PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.7065

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RESEARCH ARTICLE

Biological control of greenhouse whiteflies (*Trialeurodes vaporariorum*) using indigenous *Pseudomonas* spp.: An eco-friendly approach

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OPEN ACCESS

ARTICLE HISTORY
Received: 04 January 2025
Accepted: 15 March 2025
Available online
Version 1.0 : 17 May 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE

Kaur S, Thakur N, Yadav N, Kaur N, Puri P, Singh S, Shreaz S, Yadav AN. Biological control of greenhouse whiteflies (*Trialeurodes vaporariorum*) using indigenous *Pseudomonas* spp.: An eco-friendly approach. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.7065

Abstract

Soil deterioration and environmental degradation caused by harsh chemical pesticides have led to the search for suitable alternatives that can not only control pests but also promote plant growth. *Pseudomonas* spp. has been exploited immensely for its biocontrol and plant growth promotion attributes. This study isolated 5 indigenous bacterial strains from eight rhizospheric soil samples from Shimla and Sirmour districts of Himachal Pradesh and further screened them for traits such as siderophores production and hydrolytic enzyme (amylase and protease) activity. Screening data of isolates showed production of siderophores (19.56%), amylase (50%) and protease (71.73%). The strain EU-SIRCK1243 was positive for all three traits and was tested for indole-3-acetic acid (IAA) production yielding 2.03±0.30 µg/mL (with tryptophan) and 4.23±0.17 µg/mL (without tryptophan). It was molecularly identified as *Pseudomonas aeruginosa*, on the basis of 16S rRNA gene sequencing and evaluated for bioefficacy against *Trialeurodes vaporariorum* (greenhouse whitefly) on tomato plants over two years (2022-2023). The average adult population declined with overall percent reductions of 54.11% in 2022 and 62.04% in consecutive year 2023. In case of nymph populations the overall, reductions was 59.13% in 2022 and 62.20% in 2023. The plant growth and physiological parameters after treatment with EU-SIRCK1243 showed significant increase in shoot and root lengths, fresh, dry biomass, yield and chlorophyll content as compared to control. It is suggested that the strain EU-SIRCK1243 is a promising biocontrol agent for greenhouse whitefly and an effective plant growth promoting bacterium. In future deliberation, the better development of *Pseudomonas aeruginosa* bioproducts could be emphasized.

Keywords

bacterial biopesticides; biocontrol agents; greenhouse whiteflies; molecular characterization; plant growth promoting rhizobacteria; sustainable approaches

Introduction

The need for amelioration of problems such as climate change, environmental degradation, increasing human population, high demands of crop production, crop diseases upsurge and development of pest resistance pertaining to global food security has led to the tedious task of finding a sustainable crop production system (1). One such system incorporates beneficial microorganisms which not only promote plant growth but also help in pathogen suppression. These beneficial microorganisms are called biological control agents (BCA) (2). The use of bacterium as BCA can be an effective management strategy to reduce agrichemicals in agricultural fields (3). They offer a range of different mechanisms for the protection of plants from pests and pathogens. These BCA especially aid the management strategies of insect pests belonging to various orders, such as Lepidoptera (moths and butterflies), Coleoptera (beetles) and Hemiptera (aphids, whiteflies and scale insects) (2). Their efficacy is dependent on both biotic and abiotic factors. The biotic factors may include the microbial agent's mechanism of action, conditioning, dose, methods of application, plant pathogen's sensitivity and host plant's cultivar type and physical properties in consideration. The abiotic factors include the environmental conditions, nutrient availability, moisture, temperature and chemical residues (4).

Over the last half century, a vast array of bacteria have been isolated, characterized and exploited for their biocontrol properties against numerous insects. Families such as Bacillaceae, Enterobacteriaceae, Lactobacillaceae, Micrococcaceae and Pseudomonadaceae house the majority of entomopathogenic bacteria (5). On the biopesticide market and commercial upfront the highly exploited bacteria worldwide belong to the genus *Pseudomonas*, *Bacillus* and *Streptomyces*. Some of the important bacterial species registered and commercialized as biopesticides are *Bacillus amyloliquefaciens*, *Bacillus amyloliquefaciens* subsp. *plantarum*, *Bacillus firmus*, *Bacillus pumilus*, *Bacillus subtilis*, *Pseudomonas chlororaphis* and *Streptomyces lydicus* (6). The model strain *Pseudomonas protegens* CHA0 and Pf-5 has been used to study the relationship between Entomopathogenic pseudomonads and insects along with the different influential factors related to their insecticidal properties in the last fifteen years (4, 7).

Pseudomonas, an aerobic gram-negative fluorescent bacterium, is present worldwide (8). This bacterium can be easily mass-produced and exhibits various attributes such as secretion of growth hormones including auxins, cytokinins and gibberellins for plant growth promotion and plant disease reduction (9). Indole acetic acid (IAA) producing *Pseudomonas* tends to enhance plant root surface area for better accessibility to nutrients from soil (10). *Pseudomonas* spp. possesses several key characteristics, including the synthesis of antibiotics, phytohormones, siderophores, enzymes and antimicrobial compounds such as dialkylresorcinols, phenazines, phloroglucinols, pyrrolnitrin and pyoluteorin. Additionally, *Pseudomonas* spp. assists in atmospheric nitrogen fixation, mineral solubilization and iron-binding secretion and competes with pathogens for soil nutrients and habitat. These traits boost their biocontrol potential against diseases, nematodes and pests. A yellow green pigment called pyoverdines, which fluoresce under UV light and acts as siderophores is also secreted by *Pseudomonas* which helps in the solubilisation of iron from the surrounding environment, forming a ferric-siderophore complex effectively preventing the invasion of harmful microorganisms from the ecological niche. Furthermore, defense responses (Induced systemic resistance (ISR) in host plants through various pathways, are also elicited by these bacteria (11-13).

The biocontrol potential of several strains of *Pseudomonas* has been tested against the insect pests. The insecticidal potential of different strains of *P. fluorescens* has been tested against aphids (*Myzus persica*) (14) phytophagous ladybird beetles (*Epilachna vigintioctopunctata*) (15), fruit fly (*Drosophila melanogaster*), greater wax moth (*Galleria mellonella*) (16) and cotton bollworm (*Helicoverpa armigera*) (17). *P. maltophilia* caused growth retardation in the corn earworm (*Helicoverpa zea*) (18). The grubs of rhinoceros beetle died of septicaemia on the application of *P. alcaligenes* under stress conditions (19). The mortality of grubs was observed with the treatment of *P. aeruginosa* isolated from dead grubs of epilachna beetle (*Henosepilachna vigintioctopunctata*) (20). *Pseudomonas* isolates were isolated from untreated tomato rhizospheric soil and used as a biological control agent for silverleaf whitefly (*Bemisia tabaci*). The study was conducted under laboratory and glasshouse conditions with water treatment as a control. The results indicated that isolate Q036B showed high mortality against adults and larvae of *B. tabaci* in greenhouse. Also, all three *Pseudomonas* isolates (Q110B, Q036B and Q172B) showed significant biopotential, against *B. tabaci* under laboratory conditions (21).

Trialeurodes vaporariorum Westwood of the family Aleyrodidae in the order Hemiptera causes substantial damage to the crops grown both in open and closed spaces. Adults and nymphs both feed on the plant sap, which weakens the plant, affects the photosynthetic rate and respiration, along with causing dehydration and chlorosis (22). Greenhouse whiteflies (GHWF) also act as a vector for different plant viruses and indirectly favours the development of certain fungi such as "sooty mold" (*Cladosporium*) by the production of honeydew (23, 24). GHWF is one of the whitefly species that harms tomatoes due to their large number and causes huge yield losses in greenhouse and field cultivation. *T. vaporariorum* exclusively preferred tomato as a host plant over pepper in a study conducted in Uruguay. The study tested the developmental parameters such as time, survival, longevity, fecundity, population as well as the oviposition preference (25). GHWF causes injury to tomato plants by phloem feeding and formation of sooty mold in the honeydew produced (26). The primary management strategy is the employment of chemical insecticides and over years with prolonged chemical exposure, these insects have developed a resistance (27, 28). Therefore, their control cannot be navigated only with the help of chemical insecticides and an alternative is the need of the hour.

Pseudomonas is an important bioagent in the group of plant growth-promoting rhizobacteria which gives substantial reduction of pest and disease load in the plants, besides triggering the plant growth. Several success reports on *Pseudomonas* against the biotic stresses of several agricultural and horticultural crops have been proven worldwide by various

authors (29–32). This study is articulated to isolate and characterize the native *Pseudomonas aeruginosa*, as well as to further investigate its efficacy against the *Trialeurodes vaporariorum* (greenhouse whitefly) on tomatoes grown in a closed space system (plastic-covered greenhouses) for two years (2022 and 2023) in Baru Sahib, District Sirmour, Himachal Pradesh, India.

Materials and Methods

Area of study, sample collection and isolation of bacteria

The rhizospheric soil was collected from Sirmour (30.5628° N, 77.4702° E) and Shimla (31.1050° N, 77.1640° E) district of Himachal Pradesh, India. A total of 08 (SIRM, SIRL, SIRA, SHMA, SHMC, SHMP) samples of rhizospheric soil associated with diverse vegetation (pear, apple, lemon, marigold and cherry) were digged out a depth of 12-15 cm and collected in well labelled sterilized plastic packets. These collected soil samples were brought to the Zoology Laboratory, Department of Zoology, Eternal University, Baru Sahib, Himachal Pradesh and stored at a temperature of 4°C until further processing.

The isolation of bacterial strains was done using the spread plate method along with the 10-fold serial dilutions technique (33). The isolates were inoculated on selective media growth media King's B agar only. The plates were inoculated at 30 °C for 3-7 days. After the growth period, the colony forming unit (CFU) was recorded. The cultures were restreaked on respective media to attain the pure culture, which was further preserved in 25 % glycerol stock (80°C) and on slants (4°C).

Screening of bacteria

All the bacterial isolates were further screened for siderophore and hydrolytic enzymes (amylase and protease) production. The isolate positive for all was also checked for indole acetic acid production.

Amylase production

The production of amylase was evaluated on starch medium [5.0 g Peptone, 1.5 g Yeast extract, 2.0 g Starch, 1.5 g Meat extract, 5.0 g NaCl, 15 g Agar and 1 L ddH_2O] using the spotting method. The inoculated plates were incubated at 30 °C for the duration of 24 h. After the incubation, an iodine solution was poured onto the plate until the colony was submerged. It was then left for 1 min. A clear halo observed around the colony indicated isolate positive for amylase production (34).

Protease production

The production of protease was evaluated on skim milk agar medium [10 g Glucose, 5 g Yeast extract, 5 g Skim milk, 2 g KH_2PO_4, 10 g $Na_2CO_3$and 1 L ddH_2O] using spotting method. The inoculated plates were incubated at 30 °C for 24 h. The appearance of clear zones on skim milk agar plates was the first sign of proteolytic activity (35).

Siderophores production

The isolates were further assessed on Blue agar CAS medium for the production of siderophores. The medium was prepared by dissolving chrome azurol-S (60.5 mg) in distilled water (50 mL) and mixing it with 1 mM $FeCl_3$ solution (10 mL). The blue colour of the media was obtained after adding hexadecyl trimethyl-ammonium bromide (HDTMA) (72.9 mg) dissolved in 40 mL of water. CAS mixture was then combined with nutrient agar (300 mL) after separate sterilization. The microbial isolates cultured overnight were inoculated onto the prepared CAS plates and incubated at a temperature of 28°C for 2-10 days. The colour change of blue CAS media to deep yellowish or orange around the spotted isolates indicated positive siderophores production (36).

Indole acetic acid production

The isolate positive for both siderophore and hydrolytic enzyme production was tested for indole acetic acid production. The selected isolate was inoculated onto Luria Bertani (LB) agar supplemented with 50 µg mL^{-1} tryptophan and covered with Whatman No. 1 filter paper treated with Salkowski reagent (2% solution of 0.5 M $FeCl_3$ in 35% perchloric acid). The inoculated isolates were then incubated for 24 h at 30°C. The positive IAA production was indicated by the formation of pink colour (37). The quantitative estimation was conducted in two experimental sets, in first set 1 mL of overnight grown selected isolate was added to 50 mL of LB broth with 100 µg mL^{-1} tryptophan while in second Set II inoculated LB broth without tryptophan was used. Both sets were incubated at 30°C for a period of 14 days on shaker at speed of 180 rpm. The IAA detection was done using the centrifuged supernatant collected at 3000 rpm for 20 min, following methods by Gordon and Weber (38).

Molecular characterization

The DNeasy® Blood and Tissue Kit (Qiagen) was used to isolate the genomic DNA and the procedure was followed as per manufacturer's instructions. The quality of the DNA isolated was checked for purity using 0.8 % agarose gel. The amplification of 16S rRNA gene was carried out using the universal primers pA:5'-AGAGTTTGATCCTGGCTCAG-3'and pB:5'-TAACACATGCAAGTC GAACG-3' (39). The PCR products were checked in 1.2% agarose gels with 2-log DNA ladder as molecular standard. The gels were visualized in a UV transilluminator and the image was captured in a gel documentation system (Alpha-Imager). Selected bacterial isolate amplified 16S rRNA gene PCR product were purified using a QIA quick purification kit (Qiagen) and sequencing was performed by Molecular Forensics & DNA Technologies, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. The BLASTn result was used to determine the identity of the bacteria and phylogenetic analysis and the sequences were aligned using ClustalW alignment software (40). Using the programme MEGA 4.0.2, a phylogenetic tree was created on the matched datasets using the neighbour joining method (41). Using 100 samples from the different alignments a bootstrap analysis (42) was performed. NCBI GenBank was used to store the partial 16S rRNA sequence and accession number was assigned.

Development of Bio formulation

Bioformulation was developed by growing bacterial culture in nutrient broth at 28°C for 24h. After the bacterial culture growth, CFU of the culture was estimated as 2.2×10^7 CFU/mL. The diluted suspensions of isolate were sprayed using a hand sprayer with a capacity of 1.5 L for testing the bioefficacy against greenhouse whiteflies (Fig. 1).

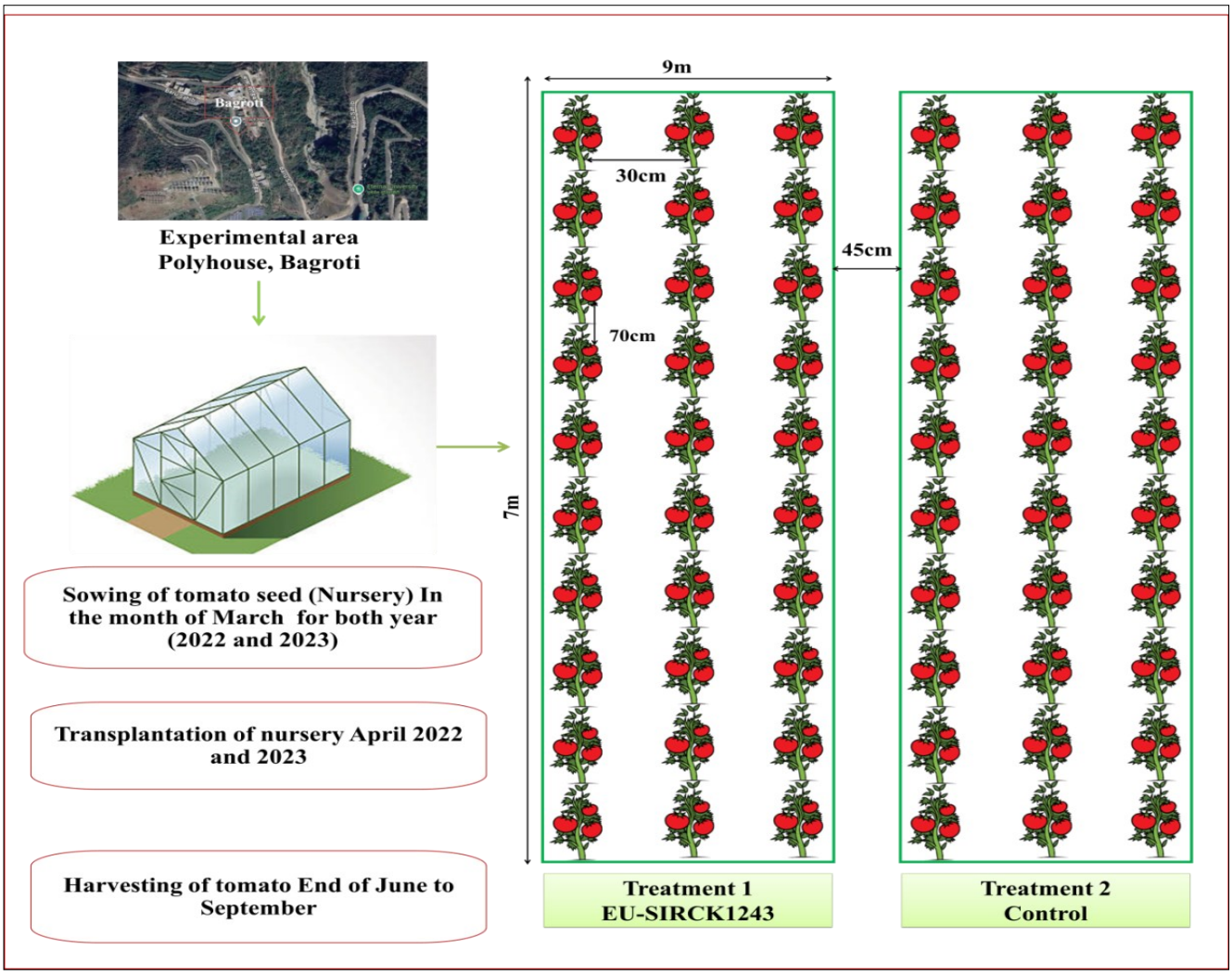

Fig. 1. Experimental design for tomato plantation under greenhouse conditions for the year 2022 and 2023.

Bio efficacy studies in the greenhouse

The experiment was conducted at the Experimental farm Bagrotti (30.7537° N, 77.2965° E), Eternal University, Baru Sahib, Himachal Pradesh, India. The tomato plant variety-Heem Sohna (hybrid) was purchased from the local market and used in the experiment. In March 2022-23, the nursery was sown in the greenhouse with a temperature of 25±2 °C and relative humidity 71±10% in loam soil. It was further transplanted in the month of April 2022-23 in randomized block design having three replications with each of two treatments. The experiment was carried out first in the year 2022 and then repeated again in the consecutive year 2023 (Fig. 1).

Three random plants were selected in each row for recording the pre-treatment data of nymphs and adults after 30 days from transplantation one day prior to spray. Post-treatment counts of nymphs and adults were recorded following three consecutive sprays, with each spray separated by an interval of fifteen days. The Leica S9i stereozoom microscope (Leica Microsystems) was used for the counting of nymph and adult populations on three leaves from each upper, middle and lower part of tomato plant. The counts of the adult population were examined in-situ. The post spray data was subjected to per cent reduction from untreated control and pre -treatment data was used as initial data. The per cent reduction in the population of whiteflies (nymphs and adults) over control was calculated, using Henderson and Tilton's formula (43).

$$\text{Corrected morality \%} = \left[\frac{\text{n in control before treatment - n in treated after treatment}}{\text{n in control after treatment - n in treated before treatment}}\right] \times 100$$

where n = whitefly population;

The growth parameters such as length, fresh/dry weight of shoot/root and physiological ones such as content of chlorophyll of the tomato plant were studied in accordance with the method given by (44). The yield was also calculated using the formula: Yield (kg/ha) = Average FW × 10000 (m^2)/ Area of each plot (m^2).

Statistical analysis

The data was subjected to analysis of variance (ANOVA), (45). The growth and physiological parameter data were analysed using the Student's t-test. The mean comparisons were conducted using the least significant difference (LSD) test (P 0.05) and critical difference (1%). Standard error and LSD results were calculated. The analysis was performed through IBM SPSS Software (version 16.0).

Results

Area of study, sample collection and isolation of bacteria

A total of 5 bacterial strains (*Pseudomonas* spp.) were isolated from collected 8 rhizospheric soil samples from 2 different districts of Himachal Pradesh. The abundance of rhizospheric bacteria observed in the media King's B ranged from 0.06×10^7 to 3.19×10^7 CFU g^{-1} soil. The maximum abundance was observed in the pear sample (3.19×10^7 CFU g^{-1} soil) and minimum in marigold and apple (0.06×10^7CFU g^{-1} soil). Out of the five isolates EU-SHMJA22321, associated with apple (SHMA) and EU-SHMTP322 and EU-SHMTP37, associated with pear (SHMP), were recovered from the Shimla district. Meanwhile, EU-SIRMM3116 and EU-SIRCK1243 (SIRM) were obtained from the Sirmour district of Himachal Pradesh.

Screening of bacteria

The isolated samples were further screened for siderophore and hydrolytic enzymes (amylase and protease). Out of 5 isolates, 2 were positive for siderophores, 3 for amylase and all 5 for proteases. Among all, 1 bacterial isolate was positive for both siderophore and amylase production, 3 isolates showed both amylase and protease activity and 2 isolates were confirmed for both siderophore and protease production. A potential strain EU-SIRCK1243 was found positive for all three traits-siderophore, siderophores, amylase and protease production. The selected isolate EU-SIRCK1243 was further analysed for the production of IAA. The isolate EU-SIRCK1243 produced IAA 2.03±0.30 µg/mL with tryptophan and 4.23±0.17µg/mL without tryptophan.

Molecular identification on the basis of 16S rRNA gene

The gene sequence analysis of 16S rRNA placed the strain EU-SIRCK1243 in genus *Pseudomonas*. BLAST search results through NCBI showed the highest similarity of more than 97% with *Pseudomonas aeruginosa* KF952246. The sequence was submitted to NCBI and the accession number granted was

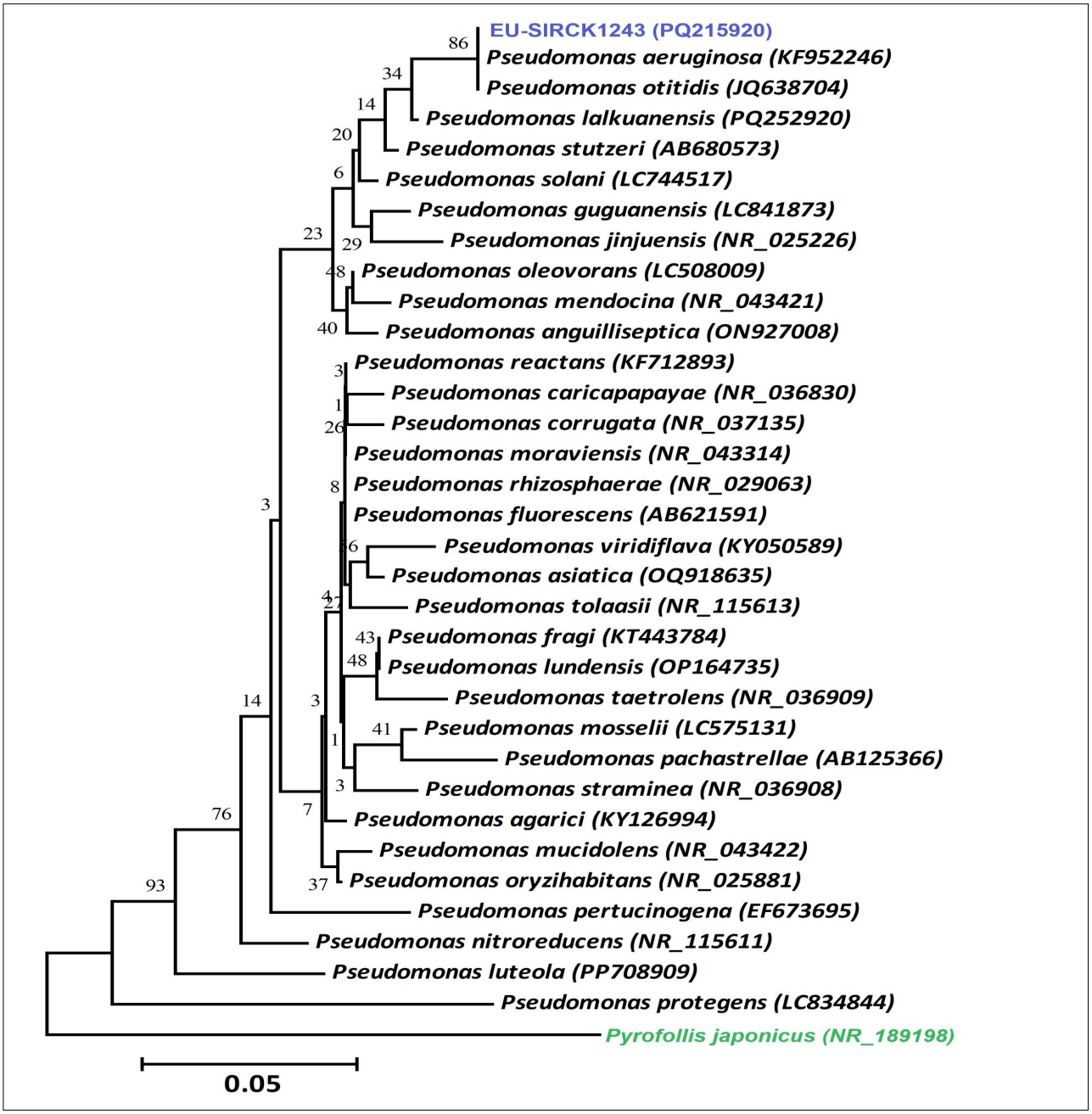

Fig. 2. Phylogenetic tree showing the relationship bacterial isolates, 16S rRNA gene sequences with reference sequences obtained through BLAST analysis. The tree was constructed using neighbor joining with algorithm using MEGA 4 software.

PQ215920 (Fig. 2).

Validation of developed bio formulation for its bio potential against Trialeurodes vaporariorum in the greenhouse

EU-SIRCK1243 was further tested for its biocontrol potential against *Trialeurodes vaporariorum*, greenhouse whiteflies on crop tomato for two year (2022-2023). The pre-treatment observations recorded during the year 2022 were 27.67 to 36.67 nymphs/plant and 22.00 to 24.00 adult/plant. The pre-treatment observations recorded during the year 2023 were 49.00 to 65.33nymphs/plant and 39.67 to 53 adult/plant (Table 1).

In the year 2022, the average adult population of GHWF recorded after 1st spray was 17.33 adults per leaf to that 32.67 of control. After first spray the per cent reduction was observed as 42.12%. The average adult population after second and third spray were recorded as 9.00 adults per leaf with 42.33 in control and 4.33 adults per leaf with 51.33 in control respectively. The per cent reduction was observed after 2nd and 3rd spray was 59.93% and 60.29% over control. The results were significant at level $p<0.05$. The nymph population recorded after 1st spray was 22.00 nymph per leaf with 50.00 nymph per leaf that of control. After second spray it was 10.00 nymph per leaf with 60.33 of control. The third spray showed a decrease 3.00 with 68.00 nymph population in control. The overall percentage reduction observed was 59.13.

Similarly in the year 2023 the average adult population of GHWF recorded after 1st spray was 25.67 adults per leaf to that 38.00 of control. After first spray the per cent reduction was observed as 49.45%. The average adult population after second and third spray were recorded as 13.33 adult per leaf that of 45.33 in control and 3.67 adult per leaf to 63.00 of control respectively. The percentage reduction was observed after 2nd and 3rd spray was 56.46% and 80.21% over control. In the year 2023, nymph population recorded after 1st spray was 32.67 nymph per leaf with 58.67 that of control. After second spray it was 15.33 nymphs per leaf with 69.00 of control. The third spray showed a decrease 5.33 nymph per leaf with 75.67 nymph population in control. The overall percentage reduction observed was 62.20. The treatment was significantly superior to the untreated control in adults and nymphs of *Trialeurodes vaporariorum*, after 1st, 2nd and 3rd sprays (Table 1).

Analysis of growth and physiological parameters (2 year pooled data)

On the analysis of growth parameters, it was observed that after spray of EU-SIRCK1243 in the year 2022 and 2023 the shoot length and root length increased by 1.02 and 0.12 fold respectively over the control. Also, an elevation in the dry and fresh biomass pertaining to 316.51g and 55.51g respectively over control was seen. In the case of physiological parameters, the chlorophyll content was improved up to 7.21 fold in comparison to control (Table 2). The yield obtained in 2022 was 1475.71± 0.44 kg/ha compared to 963.33± 0.34 kg/ha in the control and in 2023, it was 1680.48± 0.71 kg/ha compared to 1133.33± 0.39 kg/ha in the control.

Discussion

The bacteria belonging to the genus *Pseudomonas* are considered important plant growth promoting (PGP) and biological control agents for sustainable agricultural practices. They pertain to have numerous pivotal attributes contributing to PGP such as enhancing the nutrient availability, growth hormones production and also providing resilience to plants under the conditions of stress. The biopotential of species such as *P. chlororaphis, P. protegens, P. corrugata, P. aeruginosa* and *P. fluorescens* have been successfully exploited for different

Table 1. Bio efficacy of *Pseudomonas aeruginosa* EU-SIRCK1243 on adults and nymphs of whiteflies for year 2022 and 2023

2022					2023				
Treatment on adults	**NAS 1st**	**NAS 2nd**	**NAS 3rd**	**OR**	**Treatment on adults**	**NAS 1st**	**NAS 2nd**	**NAS 3rd**	**OR**
EU- SIRCK1243	17.33±0.94	9.00±0.82	4.33±1.25	54.11 ±10.39	EU- SIRCK1243	25.67±1.53	13.33±1.15	3.67±0.58	62.04±16.12
Control	32.67±2.49	42.33±2.05	51.33±0.94		Control	38.00±3.00	45.33±8.08	63.00±6.56	
F values	132.25	357.14	946.71		F values	195.57	59.08	238.23	
Treatment on nymphs	**NAS 1st**	**NAS 2nd**	**NAS 3rd**	**OR**	**Treatment on nymphs**	**NAS 1st**	**NAS 2nd**	**NAS 3rd**	**OR**
EU- SIRCK1243	22.00±1.41	10.00±1.63	3.00±0.82	59.13±16.09	EU- SIRCK1243	32.67±3.06	15.33±1.53	5.33±1.53	62.20±5.34
Control	50.00±1.63	60.33±2.05	68.00±3.27		Control	58.67±4.16	69.00±3.61	75.67±4.04	
F values	336.00	465.33	507.00		F values	169.00	925.75	1,590.04	

[Numerical values are Mean ± Standard deviation of mean of three independent observations]

*NAS-Number after spray; OR-Overall reduction in treatment over control

Significant difference $p<0.05$

Table 2. Effect of *Pseudomonas aeruginosa* EU-SIRCK1243 on different growth and physiological parameters of tomato under protected cultivation (Pooled data of two years 2022-2023)

Treatments	SL (m)	RL (m)	Shoot Biomass (g)		Root Biomass (g)		Chl a (mg g^{-1})	Chl b (mg g^{-1})	TCC (mg g^{-1})
			FW	DW	FW	DW			
EU-SIRCK1266	2.20±0.04	0.37± 0.03	387.67± 41.96	73.67± 3.77	102.17±7.78	14.50±1.18	9.21± 0.15	25.09±3.54	36.86±1.29
CONTROL	1.18±0.21	0.25± 0.01	121.00± 9.43	25.33± 1.41	52.33± 6.60	7.33±1.89	5.35± 0.10	21.73±0.15	29.65±0.28
LSD	3.22	0.28	1224.05	152.61	157.35	22.63	12.19	10.61	22.75
CD 1%	0.54	0.09	111.85	11.29	31.30	6.67	0.56	8.05	3.42
CD 5%	1.12	0.18	229.44	23.15	64.20	13.68	1.14	16.51	7.02

[Numerical values are Mean ± Standard deviation of mean of three independent observations]

(SL-Shoot length, RL-Root length, FW-Fresh weight, DW-Dry weight, Chl a-Chlorophyll a, Chl b-Chlorophyll b, TCC-Total chlorophyll content)

pest and pathogens. As biological control agents *Pseudomonas* suppress the pathogen with the help of antibiotics, secondary metabolites, competition exclusion and also by triggering the plants defence mechanism. This reduces the dependence on harmful chemical pesticides, boasts the overall crop health and increases the production. An experimental study was conducted to isolate potential indigenous *Pseudomonas* strain from the state of Himachal Pradesh and test its bicontrol activity against *Trialeurodes vaporariorum*, greenhouse whiteflies on crop tomato.

A total of 8 samples of rhizospheric soil were collected from Sirmour and Shimla district of Himachal Pradesh, India from which 05 bacterial strains were isolated using the selective King's B agar media. In a similar kind of study 206 phosphate solubilizing rhizobacteria were collected from root endospheric and rhizopheric soil samples of apple trees in 4 different districts of Himachal Pradesh and further screened for plant growth promotion traits (46).

The abundance of rhizospheric bacteria observed in the media King's B ranged from 0.06×10^7 to 3.19×10^7 CFU g^{-1} soil. The maximum abundance was observed in the pear sample (3.19×10^7 CFU g^{-1} soil) and minimum in marigold and apple (0.06×10^7CFU g^{-1} soil). The bacterial isolates were screened for siderophore, amylase and protease production. In another study conducted on the rhizospheric French bean soil samples collected from Solan and Shimla districts of Himachal Pradesh bacterial genus including *Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Rhizobium* and *Serratia* were reported for plant growth promotion. They were further screened for ammonia production, ACC deaminase activity, phosphate solubilization, IAA production, HCN production and hydrolytic enzyme (catalase) production (47).

Out of 5 isolates, 2 were positive for siderophores, 3 for amylase and all 5 for proteases. A study revealed that pyoverdine, a siderophore which was secreted by the bacterium *Pseudomonas aeruginosa*, helped in the acquisition of iron along with regulation of other virulence factors such as exotoxin A and endoprotease (48). In a similar study, it was observed that four selected Ps*eudomonas* strains *P. fragi* CC151, *P. fragi* CC275, *P. psychrophila* CC291 and *P. lactis* CC194, produced lipolytic and proteolytic enzymes (49). A potential strain EU-SIRCK1243 was found positive for all three traits-siderophore, IAA amylase and protease production. The selected isolate EU-SIRCK1243 was further analysed for the production of IAA. The isolate EU-SIRCK1243 produced IAA 2.03±0.30 µg/mL (with tryptophan) and 4.23±0.17 µg/mL without tryptophan. A similar study was conducted to analyse the PGP traits such as IAA, siderophore and phosphate solubilization of *P. fluorescens* and *P. putida*. The results showed that both *P. fluorescens* and *P. putida* produced IAA at concentrations of 89 µg/ml and 116 µg/ml, respectively (50).

The gene sequence analysis of 16S rRNA placed the strain EU-SIRCK1243 in genus *Pseudomonas* and showed highest similarity of 100.00% with *Pseudomonas aeruginosa* KF952246. Lakshmi et al (51) screened isolate KC1 from the rhizosphere of castor plants (*Ricinus communis*) in Bihar and on the basis of 16S rDNA sequencing (1450 bp) and alignment at GeneBank (NCBI, MaryLand) validated it as *Pseudomonas aeruginosa* (HM195190). They also further exploited its potential as a weed control agent (51).

EU-SIRCK1243 was further tested for its biocontrol potential against *Trialeurodes vaporariorum*, greenhouse whiteflies on crop tomato for two year (2022-2023). In the year 2022, the per cent reduction in adult population observed after 1st, 2nd and 3rd spray was 42.12%, 59.93% and 60.29% over control. The results were significant at level $p<0.05$. The overall percentage reduction in nymph population observed was 59.13%. Similarly in the year 2023 the per cent reduction in adult population was observed after 1st, 2nd and 3rd spray was 49.45%, 56.46% and 80.21 % over control. The overall percentage reduction in nymph population observed was 62.20%. The treatment was significantly superior to the untreated control in adults and nymphs of *Trialeurodes vaporariorum*, after 1st, 2nd and 3rd sprays. A study was conducted to test the formulation developed from the metabolites secreted by *Pseudomonas aeruginosa* against 3rd instar larvae of the olive fruit fly (*Bactrocera oleae*). The results show a high mortality with LC50 dosage 24.0784 µL/mL for larval forms. It was suggested that the bacterium possesses the potential of Our results natural biocides against olive fruit fly (52). In another study, *Pseudomonas* isolates were isolated from untreated tomato rhizospheric soil and used as a biological control agent for silverleaf whitefly (*Bemisia tabaci*). The study was conducted under laboratory and glasshouse conditions with water treatment as a control. The results indicated isolate Q036B showed high mortality against adult and larvae of *B. tabaci* in greenhouse. Also, all three *Pseudomonas* isolates (Q110B, Q036B and Q172B) showed significant biopotential, against *B. tabaci* under laboratory conditions (21).

On the analysis of growth parameters it was observed that after spray of EU-SIRCK1243 in the year 2022 and 2023 the shoot length and root length increased by 1.02 and 0.12 fold respectively over the control. Also, an elevation in the dry and fresh biomass pertaining to 316.51g and 55.51g respectively over control was seen. In case of physiological parameters, the chlorophyll content was improved up to 7.21 fold in comparison to control. A similar study using *Pseudomonas otitidis* isolated from Bilbis, El-Sharkyia governorate, Egypt was conducted on tomato plant. The results showed that the inoculation of isolated bacteria in tomato plants in green house led to suppression of the soil-borne pathogen, enhanced the NPK uptake and increased the plant growth (53).

Conclusion

The present investigation further contributes to the knowledge pool of *Pseudomonas aeruginosa's* biocontrol and plant growth promotion activities. Earlier this bacterium has been tested for many economically important agriculture pests and pathogens. This report particularly aids in the effective biological potential of *Pseudomonas aeruginosa* against *Trialeurodes vaporariorum* a prominent insect pest causing severe damage in open and closed plantation systems. This bacterium also enhances the growth and physiological parameters in the tomato crop. The molecular characterization and phylogenetic analysis confirms the bacterium as

Pseudomonas aeruginosa and this pertains that climatic conditions and environment of the Himalayan state Himachal Pradesh favours the survival of the bacterium in its soil. This study is a testament to the high potential, better adaptability and strengths of indigenous microflora. In future more studies should be incorporated for a better understanding of the host-insect interaction and combination of these indigenous microbes with commercial products as well as other biological control agents.

Acknowledgements

The authors are grateful to the Department of Science and Technology, Government of India for providing financial assistance to INSPIRE FELLOW Simranjeet Kaur (DST/INSPIRE Fellowship/[IF190193]). The authors also express their gratitude to the Hon'ble Vice Chancellor and Pro Vice Chancellor, Eternal University Baru Sahib, Himachal Pradesh, India for their continuous guidance and motivation.

Authors' contributions

SK conceived the study, carried out the formal analysis and investigation and drafted the original manuscript. NT conceived of the study and participated in its design and coordination. NY, NK, PP, SS and SS reviewed the manuscript. ANY reviewed and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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