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Biocontrol of citrus thrips using chitinase producing *Achromobacter xylosoxidans* bacteria

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ABSTRACT

We studied 20-bacterial isolates for their ability to produce chitinase, from these 8 were selected and 3 (B1, B5, and D2) had the highest levels of chitinase activity. Based on the activity of chitinase [which generates N-acetylglucosamine units (U)], isolate B1 had the highest enzyme activity (3.905), followed by isolates B5 (3.52) and D2 (1.35). These 3-test bacterial isolates (B1, B5 and D2) with maximum chitinase activity were evaluated for their potential as biocontrol agent against thrips of citrus (kinnow: *Citrus reticulata* Blanco) under field conditions. *C. reticulata* trees were sprayed with these 3-bacterial isolates (10^6 cfu/ml), dimethoate phosphamide and nimbicide (at 1.5 ml/L, 2 ml/L doses respectively). The B1 isolate had the maximum biocontrol efficiency of thrips (79.66 %), against the control, which caused reduction of 37.70 %. In B1 isolate, infestation was 20 % than control (36 %). In B1 the mean population density was 0.4 compared to control (0.96). The identification of isolate B1 by 16s rDNA gene sequence confirmed that it was *Achromobacter xylosoxidans*.

Keywords: Biocontrol, chitinase, *Citrus reticulata*, citrus kinnow, pesticides, population density, *Thrips* sp., *Thrips* infestation.

INTRODUCTION

Citrus fruits are India's third-largest fruits after mango and banana. Sweet orange kinnow (*Citrus reticulata* Blanco) is the second largest citrus fruit being cultivated (2,35). Citrus fruits contains carbohydrates, fiber, and citric acid, as well as vitamin C, citric acid, calcium, copper, folate, potassium, magnesium, niacin, and vitamin B6 (9). However in India, thrips (Thysanoptera) are its major insect-pest, which seriously harm leaves, blossoms and fruits, lowers the fruit's quality (6). These are controlled using insecticides, whose indiscriminate use has resulted in several health risks, polluted groundwater (32) and contaminated environment (17). Consumers anticipate high-quality residue-free fruits, safe to handle and suitable for fresh consumption.

Microorganisms are currently used as biological controls in managing diseases (11). Microbially derived enzymes are frequently used as biocontrol agents. When chitinase enzymes hydrolyze the chitin and deacylated the macromolecule chitosan, the monomer N-acetylglucosamine and disaccharide chitobiose are generated. Therefore, chitinases can dissolve chitin in the phytopathogenic fungus cell wall, exoskeleton of insects, nematodes and crab shells (3,13). Chitinase, an extracellular enzyme, binds chitin molecules to hydrolyze them into N-acetylglucosamine monomers (10). Under aerobic and anaerobic conditions, chitinolytic microbes may break down chitin. Insect's cuticle is made up of 25 to 50 % chitin, one of the major structural elements (5,19). This enzyme seriously harm the peritrophic membrane structure, when administered topically to an insect or when consumed

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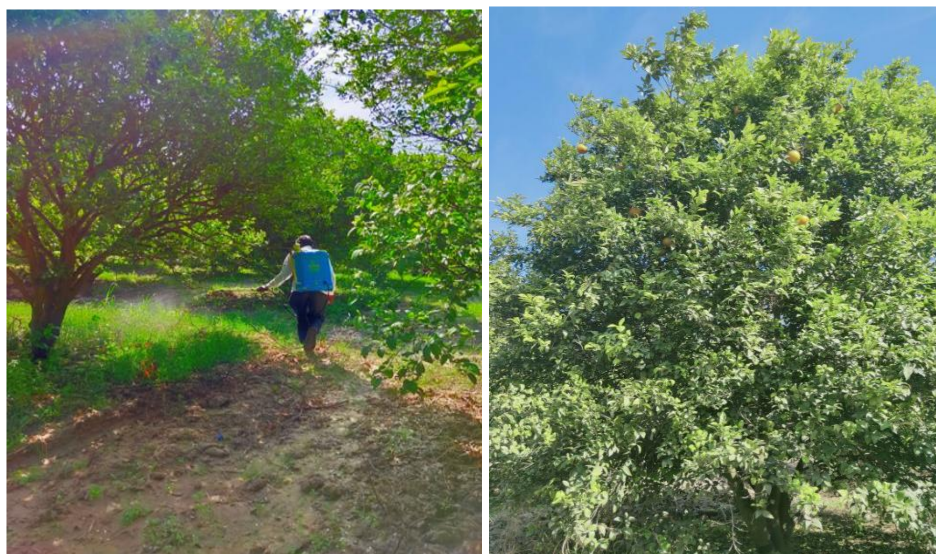

Figure 1. View of Citrus kinnow Orchard plantation

by insect larvae, which inhibits the larvae from feeding and ultimately leads to death. If applied subcutaneously, it disrupts the cuticle, which leads to abnormal molting (8). Several bacterial species (*Aeromonas, Serratia, Vibrio, Streptomyces* and *Bacillus*) contain chitinases (7). Industries use these chemical characteristics to produce biopesticides, which benefit both human society and the environment. Therefore, due to their low environmental impact and eco-friendliness, chitinases or chitinolytic microorganisms might be an appealing substitute for synthetic chemicals. Numerous bacterial genera producing chitinase have been identified to control thrips. We, therefore, isolated and analyzed the chitinase-producing bacteria. Chitinase production using bacteria is now possible under ideal conditions. These bacteria decreased the thrips' growth both *in-vitro* and *in-vivo* condidtions. In citrus trees, this application has been tested in the field (Figure 1). This study aimed to identify and classify the bacteria that produce chitinase from various microbiomes and assess bacterial isolates' biocontrol potential against thrips infestation in citrus kinnow plantations.

MATERIALS AND METHODS

The study was done in the Department of Microbiology, College of Basic Sciences and Humanities, and field study in orchard farm, Department of Horticulture, CCS Haryana Agricultural University, Hisar (longitude 75º46 E, latitude 29º 10 N and altitude of 215.2 m above mean sea level, summers temp : 45 ºC winters : 1 to 2 ºC , mean annual rainfall : 460 mm.

Colloidal chitin preparation

According to Murthy and Bleakley (24), shrimp shells were finely ground into powdered form and 20 g of this chitin powder was measured and taken in a 1000 ml beaker. After adding 150 ml of 12M HCl gradually along the sides, the mixture was agitated every 5-min for 1.0 h. After filtering the chitin-hydrochloric acid mixture using stacked layers of

muslin fabric, the filtrate was treated with 2.0 L distilled water (ice-cold) to precipitate the colloidal chitin. After overnight incubation, filtrate was passed through Whatman filter paper. Regularly running passed 3.0 L tap water through the colloidal chitin until the pH was raised to 7.0. After autoclaving, the resulting colloidal chitin was kept at 4 °C until usage.

Isolation and primary screening of bacteria for chitinase production

The soil samples were collected at depth of 8 cm to 10 cm from 3-sites : (i). Rhizosphere of congress grass, (ii). Citrus trees and (iii). Termite mound using a sterile spatula from our University Farm. Serial dilutions of soil samples were used to identify morphologically distinct bacteria, which were then plated on King's B medium and then screened on solidified 0.5 % colloidal chitin agar medium. After 48 h of incubation at 35 ºC, the isolates capable of degrading chitin surrounded by a halo zone were selected and subcultured in nutrient agar slants.

Secondary screening of bacteria on chitin agar media containing calcofluor dye

Sterilized Petri plates were filled with chitin agar media and calcofluor white M2R dye (0.001 % w/v) as an indicator (30). The isolates were spot-injected into the centre of the Petri plates after the media had solidified, and they were then incubated at 28 °C for 72 h, during which time they were checked for the development of black halos against fluorescent backgrounds using a UV transilluminator and clear zone of chitin dissolution after observing in visible light source.

Determination of chitinase assay

In a 250 ml Erlenmeyer flask, the minimal salt medium was made, supplemented with 0.5 % colloidal chitin, and autoclaved to sterilize it. An entire loop of bacterial culture was taken and injected into the medium, which was shaken and incubated at 28 °C for 72 h. The bacterial culture was then centrifuged at 10,000 rpm for 10 min after incubation. The chitinase assay was conducted using the supernatant, which was collected afterwards.

To measure chitinase activity, 3.0 ml of 50 mM citrate buffer (pH 5.05) and 1.0 ml of colloidal chitin (10 mg/ml) were combined with 1.0 ml of cell-free culture supernatant. One ml was pipetted into a screw cap tube holding 1.0 ml of distilled water after 0 and 60 min of incubation at 37 ºC. The tube was then immersed for 15 min in boiling water bath to halt the reaction. To eliminate any remaining chitin, the contents of the tubes were transferred to centrifuge tubes after being cooled with tap water. 0.1 ml of potassium tetraborate was added to the test tube. The tubes were then heated for 3.0 min in water bath of boiling water and then cooled in running water. Following the addition of 3.0 ml of DMAB reagent, the tubes were immediately immersed in a water bath heated to 36-38°C. The tubes were quickly read at 544 or 585 nm after precisely 20 min of cooling in tap water (21). A standard curve for n-acetylglucosamine was used to calculate how much of the compound was contained in 1.0 ml of the supernatant (31). Chitinase activity is expressed as the amount of N-acetyl-D-glucosamine (GlcNAc) equivalents produced by the enzyme each minute per mole of reducing sugar. The standard concentration range used for GlcNAc was 100-1000 g.

Estimation of indole-3-acetic acid (IAA) production

Bacteria were grown in Luria Bertani to produce auxins. Then, a sample of the culture's supernatant was mixed with a test tube containing Salkowski's reagent. After 30 min, the mixture was left in the dark to develop a pink colour. At 535 nm, the colour's intensity was then measured. Using the standard indole-3-acetic acid at different concentrations a calibration curve was plotted and the concentration of indole-3-acetic acid was estimated.

Morphological, physiological, biochemical and molecular identification of isolate

For morphological, physiological and biochemical identification, thorough analysis was conducted. Biologia Research India Pvt. Ltd., New Delhi, used the forward primer sequence 27F (U5F: AGAGTTTGATCMTGGCTCAG) and the backward primer sequence 1492 R (U4R: TACGGYTACCTTGTTACGACTT) to analyze the 16S rDNA gene sequence. Single Polymerase Chain Reaction (PCR) tube contained 100 ng of DNA in a 20µl reaction mixture containing 10X Buffer, each deoxy-nucleoside triphosphate at 0.1 mM, each primer at 0.5 µM, and 2 U of Taq polymerase (R001C TaKaRa Taq™). PCRs were subjected to initial denaturation for 3 mins at 95°C, 32 cycles (denaturation, 30 s at 95°C; annealing, 30 s at 55°C; extension, 1 min at 72°C) and 1 final extension cycle at 72°C for 10 min. Twenty microliters of the reaction mixtures were analyzed on 1.5% agarose/Tris-Cl-sodium acetate-EDTA in the presence of 0.5 µg of ethidium bromide per ml and photographed under UV illumination. The test amplicon of ~1300 bp was purified by Gel elution. The Purified product sequenced by Sanger's method of DNA sequencing. Using the Basic Local Alignment Search Tool (BLAST) search engine, the sequences were evaluated and compared to the nucleotide sequences kept in the NCBI (National Center for Biotechnology Information) database. Using the neighbouring ClustalW method, the phylogenetic tree was created from nucleotide sequences and implemented in MEGA XI.

Field study

The field studies on activity and control of citrus thrips using bacterial isolates were done from February 2, 2019 to March 15, 2020 in Citrus Orchard of our University. There were 6-experimental treatments: T1 (Control), T2 (Dimethoate Phosphamide, 1.5 ml/L), T3 (Nimbicide, 2 ml/L), T4 (Isolate B1, 10^6 cfu/mL), T5 (Isolate B5, 10^6 cfu/mL), T6 (Isolate D2, 10^6 cfu/mL). These were replicated five times in randomised block design. Insecticides and isolates were sprayed on 5- citrus kinnow plant flowers and fruits with a hand sprayer through a hollow cone nozzle at 3-stages : (i). pre-flowering (last week of February), (ii). flowering (second week of March) and (iii). pea stage of fruit (second week of April).

Observations on activity of thrips were recorded at 5 days interval from the last week of February (before flowering) onwards from 5 untreated citrus kinnow trees. Following the emergence of thrips, 25 flower buds and/or blooms were clipped from each tree using scissors, and kept in 250 ml 75 % alcohol in beaker. After removing flower buds and/or flowers from the beaker, alcohol solution was filtered gently and the population on filter paper was counted by magnifying lens. Thrips population on fruits was also recorded in the similar manner. The mean population at each plot was used to express the collected data. The population data was converted into a population decline percentage.

The collected information was statistically gathered in two ways: (i). Change in population density (%), (ii). efficacy (%) and fruits infestation (%) in comparison to untreated (control) plots were calculated as under :

(i). Change in population density

Change in Population Density (CPD%) = [(Xi - X_0)/ X_0] × 100

Where, X_0 : Mean number of live insects before treatment and Xi : Mean number of live insects after the treatment. Positive values in the table indicated increase in population density.

(ii). The efficacy (%) was calculated by Henderson-Tilton's formula (17):

$$\text{Population reduction (\%)} = 100 \times 1 - \left(\frac{T_a \times C_b}{T_b \times C_a} \right)$$

Where, T_a : Number of insects after treatment, T_b : Number of insects before treatment, C_a : Number of insects in control plots after treatment, C_b : Number of insects in control plots before treatment.

(iii). Infested fruits (%)

$$\text{Percentage of infested fruit} = \frac{Ni}{Nt} \times 100$$

Ni : Number of fruits infested

Nt : Total number of fruits sampled

Statistical Analysis

Using the OPSTAT and MS Excel, the acquired data were subjected to statistical analysis by one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used to compare the mean differences across treatments at a 5% level of significance.

RESULTS AND DISCUSSION

Isolation and screening of bacteria for chitinase production

Total 80-morphologically distinct bacterial isolates were retrieved from soil samples of different microbiomes on King's B medium and these were screened for chitin degradation. Based on the size of zone formed, 20-isolates showed a zone of clearance on the colloidal chitin agar plates as well as on the chitin agar medium, with calcofluor white M2R dye (0.001% w/v), when viewed under UV transilluminator and were selected for further studies (Figure 2).

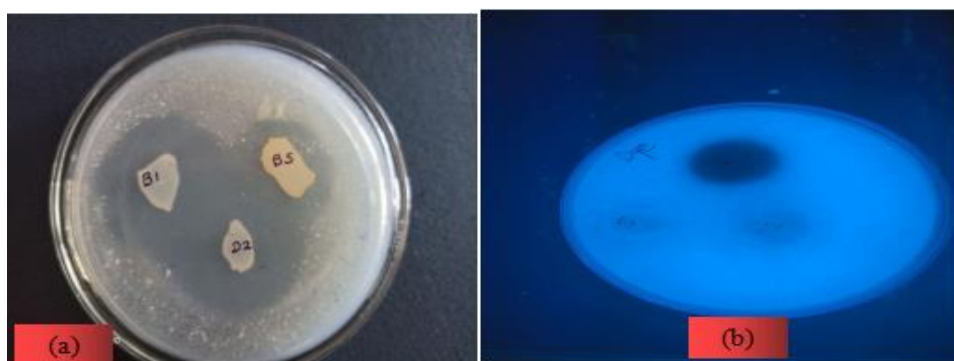

Figure 2. (a) Zone of hydrolysis of colloidal chitin by bacterial isolates, (b) Calcofluor white dye containing colloidal chitin agar plate under UV light

The earlier simple and direct method used to identify bacteria for chitinase activity required observing the clear zone surrounding the bacterial colony, developed on an agar medium supplemented with colloidal chitin as a carbon source (1). The isolate B1 had the largest zone size (38.50 mm), while, isolate A3 had the smallest zone of 12.40 mm. These isolates were examined for specific chitinase activity, enzyme activity index (EAI) (Table 1).

Table 1. Screening of bacterial isolates based on zone formation and enzyme activity

Isolates	**Zone size (mm)**	**Enzyme activity index****	**Specific activity (unit)***
A1	20.00	2.09	0.90
A2	13.60	1.92	0.76
A3	12.40	1.34	0.59
A4	24.70	2.32	1.05
A5	14.00	1.94	0.826
B1	**38.50**	**9.34**	**3.91**
B2	24.80	5.41	1.65
B3	18.30	3.6	1.17
B4	28.30	7.20	2.05
B5	**35.20**	**9.22**	**3.52**
B6	16.30	2.12	0.85
B7	25.40	5.54	1.70
B8	19.30	3.63	1.35
B9	26.00	2.91	1.16
B10	17.70	3.51	1.25
B11	21.60	4.73	1.83
D1	13.40	2.61	1.10
D2	**33.70**	**8.52**	**2.71**
D3	25.00	7.54	2.38
D4	16.30	2.19	0.91
C.D(0.05)			0.08

** **Enzyme activity index (EAI)** = Halozone diameter + Colony diameter/ Colony diameter
*one unit= one µmol of N-acetyl-D-glucosamine (GlcNAc) produced/ mg protein

Among 20 isolates, maximum chitinase enzyme activity was found in isolate B1 (3.91), which was greater than other isolates. Similarly, Shanmugaiah and his coworkers (34) isolated 39 chitinolytic bacteria from 77 rhizosphere soil samples collected from different crop fields in Tamil Nadu state, India. Among them, the strain MML2270 was selected with highest chitinolytic activity (59.05 units/ml) in primary and secondary screening in colloidal chitin agar and later identified as *Bacillus laterosporus*. A total of 20 chitinolytic bacterial isolates showed an EAI of 1.34 to 9.34. The maximum enzyme activity index was recorded for B1 (9.34) and the minimum for isolate A3 (1.34). The zone around the bacteria varied in size, indicating the bacteria's ability to utilize chitin as the sole energy source. Similar findings were reported by Mubarik *et al.* (22), they did comparable screening experiment on bacteria, isolated from the rhizosphere soil of chili pepper, to study their chitinase assay by hydrolyzing the chitin of whitefly insect (major pest of crops, like chili pepper). Twenty five rhizobacterial isolates showed zone of hydrolysis on colloidal chitin agar media. The enzyme chitinolytic index was highest in two isolates B1 (9.34 mm) and B5 (9.22 mm) and specific activity was 3.91 U for B1 and 3.52 U for B5 isolate.

Morphological, biochemical, physiological and molecular analysis

Different morphological characters of all the rhizobacterial isolates were evaluated (Table 2). Size of colonies and their colours differed, Rani *et al.* (27) also reported differences in colony morphology, staining, colony shape and pigmentation among 65 isolates from rhizosphere of pigeon pea. Based on the preliminary screening of selected chitinolytic bacteria, three most promising and effective chitinolytic bacterial isolates B1, B5 and D2, were characterized for biochemical properties. Bacterial isolate B5 was found positive for various biochemical tests (Table 3). [citrate utilization test, hydrogen cyanide production, urease activity, Voges Proskauer (VP) test and negative for methyl red test].

Table 2. Microscopic and biochemical examination of potent bacterial isolates

Microscopic examination of selected bacterial isolates						
Isolates	**Colony morphology**	**Gram's Reaction**	**Shape**	**Arrangement**	**Capsule Staining**	**Spore staining**
A1	Cream, flat, entire,	+ve	Cocci	Chains	-ve	-ve
A2	White, smooth, shiny	+ve	Cocci	Chains	-ve	-ve
A3	Yellow, flat, gummy	-ve	Rods	Single	-ve	-ve
A4	Cream, irregular, wrinkled	+ve	Rods	Single	+ve	-ve
A5	Red, circular, flat	-ve	Cocci	Single	-ve	-ve
B1	White, circular, flat, entire	-ve	Rods	Single	-ve	-ve
B2	Yellow, circular, flat, glistening	+ve	Cocci	Chains	-ve	-ve
B3	Cream, circular, convex	+ve	Cocci	Diplococcus	-ve	-ve
B4	White, irregular, flat	-ve	Rods	Chains	+ve	-ve
B5	Cream, circular, mucoid	-ve	Rods	Single	+ve	-ve
B6	Yellowish, irregular, flat	+ve	Cocci	Cluster	-ve	-ve
B7	Red, circular, flat	-ve	Cocci	Single	-ve	-ve
B8	Small creamish, irregular, flat	-ve	Cocci	Chains	-ve	-ve
B9	Cream, irregular, convex	+ve	Cocci	Chains	+ve	-ve
B10	Small creamish, irregular, flat	+ve	Rods	Single	-ve	-ve
B11	Cream, irregular, mucoid	-ve	Rods	Single	-ve	-ve
D1	Cream, flat, entre	-ve	Rods	Single	-ve	-ve
D2	Brown, circular, convex, smooth	+ve	Cocci	Single	-ve	-ve
D3	White, circular, flat	+ve	Rods	Chains	-ve	-ve
D4	Yellow, flat, gummy	-ve	Cocci	Chains	-ve	-ve

+ Positive for test, - Negative for test

With alignment and cladistics analysis of a homologous sequence of known bacteria, the phylogenetic evaluation of best isolate B1 was completed. 16S rDNA sequences were uploaded to GenBank (accession number OQ186718) in NCBI and BLAST was used to determine their identity. The bacterium was identified as *Achromobacter xylosoxidans* strain NSJB1 as per the phylogenetic tree of the strain that was submitted to the NCBI (Figure 3).

Table 3. Biochemical characterization of three selected bacterial isolates

Bacterial Isolate	Citrate utilization	Indole test	Methyl red test	Urease test	Voges Proskauer test	Motality
B1	-ve	+ve	+ve	-ve	-ve	+ve
B5	+ve	+ve	-ve	+ve	+ve	-ve
D2	+ve	+ve	-ve	-ve	-ve	+ve

+ Positive for test, - Negative for test

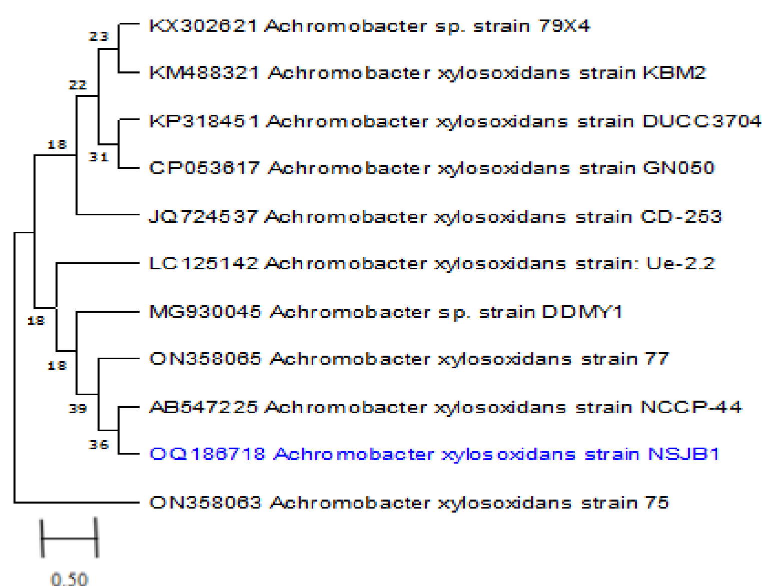

Figure 3. The phylogenetic tree of *Achromobacter xylosoxidans* strain NSJB1, which was produced using a neighbour joining tree, demonstrates how evolutionary distances connect chitinase-producing NSJB1 bacteria to known sequences of related families (MEGA XI programme)

Estimation of Indole Acetic Acid production and Hydrogen Cyanide production

After 72 h of incubation, the IAA synthesis of selected chitinase-producing bacterial isolates was evaluated in tryptophan-added Luria Bertani broth. The B1 bacterial isolate had the highest amount of IAA (4.37 g/ml) and B5 had the lowest IAA (2.23 µg/ml). In earlier studies, different bacterial strains generated different amounts of IAA (15,26). Due to higher IAA production, some antagonistic rhizobacteria showed inhibitory effects. The IAA production varies with growth conditions, species and strains (23,25,36). Thirty bacterial isolates were examined for various biochemical tests viz., Citrate, Urease, Indole test, Methyl red and Voges Proskauer test. Selected bacterial isolates were tested for HCN production on King's B medium (Table 3). HCN is a common secondary metabolite produced by rhizospheric *Pseudomonas* and *Bacillus* (28,33) but its role for biocontrol activity is not clear (4).

Thrips population

The average number of thrip fruit in different treatments ranged from 1.24-0.12 than 1.44 thrips in untreated control (T1). Among the insecticidal treatments (T2) dimethoate phosphamide was most effective against thrips population. In treatments with isolate B1 (T4) thrips population ranged from 1.2 - 0.4 thrips/fruit, besides it had also highest chitinolytic index than other bacterial isolate (Table 4).

Table 4. Mean population density of thrips on citrus fruit before and after spray

Treatment	Before Spray	3 DAS	7 DAS	14 DAS
T1	1.44	1.48	1.32	0.96
T2	1.24	0.24	0.16	0.12
T3	1.32	0.36	0.32	0.28
T4	1.2	0.8	0.52	0.4
T5	1.12	0.96	0.76	0.44
T6	1.4	1.32	1.2	0.8
C.D.	N/S	0.29	0.29	0.21

DAS = Days After Spraying

The overall trends in reduction of mean population density of thrips (Figure 4) in different treatments decreased at different time intervals *i.e.,*7, 10 and 14 DAS (Days After Spraying). We also examined the capability of bacterial isolates to degrade thrips exoskeleton. Application of bacterial isolates and chemical pesticides decreased the number of thrips. Chitinolytic action of bacterial isolates treatments hydrolyzed to chitin of thrips exoskeleton. These results agreed with study (18) of greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouche) population was higher in untreated control than any treatment *S. citri* population in untreated orchards was higher than in treated orchards (16). Similar results were found in entomopathogen treatment, with a mean population of 8.3 to 10.0 thrips/plant than 18.4 in control (12).

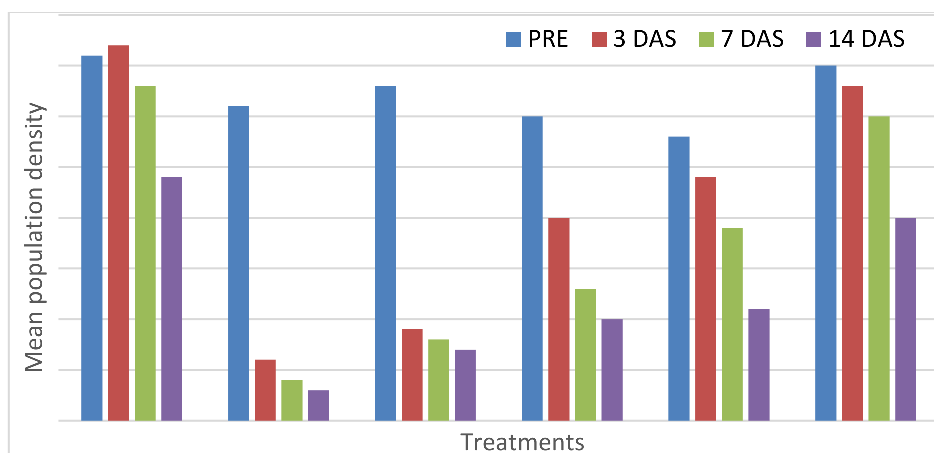

Figure 4. Mean population density of thrips in citrus kinnow field. (T1 Control; T2 Dimethoate Phosphamide 1.5 ml/L;), T3 Nimbicide, 2 ml/L; T4 Isolate B1, 10^6 cfu/mL; T5 Isolate B5, 10^6 cfu/mL; T6 Isolate D2, 10^6 cfu/mL)

Infestation in citrus kinnow fruit

In dimethoate phosphamide spray (T2), before treatment infestation was 28 % and it was continuously reduced. After 7 and 14 days, the infestation was only 12 % and 8 %, respectively. In nimbicide spray (T3) before treatment infestation was 32 % and at 3,7,14 days after spray the infestation was only 16 %. In B1 isolate spray (T4) before treatment infestation was 36 % and after 3-sprays was reduced to 32,24,20 % respectively (Figure 5 and 6).

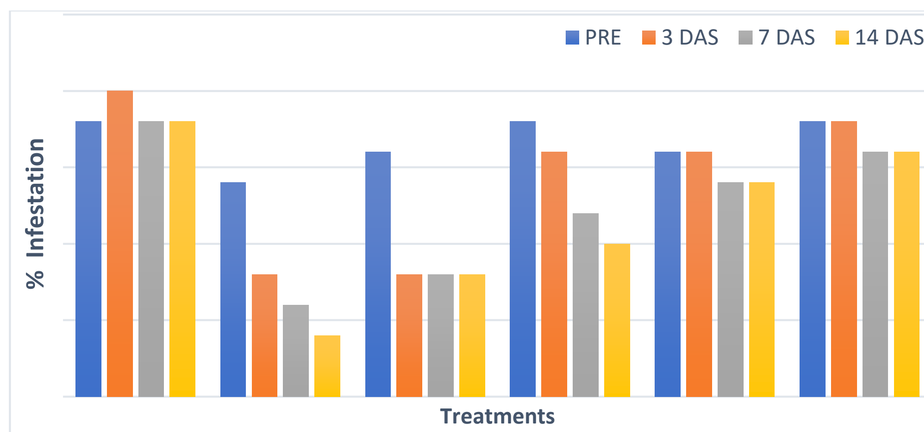

Figure 5. Infestation in citrus kinnow fruit. (T1 Control; T2 Dimethoate Phosphamide 1.5 ml/L;), T3 Nimbicide, 2 ml/L; T4 Isolate B1, 10^6 cfu/mL; T5 Isolate B5, 10^6 cfu/mL; T6 Isolate D2, 10^6 cfu/mL)

Figure 6. Visual observations of thrips infestation on citrus kinnow fruits

Therefore, the chitin hydrolyzing enzyme of bacterial origin, which acts as an antagonistic against insect pests, was responsible for the decrease in the thrips infestation in citrus kinnow fruit. These chitinases play a crucial role in controlling the defence and attack mechanisms of numerous fungi and insects. Rao *et al.* (29) observed that the infestation of Nagpur mandarin fruits by citrus thrips, *S. citri* was significantly less at 2.5 % dose (6.2-

32.6 % infested fruits) than 1.0, 0.5 and 0.1 % dose (11.2-65.6 % infested fruits) of mark all season HMO (Horticulture Mineral Oil), which corroborate the present study in terms of reduction of thrips population in citrus kinnow fruits.

Efficacy of chemical pesticides and bacterial isolates

The effectiveness of dimethoate phosphamide (T2) treatment after 3,7,14 days was 81.17 %, 85.92 %, and 85.48 %, respectively (Figure 7).

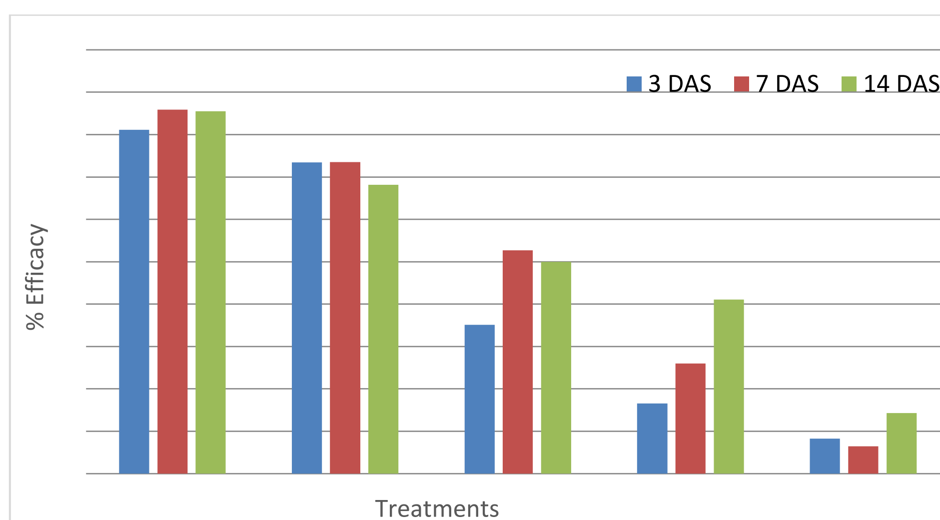

Figure 7. Efficacy of chemical pesticides and bacterial isolates. (T1 Control; T2 Dimethoate Phosphamide 1.5 ml/L;), T3 Nimbicide, 2 ml/L; T4 Isolate B1, 10^6 cfu/mL; T5 Isolate B5, 10^6 cfu/mL; T6 Isolate D2, 10^6 cfu/mL)

The effectiveness of (T3) nimbicide spray treatment after 3,7,14 days was 73.46 %, 73.55 %, and 68.18 %, respectively. B1 isolate treatment (T4) at 3,7,14 days sprays showed efficacy of 35.14 %, 52.73 %, and 50.00 %, respectively. The effectiveness of (T5) B5 isolate treatment after 3,7,14 days was 16.60 %, 25.97 % and 41.07 %, respectively. D2 isolate treatment (T6) after 14 days, showed the lowest efficacy of 14.29 %. The isolate B1 treatments effectively reduced the damage caused by thrip similar to chemical pesticides. To combat thrips infestation in citrus kinnow plantations, chitinase-producing isolates were used as a biocontrol alternative. The severity of damage done by *T. tabaci* in onion was greatly reduced by the application of dimethoate and *M. anisopliae* by 25 % and 50 %, respectively (20).

Reduction in population density of thrips

Dimethoate phosphamide (T1) spray at 3, 7, 14 days decreased thrips population by 80.65 %, 87.10 % and 91.65 %, respectively. Nimbicide spray (T3) spraying at 3, 7, 14 days decreased thrips population by 72.73 %, 75.76 % and 80.56 %, respectively. In contrast, these values with B1 spray (T4) at 3, 7, 14 days decreased thrips population by 33.33 %,

Table 5. Effects of treatments on the change in thrips population density on citrus kinnow fruits

Treatment	**CPD** (Change in population density **(%)**		
	3 DAS	**7 DAS**	**14 DAS**
T1 (Control)	2.78	-8.33	-33.33
T2 (Dimethoate phosphamide, 1.5 ml/L)	**-80.65**	**-87.10**	**-91.67**
T3 (Nimbicide, 2 ml/L)	-72.73	-75.76	-80.56
T4 (Isolate B1, 10^6 cfu/mL)	**-33.33**	**-56.67**	**-72.22**
T5 (Isolate B5, 10^6 cfu/mL)	-14.29	-32.14	-69.44
T6 (Isolate D1, 10^6 cfu/mL)	-5.71	-14.29	-44.44
C.D. (5%)	1.996	0.95	2.398

DAS = Days After Spraying

56.67 % and 72.22 %, respectively. D2 (T6) spray at 3, 7, 14 days caused minimum decrease of 5.71 %, 14.29 % and 44.44 %, respectively (Table 5). Thus isolate B1 i.e *Achromobacter xylosoxidans* strain NSJB1, has the potential for use as biocontrol agent for thrips. The lowest thrips/plant population on onion was recorded by *M. anisopliae* at 1×10^9 concentration spores/ml with adjuvant (sunflower and Triton-X), reducing 58 % population and a 49.12 % improvement in yield than control (12).

CONCLUSIONS

This investigation of bacterial isolate as a potential biocontrol agent for the thrips in citrus kinnow plantation showed that under the field conditions the B1 bacterial isolate had the maximum biocontrol efficacy of 52.73 % on thrips. B1 bacterial isolate was identified as *Achromobacter xylosoxidans* strain NSJB1by 16s rDNA gene sequencing provides an alternative biocontrol approach as compared to chemical insecticides.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

DECLARATION

We declare that all authors of this Ms have made substantial contributions. We have not excluded any author that substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

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