show the statistical relationship between isolates based on ANOVA and Duncan multiple post-hoc test ($P \leq 0.05$).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29692.

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