Full Length Research Article

CARAS

Research Journal of Agricultural Sciences
Volume 16; Issue 03 (May–Jun 2025); pp 286–293

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Contribution of Cowpea Rhizosphere Microflora in Plant's Resilience in Imidacloprid Stress

Radhika S. Velankar*[1] and R. S. Zunjarrao[2]

[1] Department of Biotechnology, Modern College of Arts, Science and Commerce (Autonomous), Shivajinagar, Pune - 411 005, Maharashtra, India
[2] Department of Botany, Modern College of Arts, Science and Commerce (Autonomous), Shivajinagar, Pune - 411 005, Maharashtra, India

Received: 30 Mar 2025; Revised accepted: 07 May 2025

Abstract

Accumulation of applied imidacloprid in agricultural fields is responsible for reducing the quality of crops. The rhizosphere microflora of plants can contribute in improving the performance of the plants in stressful conditions and make them strong to survive in high imidacloprid load. The aim of the present work was to isolate microorganisms from cowpea (*Vigna unguiculata* L. Walp) rhizosphere containing high doses of imidacloprid and study its role in providing resilience to cowpea in imidacloprid induced stress. From seventeen isolates, one organism was identified as *Pseudomonas aeruginosa.* This organism was inoculated in soil spiked with imidacloprid and used for cowpea cultivation. The effect of these soil treatments on the growth and physiological parameters of cowpea was assessed. Results revealed that the isolated organism was successful in providing conducive growth conditions for Cowpea in imidacloprid induced stress by eliciting enhanced levels of its vegetative and physiological parameters like germination percentage, plant height, root length, fresh weight, leaf area, total chlorophyll, and proline content. The results of the work suggests that *Pseudomonas aeruginosa* can be used as a biofertilizer to improve the production of agronomically important crops like cowpea in stress conditions by making it resilient to imidacloprid presence in soil.

***Key words*:** Biofertilizer, Cowpea, Imidacloprid induced stress, *Pseudomonas aeruginosa,* Vegetative growth

Soil pollution is a major global problem in modern agriculture which can pose a threat to the ecosystem and eventually to humans [1]. The use of pesticides like imidacloprid in increasing amounts has raised a concern as these chemicals accumulate in the environment and cause pollution [2]. Imidacloprid is usually applied to the seeds before sowing them or can be added in the soil near the roots of the affected plant. This pesticide tends to remain in the soil for a long time and can cause negative impacts to the soil flora and fauna and to other nearby resources like water bodies, animals, and other plants [3]. The pesticide accumulation in soil reduces its quality making it weak in providing nutrition for crops [4]. Consequently, the production of crops is no longer increasing like past as they are threatened by irrational use of pesticides in fields to control the pest attacks. Increased incidences of biotic and abiotic stresses on agricultural resources like soil makes it necessary to search for innovative strategies to protect our food systems for sufficient supply in the future [5].

Inoculation of useful rhizosphere microorganisms in stress induced soil under crop cultivation can be an environmentally friendly and low-cost solution that can manage the effects of pesticide stress on plant growth and hence the crop yield [6-7]. It is a belief that those plants which survive in high pesticide concentrations can bring about degradation of pesticide mixture in soil due to the intensive microbial activity in their rhizosphere [8]. Rhizosphere is the region of soil where microorganisms mediated processes are specifically influenced by the root system of the plant. This includes the soil associated to the plant roots and it usually extends a few millimeters off the root surface which proves to be an important area for plant and microbial interactions [9]. Interaction between microorganisms and plants in the rhizosphere area and their potential to remediate pesticide polluted soil has been a popular topic of research [10].

Cowpea (*Vigna unguiculata* L. Walp) is one of the major leguminous plants which has got a better nitrogen fixing ability then other leguminous members and is more resilient to stress conditions. This crop is known to grow in less fertile soils, and can withstand a wide range of soil pH. It can associate itself with distinct microorganisms including the nitrogen fixing bacterial populations and fungal species [11-12]. Due to these properties, Cowpea is considered as a good legume crop for facing the predictive environmental changes which includes changes due to pollution [13-14].

The existing body of the present research work aims at underlining the importance of plant growth promoting microorganisms in improving the overall health of Cowpea plant in response to imidacloprid stress considering its economic value as an important legume. The work comprises of isolation and characterization of microorganisms from

***Correspondence to:** Radhika S. Velankar, E-mail: radhikaoke@gmail.com; Tel: +91 9881001172

Citation: Velankar RS, Zunjarrao RS. 2025. Contribution of cowpea rhizosphere microflora in plant's resilience in imidacloprid stress. *Res. Jr. Agril. Sci*. 16(3): 286-293.

Cowpea rhizosphere treated with imidacloprid concentration higher than the standard dose usually mixed in the soil. From seventeen isolated organisms, only one identified as *Pseudomonas aeruginosa* (*P. aeruginosa*) was selected for further studies. This organism was used as a microbial inoculant in the soil to check its effect on Cowpea seed germination and plant's vegetative and physiological parameters. Microbial inoculants, working singly or in consortium, are microorganisms (or their product) which can be directly applied in soil or to plant. They have a positive effect on both plant and soil by restoring fertility of soil and improving the performance of plants. These microbes can help plants to grow and get protection against biotic and abiotic stress by producing antimicrobial, or bio stimulatory compounds [15].

The results of this study demonstrated that the isolated *Pseudomonas* strains can be employed as plant growth promoting inoculant to enhance Cowpea crop growth under high imidacloprid stress in agricultural fields. This organism may be removing excessive imidacloprid from soil, improving its fertility and making it a better place for the growth of healthy plants and hence increasing the crop productivity. The isolated microorganism when re-introduced in soil can have mutually beneficial interactions with the host Cowpea crop, enhancing the metabolic activity of rhizosphere and promote plant growth [16]. This study attempts to lay the foundations for more targeted use of plant growth promoting microbes in Cowpea cultivation under challenging imidacloprid induced stress conditions and increase the crop yield.

MATERIALS AND METHODS

Chemicals, plant seeds and soil

Confidor Imidacloprid 200 SL (17.8 % w/w) (Bayer Crop Science) was procured from a local agricultural store in Pune. All the bacteriological media and medium components that were used in the experiment were purchased from HiMedia. The research grade Cowpea seeds (Amar Kunti) were procured from Amar Seeds Pvt. Ltd, Pune. Black cotton soil was purchased from a local farm in Manjari, Pune.

Choice of pesticide concentration used in the study

Survey conducted among farmers who use imidacloprid in the field confirmed the standard dose of imidacloprid added in soil was ~ 42 mg/kg. Imidacloprid concentration selected in the study was 1280 mg/kg, ~30 times higher than the standard dose. The higher dose was selected as we wanted to give abiotic, imidacloprid induced stress to Cowpea and the microbes present in its rhizosphere.

Isolation of microbes from rhizosphere region of germinated Cowpea plants

The Cowpea seeds were washed with distilled water and sown in 5 kg black cotton soil in pots spiked with 1280 mg/kg Confidor pesticide. After one month of Cowpea growth, the plants were uprooted and soil stuck near their roots was used for rhizosphere microflora isolation. The microbial isolation was done using enrichment method as described by Jariyal *et al.* [17]. Mineral Salt Medium broth (MSM) with 1280 ppm imidacloprid as a carbon source was used for isolation. The Cowpea root soil was collected in phosphate buffer, mixed well, centrifuged, and the pellet was inoculated in fresh MSM broth containing 1280 ppm imidacloprid. The inoculated broth was kept at 37°C on a shaker incubator at 100 rpm for 48 h. As these plants were uprooted young, they could not be used for further growth experiments, hence new Cowpea seeds were sown for germination experiments.

Biochemical and Physiological characterization of the selected culture

Biochemical tests like Gram staining, catalase test, oxidase test, endospore staining, motility test, sugar utilization test and IMViC (Indole, MRVP, Citrate utilization) assay were performed on the selected bacterial isolate as described by Dev *et al.* [18]; Nagar *et al.* [19] with minor changes. In all the tests, 18h old bacterial culture was inoculated in the respective test medium and appropriate detecting reagents were used for recoding the positive changes in the tests. Physiological studies of selected organisms were performed as described by Dastager *et al.* [20] with modifications. 18h old organisms were inoculated in Luria Bertani (LB) broth with different components (different NaCl concentrations and pH values) and incubated for 24-48h. Growth was observed in the tubes after the incubation period and recorded.

Identification of the selected bacterial isolate

The bacterial isolate was identified using the 16S rRNA gene sequencing method. The identification report was generated using EzBioCloud Database and the extent of homology was ~1200 bp. The sequence was deposited in the GenBank database [21] and accession number was generated.

Effect of identified bacterial isolate on the germination of cowpea seeds

To study the effect of bacterial isolate and imidacloprid on Cowpea growth, an experiment was performed as described by Akbar and Sultan [22] with slight modifications. Twenty Cowpea seeds were put in each pot containing sterilized 5 kg black cotton soil with no previous history of imidacloprid. The pots were arranged in a randomized block design in triplicate. In each replicate, the soil was subjected to four different treatments of imidacloprid (1280 mg/kg) and isolated bacterial culture (~1.5 X 10^8 cells/g soil) as follows:

CP1: Black cotton soil with imidacloprid and bacterial culture;
CP2: Black cotton soil with imidacloprid and no bacterial culture;
CP3: Black cotton soil without imidacloprid and no bacterial culture;
CP4: Black cotton soil without imidacloprid and bacterial culture.

This experiment was carried out at 28°C temperature and 45% humidity. Every week for a month, 500 mL of imidacloprid solution and 50 mL of bacterial culture was applied to appropriate pots. These applications ensured that the plant will be under stress during the growth period and isolated organism will be replicating in these stress conditions in soil along with the growth of plant. Soil with CP3 treatment was watered regularly as it did not have either imidacloprid or the selected isolate.

Measurement of vegetative growth of Cowpea

The germination of seeds from all the pots was monitored daily for a period of 60 days. After multiple shoots and ample of leaves were visible, a random sample of 15 plants from each pot was considered for further studies. The vegetative attributes of all 15 plants like plant height, root length, plant fresh weigh, and leaf area were measured. The average value of all the vegetative parameters was calculated and analyzed. For chlorophyll and proline content estimations, the plant material was weighed as per protocol and used for analysis. Similar exercise was carried out for other two replicates and graphs were drawn based on the average value of three replicates (Fig 2-6). In all the types of soil treatments, Cowpea seeds were sown at the same time and kept at same environmental conditions.

Measurement of physiological characters of cowpea

Total chlorophyll content of plants

For estimation of total chlorophyll content, leaf sample was crushed in acetone and centrifuged at 5000 rpm for 7 minutes. The absorbance of supernatant was recorded at 645 nm and 663 nm and chlorophyll content was calculated according to Kamble *et al.* [23].

Proline content of plants

The proline content of plant was estimated from the fresh leaves as described by Bates *et al.* [24] by taking the absorbance of sample at 520 nm using a spectrophotometer.

Data analysis

Statistical analysis was performed using the GraphPad Prism Software (version 10). The effect of imidacloprid and the bacterial inoculation on the growth of Cowpea plant was analysed by one way ANOVA at a significance level ($p < 0.0001$).

RESULTS AND DISCUSSION

Isolation of microbes from rhizosphere region of germinated cowpea plants

Total seventeen bacterial cultures were isolated from the rhizosphere region of Cowpea plant germinated in black cotton soil spiked with 1280 mg/kg imidacloprid. Based on the growth of all isolates, only one organism (isolate 1.2) was selected for further studies.

Biochemical characterization and identification of the selected culture

Selected isolate 1.2 was Gram negative, rod shaped, motile, greenish pigmented organism with an ability to utilize citrate, produce catalase and oxidase enzyme. It was able to break down glucose, sucrose, maltose, lactose and produce acid and gas (Table 1).

Table 1 Results of biochemical tests for isolate 1.2

S. No.	Biochemical test	Isolate 1.2
1.	Shape and Gram nature	Rod, Gram Negative
2.	IMViC tests: Indole production test	-
3.	Methyl red test	-
4.	Vogus Proskauer test	-
5.	Citrate utilization test	+
6.	Catalase test	+
7.	Oxidase test	+
8.	Endospore staining	-
9.	Motility test	+
10.	Pigmentation	+
11.	Sugar fermentation (Acid and gas production)	+

Isolate 1.2 grew at temperature of 35±2°C, and at pH of 7.0±0.2. It could tolerate salt concentration up to 15%.

The molecular analysis of the isolate based on 16s rRNA gene sequencing revealed that the organism had 99.93% similarity with *Pseudomonas aeruginosa* JCM 5962(T). The sequence data was deposited in the GenBank database (NCBI). The accession number assigned to the identified organism; *P. aeruginosa* was MW009677.

CP1 CP2 CP3 CP4

Fig 1 Habit of Cowpea plants as affected by different soil treatments (CP1-CP4). CP1: soil spiked with imidacloprid and inoculated with *P. aeruginosa*; CP2: soil spiked with imidacloprid but no *P. aeruginosa*; CP3: soil without imidacloprid and no *P. aeruginosa*; CP4: soil without imidacloprid and inoculated with *P. aeruginosa*

Vegetative growth of cowpea in presence of P. aeruginosa

Representative cowpea plant, one from each pot was uprooted and a standard measuring scale was used to measure the height of plant and root length in centimeters (Fig 1). This gave an idea about the difference in plant height and root length of the plants grown in different soil treatments, CP1-CP4. The figure clearly shows that the plant grown in soil with imidacloprid and without *P. aeruginosa* (CP2) was shortest in height and the plant grown in soil without imidacloprid and inoculated with *P. aeruginosa* (CP4) was the tallest.

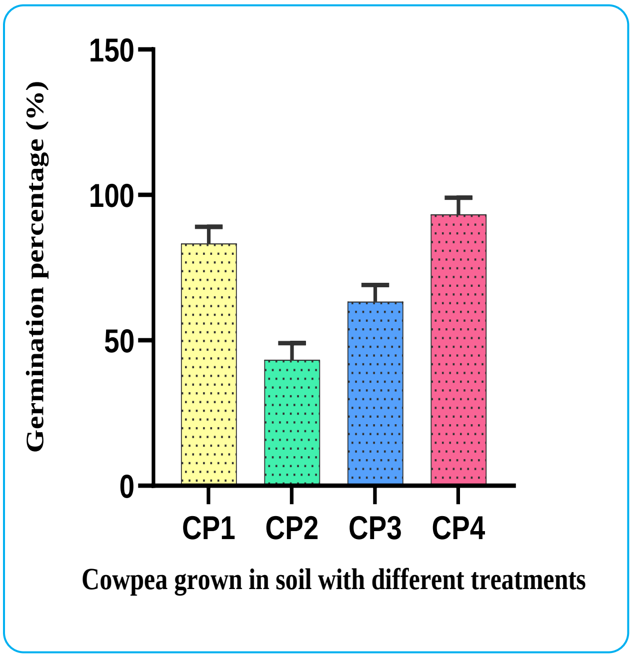

Fig 2 Germination percentage of Cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0002% significance level as per ANOVA test

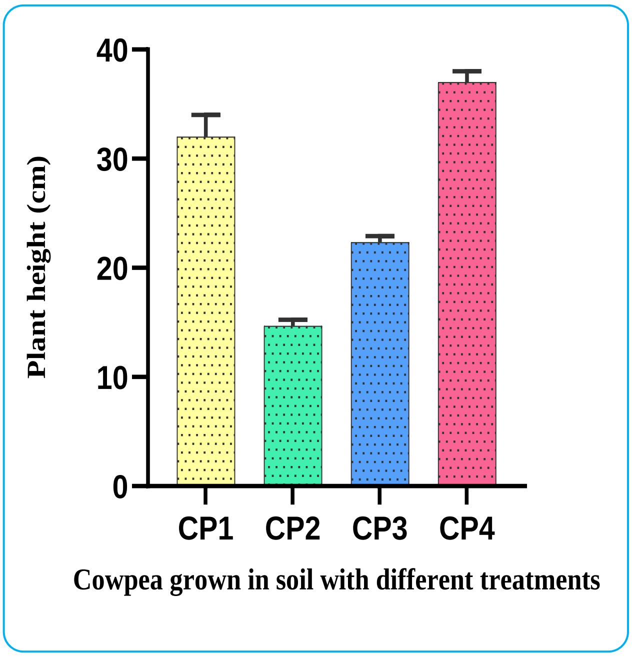

Fig 3 Height of Cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0001% significance level as per ANOVA test

The CP1 soil treatment with imidacloprid and *Pseudomonas aeruginosa aeruginosa* supported a germination percentage of 83% while seeds sown in CP4 soil without imidacloprid and with *Pseudomonas aeruginosa aeruginosa* had 93% germination. The 10% difference in the CP1 and CP4 treatments was there as CP1 soil was treated with imidacloprid while CP4 soil did not have imidacloprid in it and both the soils had active *Pseudomonas aeruginosa aeruginosa* culture giving successful chances of germination. These values were higher as compared to seed germination in soils with other two treatments in which the culture was not present (CP2, 43% and CP3, 63%) (Fig 2). Least seed germination (43.3%) was measured in CP2 treatment of soil with imidacloprid and no bacterial culture suggesting the negative impact of only imidacloprid on the seed germination. This suggested that the germination percentage was dependent mainly on the presence of *Pseudomonas aeruginosa aeruginosa* in soil and it helped majority of the seeds to germinate in imidacloprid induced stress condition. In other words, when *Pseudomonas aeruginosa aeruginosa* was used as bacterial inoculant, 84% seeds could germinate properly even in the presence of imidacloprid, while in soils with no culture, the germination percentage reduced.

The plants grown in soil inoculated with *P. aeruginosa* and spiked with imidacloprid (CP1) and no imidacloprid, but with culture (CP4) were 32 cm and 37 cm in height respectively. This height was more than the plants grown in soil without the culture (CP2 and CP3) which had the height of 14.6 cm and 22.3 cm respectively (Fig 3). CP2 soil treatment was with the imidacloprid while CP3 was without any pesticide treatment. Here, the presence of *P. aeruginosa* in soil increased the plant height by ~2.2 times in soils with imidacloprid (from 14.6 cm to 32cm) and by ~1.7 times in soil without imidacloprid (from 22.3 cm to 37 cm). This showed that there was more increase in height of those plants exposed to imidacloprid stress as compared to those plants which did not have any imidacloprid exposure due to the presence of *P. aeruginosa*. Hence the importance of *P. aeruginosa* in enhancing the plant height in imidacloprid induced stress conditions was highlighted.

Maximum root length (7.33 cm) was seen in plants grown in soil spiked with imidacloprid and inoculated with bacterial culture (CP1) while minimum length (4.6 cm) was observed in that plant which grew in soil without imidacloprid and no *P. aeruginosa* (CP3) (Fig 4). More length of Cowpea roots in CP1 soil treatment as compared to plant roots in CP3 can be attributed to the presence of *Pseudomonas aeruginosa aeruginosa* in CP1 which was not there in CP3 soil treatment. Along with variable length, a morphological change was also seen in the roots of plants grown in imidacloprid spiked soil with bacterial inoculant (CP1). It had more fibrous roots while the plants grown other soil treatments (CP2-CP4) had tap root system (Fig 4). The possible reason for the difference in root morphology in soil spiked with imidacloprid and inoculated with *Pseudomonas aeruginosa aeruginosa* can be that the plant increased its diameter in the subsurface layer of soil so that it can harbor more bacterial species which can help the plant to mitigate the stress induced by imidacloprid. The cowpea plant is known to alter its root morphology depending upon the type of treatment given to the plant during its growth stages [25].

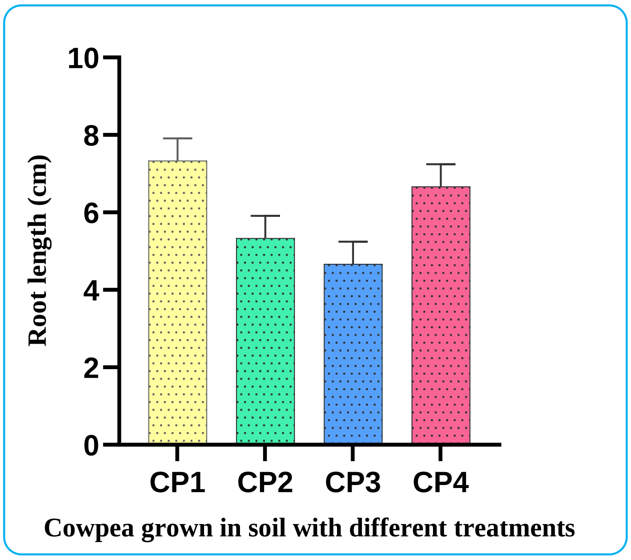

Fig 4 Root length of Cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0001% significance level as per ANOVA test

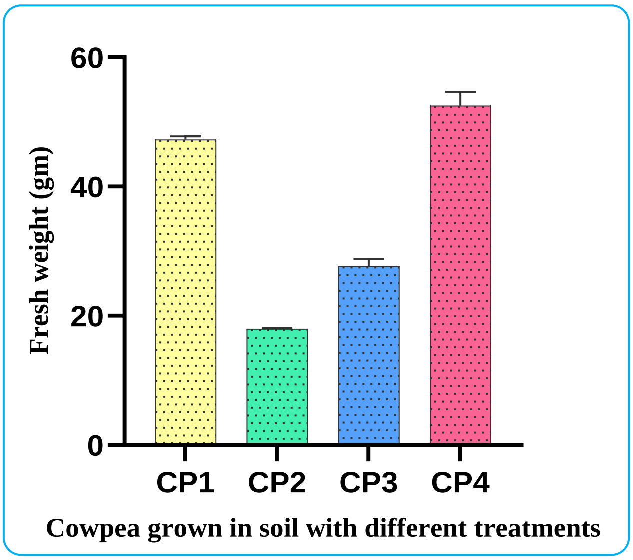

Fig 5 Fresh weight of Cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0001% significance level as per ANOVA test

Plant fresh weight gives an idea about the biomass of the plants. A substantial fresh weight of 52.5 gm was observed for the plants grown in soil with the bacterial inoculant and without imidacloprid (CP4). In CP1 soil treatment which had both, the imidacloprid and bacterial culture, the fresh weight of growing plant was 47.29 gm. These observations suggested that the presence of *Pseudomonas aeruginosa aeruginosa* in soil helped to maintain the biomass of the plant in soil treated with imidacloprid. In CP3 soil treatment, which did not have imidacloprid or the organism, the grown plants had a fresh weight of 27.65 gm while in soil without *Pseudomonas aeruginosa aeruginosa* and only imidacloprid treatment (CP2), the weight was minimum (18 gm) (Fig 5). This suggested that the presence of only imidacloprid in soil had detrimental effect on plant biomass.

Maximum leaf area (44.23 cm^2) was observed in plants grown in soil inoculated with *P. aeruginosa* and spiked with imidacloprid (CP1). To ensure proper photosynthesis in imidacloprid stress conditions, the leaf area of plants increased in CP1, but, in CP2 soil treatment with only imidacloprid and no *P. aeruginosa*, the leaf area was minimum (23.35 cm^2). Slightly reduced leaf area (~39 gm) was observed in CP4 as compared to CP1 soil treatment, with culture and no imidacloprid. This can be an indication that *P. aeruginosa* was helping the plant leaf area to increase, making it capable enough to prepare food and thrive well in stress conditions (Fig 6).

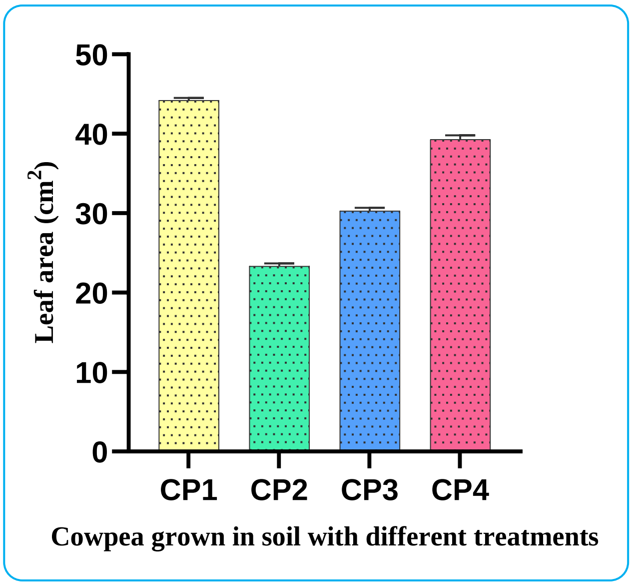

Fig 6 Leaf area of Cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0001% significance level as per ANOVA test

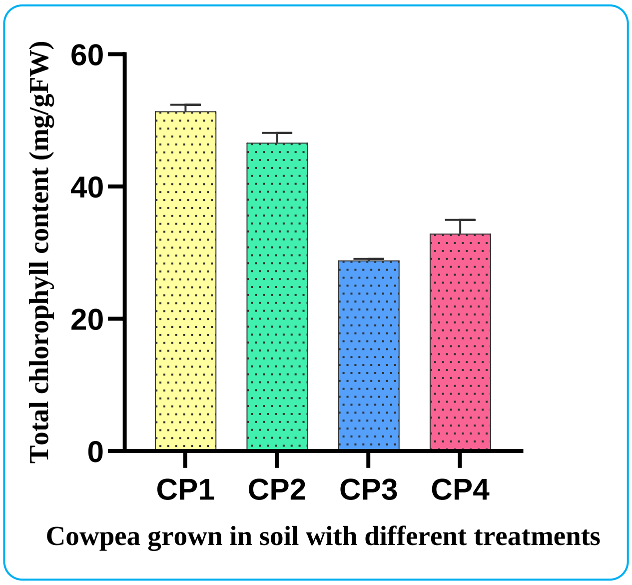

Fig 7 Total chlorophyll content of cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). A significant difference between the germination percentage of Cowpea at different soil treatments was at 0.0001% significance level as per ANOVA test

Physiological characters of cowpea plant

Total chlorophyll content

Maximum chlorophyll content of 51.3 mg/gFW was observed in plants grown in soil spiked with imidacloprid and inoculated with *P. aeruginosa* (CP1). In the CP2 treatment of soil, plants with a chlorophyll content of 46.6 mg/gFW were grown. This treatment was with imidacloprid but no culture. Here, the chlorophyll content reduced by ~5 mg/gFW which can be due to absence of the identified culture from the soil (CP2). Other two treatments i.e., CP3 and CP4 had low chlorophyll content, 28.8 mg/gFW and 32.8 mg/gFW respectively (Fig 7). Here, the soil treatments were without imidacloprid and CP3 treatment comprised of no *P. aeruginosa* while CP4 had the bacterial inoculant in the soil. The difference in chlorophyll content was mainly altered according to the imidacloprid stress conditions. Soil treatments with imidacloprid had more chlorophyll content (CP1 and CP2) as compared to the soil without any imidacloprid (CP3 and CP4). A further change in the chlorophyll of the plants was dictated by the inoculation of the bacterial culture in the soil. In imidacloprid induced stress conditions, the chlorophyll content was elevated due to *P. aeruginosa* suggesting its positive impact on the chlorophyll levels in plants. Hence, the plants were able to fix more sunlight in stress conditions due to bacterial culture and remain healthy.

Proline content

Soil treatments CP1 and CP2 which were under imidacloprid stress had high levels of proline 42.3 mg/gFW and 39 mg/gFW respectively as compared to the soil treatments CP3 and CP4 (without imidacloprid) had proline content of 19.5 mg/gFW and 27.3 mg/gFW respectively. This clearly indicated that proline content was higher in those plants which were grown in soil with imidacloprid suggesting that the plants were experiencing stress. The plants grown in soil inoculated with *P. aeruginosa*, further, saw a rise in proline content in imidacloprid treated soil (CP1) suggesting the possible role of this organism in making the plant more fit for survival in stress conditions created by imidacloprid (Fig 8).

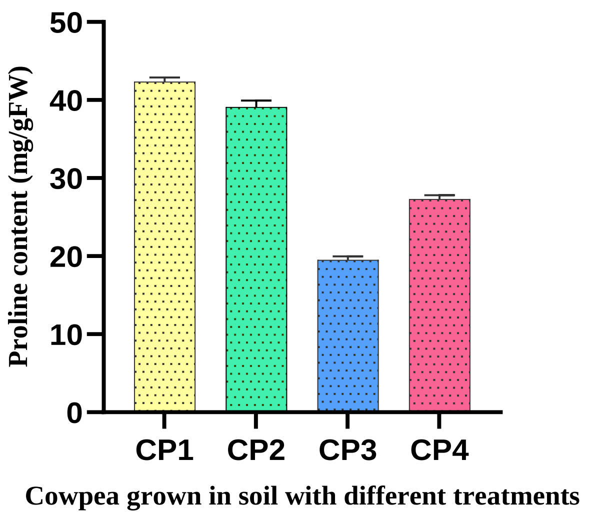

Fig 8 Proline content of cowpea plants grown in soil with different treatments (CP1-CP4). Each bar represents the mean value of three experiments and the error bars shows the Standard Deviation. The graph is drawn using GraphPad Prism Software (version 10). Difference between the germination percentage of Cowpea in different soil treatments was at 0.0001% significance level as per ANOVA test

The use of excessive imidacloprid in agricultural field creates stressful conditions for the growth of crops. It is reported that in stress conditions, nourishment and development of the plant is hampered due to the increased production of reactive oxygen species, elevated ethylene production, increased lipid peroxidation, and accumulation of free radicals. These biochemical changes eventually cause cell death. Abiotic stresses are also known to reduce the seed germination, plant height, development of roots, reduction the plant biomass and are responsible for causing losses in productivity of crop plants [26].

In such extreme situations, the beneficial microbes that live near plant roots can help to improve plant's health by increasing their nutrient uptake capacity, and by promoting tolerance to plants against biotic and abiotic stresses [26-28]. Microorganisms are known to have other unique properties like ubiquitous nature, genetic diversity, and their association with crop plants [29]. Hence some rhizosphere microbes can promote high seed germination, plant height, foliage cover, leaf area, total chlorophyll of plant, and the photosynthetic rates.

Cowpea shows good seed germination in high imidacloprid concentration and its root region harbours a rich microflora which helps the plant fix atmospheric nitrogen [30]. It is a nutritionally rich legume, which shows high levels of protein and carbohydrates, less amount of fat, and a complementary amino acid profile which pairs properly cereal grains, making it a useful meat substitute. In addition to these nutritional enrichments, Cowpea exhibits resilience to abiotic stresses like pesticide presence, drought, and salinity, which has led to its widespread utility in cropping systems in arid and semiarid regions [31]. In the present work, the microbe isolated from imidacloprid spiked Cowpea rhizosphere was identified as *P. aeruginosa* and it was inoculated in soil spiked with imidacloprid which was used for Cowpea seed germination.

P. aeruginosa in imidacloprid spiked soil aided the enhancement of vegetative parameters like seed germination, plant height, root length, plant fresh weight, and leaf area as compared to plants grown in normal soil without any imidacloprid. All these parameters were enhanced despite the imidacloprid stress induced exposure to the Cowpea seeds. Some physiological parameters like plant fresh weight, root length, germination percentage and leaf area of plants grown in only imidacloprid spiked soils were seen to reduce as they did not receive the help of the isolated culture during their growth period. Hence, this proved that Cowpea took the help of *P. aeruginosa* to stay healthy in stress conditions.

The results of the present work are like the ones reported by Akbar and Sultan [22] who demonstrated that chlorpyrifos pesticide had detrimental effect on Cowpea plant growth and caused a decrease in percentage germination, plant height and biomass. But, inoculation of chlorpyrifos degrading microbes like A*chromobacter xylosoxidans* and *Ochrobactrum* sp in soil used for plant growth, enhanced the plant growth parameters like plant length and weight. Similar results were also reported by Dastager *et al.* [20] for black pepper plant as its growth enhanced in the presence of *B. tequilensis* in acidic and alkaline soils. The extreme acidity and alkalinity created stress conditions in the soil but the Black pepper plant was able to grow well in such soils due to presence of *B. tequilensis* suggesting its plant growth promoting capabilities.

Proline which is one of the most important metabolites, protects plant integrity, and proteins stabilization in stress conditions [32]. Synthesis of proline provides NADPH in plants which activates the ascorbate–glutathione cycle and helps plant to cope up with stress. In the present work, the physiological parameters like total chlorophyll, and proline content were increased due to *Pseudomonas aeruginosa* inoculation in soil spiked with imidacloprid. Hence, this organism proved itself to be a plant growth promoting microbe even in stressed conditions. The increase in proline content of imidacloprid treated plants can be related to the activation of enzyme activities and elevation in other non-enzymatic parameters like phenolic content. The increased proline concentration in imidacloprid exposed plants clearly indicated that the cowpea was under some kind of stress.

These results partially agree with the once reported Huang *et al.* [33] who showed that in seedlings treated with imidacloprid, there was a significant decrease in the shoot/root ratio at the two-leaf stage of *Brassica napus* (oilseed rape) plant. Other growth parameters like plant height, leaf area, weight of stem, leaf, and root did not deviate much in the treatments as compared to the control plants without any imidacloprid exposure. In another work by Sharma *et al.* [34] it was reported that imidacloprid residues in soil limited mustard plant's growth and development, induced oxidative stress in it, impaired plant's photosynthetic capacity, and promoted buildup of enzymes and non-enzyme antioxidants in the plant.

In a work by Yumnam *et al.* [35], the effect of imidacloprid was studied on rhizosphere microbes of tea plants and it was observed that the bacterial population was badly affected by increased imidacloprid concentration in soil while the actinomycetes and fungal culture were seen to be resistant to the presence of imidacloprid as their growth was unaffected. These findings partially agree with the ones reported in the present work. Ahemad and Khan [36] studied dose dependent effect of different insecticides like pyriproxyfen, imidacloprid, thiamethoxam, and fipronil on the growth of mustard rhizosphere microbe, *Klebsiella sp*. PS19 and concluded that with increased doses of insecticides, the rhizosphere microbes showed a reduction in their number with compromised plant growth promoting abilities. Similarly, Li *et al.* [37], showed that excessive concentration of imidacloprid hampered the plant growth and reduce the number of rhizosphere microbes of pepper plant. These results are contradictory to the results presented in the current work which suggested enhanced Cowpea growth and physiological parameters in imidacloprid induced stress by the virtue of *Pseudomonas aeruginosa.*

All these previous published papers talk about negative effect of different pesticides on the overall health of plants and the rhizosphere microbial population, but our work is slightly different as it has showed enhancement of plant growth parameters and photosynthetic efficiency in imidacloprid stress conditions due to the activity of isolated microbe, *Pseudomonas aeruginosa*. A more intensive work studying the role of soil native microbes in alleviating imidacloprid stress for plants should be performed in future to establish a connection between the activities of deliberately inoculated culture (like *Pseudomonas aeruginosa* in the present study) and the soil natural microbes which will be present in the soil by default.

CONCLUSION

Contribution of *Pseudomonas aeruginosa* in helping the Cowpea plant to exhibit resilience against abiotic stress caused by imidacloprid in soil was studied in the present work. Cowpea rhizosphere microorganism was isolated, identified and its role in the plant growth enhancement in soil treated with high dose of imidacloprid (1280 mg/kg) was established. The dose of 1280 mg/kg was higher than the standard dose of imidacloprid used in the fields by farmers, and it caused stress in soil. Along with imidacloprid, the soil was inoculated with the identified microorganism, *Pseudomonas aeruginosa* which was used for cultivation of Cowpea. All the vegetative and physiological parameters of Cowpea grown in soil spiked with imidacloprid were seen to enhance due to the presence of *Pseudomonas aeruginosa*, while in soil without the culture and only imidacloprid, some growth parameters were negatively affected. Thus, this organism was supporting a healthy development of cowpea plant in very high imidacloprid concentration in soil. The isolated microbe can provide Cowpea with an ability to recovery quickly from imidacloprid stress and highlight its resilient nature in abiotic stress. The earlier published papers discussed here mainly reports the negative impacts of high pesticide concentrations on the growth of plants and the number of rhizosphere microbes present in such soils, however, our work is reporting positive impacts of the isolated cultures on the growth of cowpea growing in soils stressed with imidacloprid. This research offers a good opportunity for using *Pseudomonas aeruginosa* as a biofertilizer to increase the growth and yield of agronomically important crops in soils polluted with pesticides.

Acknowledgement

The authors would like to thank National Centre for Microbial Resource (NCMR), Pune for sequencing the selected bacterial culture. Special thanks to Dr. Pradnya Kanekar for extending her guidance in doing the experiments.

LITERATURE CITED

1. Elumalai P, Gao X, Parthipan P, Luo J, Cui J. 2025. Agrochemical pollution: A serious threat to environmental health. *Curr. Opin. Environ. Sci. Health* 43: 100597.
2. Shakir SK, Kanwal M, Murad W, ur Rehman Z, ur Rehman S, Daud MK, Azizullah A. 2015. Effect of some commonly used pesticides in seed germination, biomass production and photosynthetic pigments on tomato (*Lycopersicon esculentum*). *Ecotoxicology* 25(2): 329-341.
3. Zaharia R, Trotuș E, Trașcă G, Georgescu E, Șapcaliu A, Fătu V, Petrișor C, Mincea C. 2023. Impact of seed treatment with imidacloprid, clothianidin and thiamethoxam on soil, plants, bees and hive products. *Agriculture* 13(4):830.
4. Tudi M, Ruan HD, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT. 2021. Agriculture development, pesticide application, and its impact on the environment. *Int. Jr. Environ. Res. Public Health* 18(3): 1112.
5. Rhee SY, Anstett DN, Cahoon EB, Covarrubias-Robles AA, Danquah E, Dudareva N. 2025. Resilient plants, sustainable future. *Trends Plant Science* 30(4): 382-388.
6. Numan M, Bashir S, Khan Y, Mumtaz R, Shinwari ZK, Khan AL, Khan A, AL-Harrasi A. 2018. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: A review. *Microbiol. Research* 209: 21-32.
7. Kong Z, Liu H. 2022. Modification of rhizosphere microbial communities: A possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. *Front. Plant Science* 13: 920813.
8. Tarla DN, Erickson LE, Hettiarachchi GM, Amadi SI, Galkaduwa M, Davis LC, Nurzhanova A, Pidlisnyuk V. 2020. Phytoremediation and bioremediation of pesticide-contaminated soil. *Applied Science* 10(4): 1217.

9. de Souza R, Ambrosini A, Passaglia LMP. 2015. Plant growth promoting bacteria as inoculants in agriculture. *Genet. Mol. Biology* 38: 401-419.
10. Kafle A, Timilsina A, Gautam A, Adhikari K, Bhattarai A, Aryal N. 2022. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances* 8: 100203.
11. Abebe BK, Alemayehu MT. 2022. A review of the nutritional use of cowpea (*Vigna unguiculata* L. Walp) for human and animal diets. *Jr. Agric. Food Research* 10: 100383.
12. El-Taher AM, Abd El-Raouf HS, Osman NA, Azoz SN, Omar MA, Elkelish A, Abd El-Hady MAM. 2022. Effect of salt stress and foliar application of salicylic acid on morphological, biochemical, anatomical, and productivity characteristics of cowpea (*Vigna unguiculata* L.). *Plants* 11: 115.
13. Bondok AAT, Saker HM, Abo-Marzoka SA, Saad-Allah KM, Rady AMS. 2024. Cowpea (*Vigna unguiculata* L. Walp) morpho-physiological and yield responses to chemical, organic, and biofertilizers at various watering levels utilizing drip irrigation system. *Plant Prod. Science* 27(2): 110-124.
14. Kirarei E, Kipsumbai P, Pascaline J, Ezekiel K. 2025. An overview on the taxonomy, distribution, production and economic importance of cowpea (*Vigna unguiculata* L. Walp). *Journal of Crops, Livestock and Pests Management* 2(3): 1-14.
15. Hajji L, Dali S, Rhouma A, Al-Ani L, Chihani-Hammas N, Khlif A, Bargougui O. 2025. Microbial inoculants for plant resilience performance: roles, prospects, and challenges. *Jr. Plant Dis. Protection* 132: 88.
16. Yu S, Shen Y, Yang Y, Zhang Z, Zhu J, Xia Q, Song M, Lv B, Sun L, Qian H, Lu T. 2025. Inoculation of soybean rhizosphere microorganisms could promote corn growth. *Rhizosphere* 33: 10104.
17. Jariyal M, Gupta VK, Mandal K, Jindal V, Banta G, Singh B. 2014. Isolation and characterization of novel phorate-degrading bacterial species from agricultural soil. *Environ. Sci. Pollution Research* 21: 2214-2222.
18. Dev SS, Nisha EA, Venu A. 2016. Biochemical and molecular characterization of efficient phytase producing bacterial isolates from soil samples. *Int. Jr. Curr. Microbiol. Applied Science* 5: 218-226.
19. Nagar A, Kamble A, Singh H. 2021. Preliminary screening, isolation, and identification of microbial phytase producers from soil. *Environ. Exper. Biology* 19: 11-22.
20. Dastager SG, Deepa CK, Pande A. 2011. Growth Enhancement of black pepper *(Piper nigrum)* by a newly isolated *Bacillus tequilensis* NII-0943. *Biologia* (*Section Cellular and Molecular Biology*) 66: 801-806.
21. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Research* 41(D1): D36-D42.
22. Akbar S, Sultan S. 2016. Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. *Braz. Jr. Microbiology* 43: 563-570.
23. Kamble P, Giri S, Mane R, Tiwana A. 2015. Estimation of chlorophyll content in young and adult leaves of some selected plants. *Univers. Jr. Environ. Res. Technology* 5: 306-310.
24. Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil* 39: 205-207.
25. Dukare A, Kale S, Kannaujia P, Indore N, Mahawar M, Singh R, Gupta R. 2017. Root development and nodulation in cowpea as affected by application of organic and different types of inorganic/plastic mulches. *Int. Jr. Curr. Microbiol. App. Science* 6(11): 1728-1738.
26. Khoshru B, Mitra D, Khoshmanzar E, Myo EM, Uniyal N, Mahakur B. 2020. Current scenario and future prospects of plant growth-promoting rhizobacteria: an economic valuable resource for the agriculture revival under stressful conditions. *Journal of Plant Nutrition* 43: 3062-3092.
27. Varma A, Tripathi S, Prasad R. 2019. Plant Biotic Interactions State of the Art. Springer. ISBN 978-3-030-26656-1 ISBN 978-3-030-26657-8 (eBook).
28. Khan N, Bano A, Ali S, Babar Md A. 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. *Jr. Plant Growth Regulation* 90: 189-203.
29. Wani AK, Akhtar N, Sher F, Navarrete AA, Américo-Pinheiro JHP. 2022. Microbial adaptation to different environmental conditions: molecular perspective of evolved genetic and cellular systems. *Arch. Microbiology* 204(2): 144.
30. Oke R, Zunjarrao R. 2022. Effect of imidacloprid on germination and biochemical nature of cowpea (*Vigna unguiculata* L. Walp) and sunflower (*Helianthus annuus* L.). *Ecol. Environ. Conservation* 28: 62-67.
31. Halo BA, Aljabri YAS, Yaish MW. 2025. Drought-induced microbial dynamics in cowpea rhizosphere: Exploring bacterial diversity and bioinoculant prospects. *PLoS One* 20(3): e0320197.
32. Fedotova MV. 2019. Compatible osmolytes-bioprotectants: is there a common link between their hydration and their protective action under abiotic stresses? *Journal of Molecular Liquids* 292: 1-11.
33. Huang L, Zhao C, Huang F, Bai R, Lü Y, Yan F, Hao Z. 2015. Effects of imidacloprid and thiamethoxam as seed treatments on the early seedling characteristics and aphid-resistance of oilseed rape. *Jr. Integr. Agriculture* 14(12): 2581-2589.
34. Sharma A, Yuan H, Kumar V, Ramakrishnan M, Kohli SK, Kaur R, Thukral AK, Bhardwaj R, Zheng B. 2019. Castasterone attenuates insecticide induced phytotoxicity in mustard. *Ecotoxicol. Environ. Safety* 179: 50-61.
35. Yumnam D, Dutta BK, Paul SB, Choudhury S. 2014. Effect of imidacloprid on the soil and rhizosphere microflora of tea agro-ecosystem. *Global Journal of Biotechnology and Biochemistry* 9(2): 35-40.
36. Ahemad M, Khan MS. 2011. Effects of insecticides on plant-growth-promoting activities of phosphate solubilizing rhizobacterium *Klebsiella* sp. strain PS19. *Pestic. Biochem. Physics* 100(1): 51-56.
37. Li D, Zhou C, Wang S, Hu Z, Xie J, Pan C, Sun R. 2023. Imidacloprid-induced stress affects the growth of pepper plants by disrupting rhizosphere-plant microbial and metabolite composition. *Science of the Total Environment* 898: 165395.