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Degradation of Imidacloprid from Polluted Soil by Rhizosphere Microflora, *Bacillus safensis* Isolated from Sunflower (*Helianthus annuus*)

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Abstract Imidacloprid is a systemic pesticide which is used on many plants to kill pests. Due to its excessive use, it is known to be a soil contaminant which can reach other non-target areas in a short period of time. Hence, it becomes important to remove this pesticide from soil. The ability of microflora isolated from the Sunflower rhizosphere to degrade imidacloprid was studied in the liquid growth medium. Subsequently, the isolated organism was used as a 'bio-remediator' to degrade excessive imidacloprid from contaminated soils. The microbial culture present in Sunflower rhizosphere spiked with imidacloprid was isolated and enriched in Mineral Salt Medium containing imidacloprid as a sole source of carbon and maintained at 35±2°C. Organism was selected based on its ability to grow in highest imidacloprid concentration and was identified as *Bacillus safensis*. The soil was further amended with the isolated culture at a concentration of 36 X 10^8 cells in two set ups, autoclaved soil and unautoclaved soil. The imidacloprid removal efficiency of the culture was studied using High Performance Liquid Chromatography. Soil samples were taken out at different time intervals of 5, 10, 20, 40 and 80 days. In soil amended with *B. safensis*, along with imidacloprid residue, one metabolite of the imidacloprid degradation pathway, 6-Chloronicotinic Acid was detected in both the set ups. The imidacloprid residues followed Pseudo first-order kinetics in both the soils. The isolated culture showed good imidacloprid degradation (53-60%) from broth and soils suggesting the role of the organism as a bio-remediator. This is the first-time study of the potential role of *Bacillus safensis* in remediation of soils contaminated with excessive amounts of pesticides containing imidacloprid.

Keywords: *Bacillus safensis, Bio-remediator, High Performance Liquid Chromatography, Imidacloprid, Rhizosphere Microflora*

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1. Introduction

Pesticides that are used in agriculture tend to remain in soil causing pollution and are toxic to non-targets. These non-target organisms can be economically important like pollinators or some insects beneficial to the farmers and disruption of food chain and biogeochemical cycles [1,2,3]. Recently, the ill effects of antimicrobial activity of pesticides on the non-target microorganisms like rhizosphere microflora is reported [4]. It should be ensured that the used pesticide is biodegradable after its action is exerted and it does not affect the non-target plants and animals [5]. In nature, the metabolic fate of pesticide depends upon its properties, biotic factors like microbial population, cultivated plants and the abiotic factors like soil contents, pH, temperature, humidity etc.

Imidacloprid, a neonicotinoid accumulates in soil and some control measures for its removal from the environment must be addressed on priority [6]. Due to its environmental toxicity and persistence, the World Health Organization (WHO) has categorized it as Class II hazardous pesticide. One way to control the spread of imidacloprid is to use the soil indigenous microflora like bacteria and fungi from imidacloprid contaminated areas as bio-degraders as they can efficiently degrade imidacloprid from the agricultural ecosystem [7]. Even though microbes catalyse similar metabolic reactions as plants or animals, they have an extra ability to fully mineralize many heterocyclic, organic, aromatic, and aliphatic compounds [8].

In the view of this, it becomes necessary to isolate and identify bacterial cultures that can degrade imidacloprid present in high quantities from soil. At present, around 18 bacteria, 10 enzymes and 29 genes are known to degrade imidacloprid. Bacterial genera like *Flavobacterium*,

Brevundimonas, Pigmentiphaga, Stenotrophomonas, Arthrobacter, Xanthomonas and *Bacillus* have shown the ability to degrade pesticides from soil [9,10] but imidacloprid degradation capability of one of the species of *Bacillus, Bacillus safensis* has not been reported earlier.

In the present work, biodegradation potential of *B. safensis* to degrade imidacloprid and identification of the metabolite formed in the imidacloprid degradation pathway was studied. Black cotton soil spiked with different imidacloprid concentrations, which was used for cultivation of Sunflower, provided the necessary source for isolation of rhizosphere microflora. Hence, this paper highlights the role of *B. safensis*, individually and along with the indigenous soil microorganisms, as a 'bio-remediator' in removal of excessive imidacloprid from contaminated soils. This is the first-time report of the degradation of imidacloprid by *B. safensis* from broth and soil containing imidacloprid in high concentrations.

2. Materials and Methods

2.1. Chemicals and Seeds

Confidor [Imidacloprid 17.8% SL (Bayer)] pesticide and research variety Sunflower seeds were procured from the local market. Pure Imidacloprid (98%), Acetonitrile (HPLC grade) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and 6-Chloronictoinic acid (6-CNA) was purchased from Dr. Ehrenstorfer, LGC GmbH standards (UK). Pure imidacloprid and 6-CNA were used as standards.

2.2. Choice of Pesticide Concentrations Used in the Study

A general discussion with farmers confirmed that the maximum concentration of Confidor pesticide that is used in agriculture fields is 40 mg/kg. Since the focus of our study was to remediate imidacloprid contaminated soils, we have chosen 8 times (320 mg/kg), 16 times (640 mg/kg) and 32 times (1280 mg/kg) more concentration of imidacloprid than standard dose (40 mg/kg). The selected concentrations were so high that they affected the soil quality under study.

2.3. Seed Germination and Seedling Growth

The rhizosphere soil of the Sunflower plant was used to isolate microorganisms. Black cotton soil spiked with 1280 ppm imidacloprid was used to grow Sunflower. After 1 month, the plants were uprooted without cleaning the soil from their roots and used for microflora isolation.

2.4. Isolation of Rhizosphere Microflora and Culture Media

Isolation of rhizosphere soil microflora was performed with minor modifications [11]. For enrichment of cultures, the rhizosphere soil was homogenized in phosphate buffer (pH 7) with 1280 ppm imidacloprid and centrifuged. The pellet was resuspended in fresh phosphate buffer with imidacloprid and kept on shaker incubator for 48h at 37°C. Absorbance of the culture was taken at 600 nm to decide the cell number and then it was plated on Mineral Salt Medium (MSM) with Imidacloprid as Carbon source with following components: K_2HPO_4,1.5g/L; KH_2PO_4, 0.5g/L; $MgSO_4.7H_2O$, 0.2g/L; NaCl, 1.0g/L; Agar, 3.0% pH: 7. After 2 days, out of a few colonies grown on plate, only one colony was selected as representative of those cultures which can withstand high imidacloprid concentration. The selected bacterial culture (R1) was characterized biochemically and then identified using 16S rRNA sequencing.

2.5. Study of Imidacloprid Degradation by Isolated Culture in Liquid Medium

To study the imidacloprid degradation capacity of isolated culture from medium, 50 ml of MSM broth supplemented with three imidacloprid concentrations (320, 640 and 1280 mg/kg) was inoculated with 500 µl (~10^8 cells) of overnight grown culture in separate flasks. Two controls were kept, one with the culture and without imidacloprid and another with different imidacloprid concentrations and without any inoculum. The flasks were kept at 37˚C in a shaker incubator for 80 days. Aliquot of the broth was taken on 5^{th}, 10^{th}, 20^{th}, 40^{th} and 80^{th} days after the treatment and centrifuged. To the supernatant, sodium sulphate and acetonitrile were added. Out of the two separate layers formed, the top layer was collected and evaporated to dryness. The dried residue was mixed with acetonitrile, filtered, and used for HPLC analysis [12].

2.6. Imidacloprid Degradation from Black Cotton Soil Amended with Selected Bacteria

Black cotton soil was sieved to remove larger stones, shade dried and was divided into two sets. One set of soil was autoclaved at 120°C for 20 minutes to kill any indigenous microorganisms present in soil which can degrade imidacloprid before starting the experiment. The second set of soil was not autoclaved with an intention to study the interaction of indigenous soil microbes with the pure culture of the selected organism. 800 g of soil from both the sets was fortified with three imidacloprid concentrations i.e., 320, 640 and 1280 mg/kg separately and inoculated with ~36 X 10^8 cells. From each imidacloprid fortified soil samples (microbes+ imidacloprid), 100 g was taken in eight separate pots. The pots were kept at room temperature and moistened with distilled water every 3-4 days. 10g soil samples were taken at different time intervals (5, 10, 20, 40 and 80 days) from each pot to check for imidacloprid degradation. Two controls, soil without imidacloprid but with microorganism and soil without microbial culture spiked with three imidacloprid concentrations was used.

2.7. Imidacloprid Residue Analysis from Soil

The extraction of imidacloprid from soil samples was done with some changes [12]. 10 g of soil was thoroughly mixed with acetonitrile and centrifuged. To the supernatant, sodium sulphate and hexane were added.

After two separate layers were formed, the top layer was separated and air dried. The dried residue was resuspended in acetonitrile, filtered, and analyzed for imidacloprid residues using HPLC.

2.8. Analytical Instrument and Standard Operating Conditions Used in the Study

HPLC (Shimadzu, Kyoto, Japan) equipped with a C18 column and UV detector set at 270 nm wavelength was used to detect imidacloprid residues from broth and soil. Mobile phase of Acetonitrile: Water (10:90 v/v) with a flow rate of 1 ml/min, 400 psi pressure in the column and a run time of 15 minutes were used to analyze the samples [13]. The pure standards of Imidacloprid, 6-Chloronicotinic acid and pesticide formulation were also analyzed at the same conditions. The compounds in the samples were identified and quantified by comparing the retention times and peak areas of the sample chromatograms with that of standard compounds.

Under these conditions, pure Imidacloprid and 6-Chloronicotinic acid chromatograms showed a maximum peak area of 2824 at 3.417 minutes and 684117 at 7.492 minutes respectively. The pesticide solution showed three peaks at 3.442, 4.283 and 6.533 minutes with peak areas of 83376, 21147 and 18939 respectively. Out of these, peak at 3.442 minutes had similar retention time as that seen in pure imidacloprid solution (3.417 minutes). Hence, this peak in pesticide chromatogram was of imidacloprid. Apart from peaks at similar retention times like standard solutions, the chromatograms of the samples showed many unidentified peaks.

10 g soil was cleaned and extracted, and a final volume of 2 ml was made. From this, when 20 µl (equivalent to 100 mg sample) of sample was injected in the HPLC instrument, it did not produce any background interference. From this, the limit of Quantification (LOQ) was 0.01mg/kg and Limit of Detection (LOD) was 0.003 mg/kg [14]. The imidacloprid residues present in broth and soil were calculated using the following formula [15]:

$$\text{Concentration of imidacloprid residue } (mg/kg) = \frac{\begin{pmatrix}\text{Peak Area of the sample} \\ \text{X Volume of cleaned extract } (L) \\ \text{X Dilution factor} \\ \text{X Concentration of standard } (mg/L)\end{pmatrix}}{\text{Peak Area of the standard X Weight of sample } (kg)}$$

From residue values, the percentage degradation of imidacloprid and formation of its metabolites in broth and soil were calculated.

2.9. Data Analysis

All the experiments were performed in triplicate and presented as Mean ± Standard Deviation (SD). Data analysis to study the imidacloprid degradation by isolated microbe in broth and soil was done using ANOVA and Duncan's Multiple Range Test (DMRT) at 0.05% level of significance using SPSS software version 24.

3. Results

3.1. Identification of Selected Rhizosphere Microbe

Based on nucleotide sequence-based homology of 16S rRNA with GenBank Database (NCBI), the selected culture was identified as *Bacillus safensis*. The organism was Gram positive in nature and formed thin purple rods. The sequence of the identified organisms was deposited to National Centre for Biotechnology Information (NCBI) and GenBank Accession Number was obtained (Table 1).

Table 1. Identification of the Isolated Bacterial Culture

Strain no.	Taxonomic Designation	% Similarity	GenBank Accession Number
R1	*Bacillus safensis* subsp. *safensis* FO-36b(T)	100	MW790238

3.2. Degradation of Imidacloprid from Fortified MSM Broth

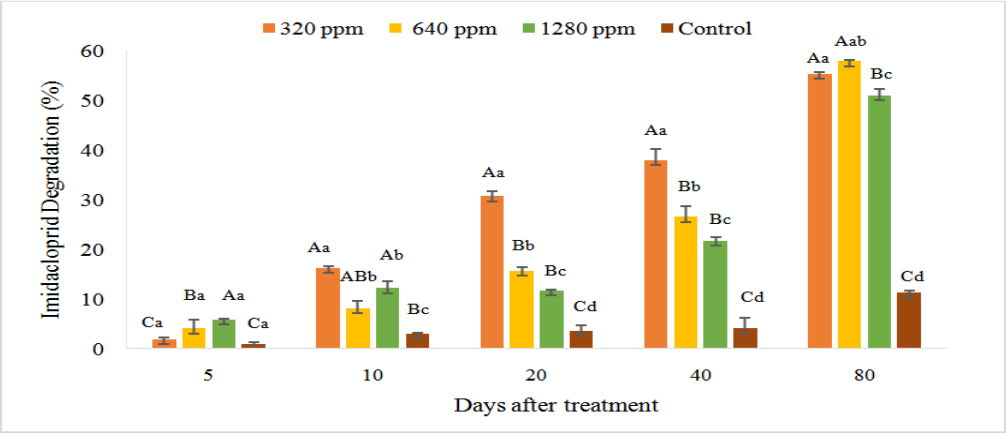

Figure 1. Imidacloprid degradation by *B. safensis* from MSM fortified with three imidacloprid concentrations at different time intervals (Each bar is the mean value of triplicates and the error bar shows the SD. Capital alphabets denote significant difference among the imidacloprid concentrations on the same day after the treatments and small letters denote significant difference among the same imidacloprid concentration on different days at 0.05% significance level as per DMRT)

In MSM broth, *B. safensis* degraded imidacloprid in one of its metabolites, 6-Chloronicotinic acid. However, due to less concentration, after 40th day, the presence of 6-CNA could not be detected. 6-CNA residues obtained from broth on 5th day were 0.89 mg/kg, and 0.12 mg/kg on the 20th day. These residues were 0.28% and 0.04% respectively of the total imidacloprid residues obtained from broth. Imidacloprid degradation shown by *B. safensis* was 55.26%, 57.86% and 50.98% on 80th day after the treatment from medium spiked with 320, 640 and 1280 mg/kg imidacloprid respectively. On the 5th day, imidacloprid degradation was less as compared to the degradation seen from 20th to 80th day at all concentrations. One probable reason for this can be the time taken by bacteria to adapt to the changing environment before it starts growing and degrading imidacloprid [16]. In control broth, very little degradation was seen from 0.9% on 5th day to 11.23% on 80th day after the treatment (Figure 1, Table 2). The degradation of imidacloprid thus was more than 50% in all the media spiked with different imidacloprid concentrations. On the 5th day, no significant difference was seen in the degradation of imidacloprid from any medium. However, on 10th, 20th and 40th day, significant difference in the degradation of imidacloprid from media spiked with different imidacloprid concentration was seen, with prominent degradation was seen in media spiked with 320 mg/kg imidacloprid.

3.3. Imidacloprid Degradation in Black Cotton Soil Amended with *B. safensis*

Pesticide metabolism studies are important to understand the fate and persistence of pesticide and the degradation products that are formed. This information can be helpful in predicting the potential risk of using any pesticide [17]. As mentioned earlier, the imidacloprid degradation capability of *B. safensis* was studied in autoclaved and non-autoclaved soils.

3.3.1. Imidacloprid Degradation in Autoclaved Black Cotton Soil

In autoclaved soil, *B. safensis* was able to degrade imidacloprid into 6-chloronicotinic acid, one of its metabolites. The concentration of 6-CNA was measured till the 20th day after which it could not be measured. About 0.12 mg/kg of 6-CNA was formed in the soil on the 5th day and 0.14 mg/kg of 6-CNA residue was obtained on the 20th day. *B. safensis* was successful in degrading imidacloprid from soil by 50.05%, 46.9% and 48.87% on 80th day after treatment from soil spiked with three imidacloprid concentrations. These percentage values were high as compared to the ones observed in control soil without any inoculum, where maximum degradation of 12.06% was seen on the 80th day (Figure 2, Table 2). The three imidacloprid concentrations did not seem to have any major effect on the degradation potential of *B. safensis*, suggesting the potential of the organism to survive in all the imidacloprid concentrations.

3.3.2. Imidacloprid Degradation in Non-autoclaved Black Cotton Soil

In non-autoclaved soil, *B. safensis* could form 6-Chloronicotinic acid (6-CNA) from imidacloprid. The residues of this metabolite were 1.02 mg/kg on the 5th day and 0.09 mg/kg on the 20th day in the soil. The concentration of 6-CNA was below the detection limit after 20 days of treatment. The 6-CNA later can get converted to carbon dioxide in the soil [18]. Non-autoclaved soil experienced a degradation of imidacloprid by 60.31%, 61.9% and 53.13% at spiking levels of 320, 640 and 1280 mg/kg imidacloprid respectively. This degradation was high as compared to degradation in autoclaved soil suggesting the role of indigenous organisms in removing imidacloprid from non-autoclaved soil. The control soil spiked with three imidacloprid concentrations was able to degrade imidacloprid from 2.87% on 5th day to 29.81% on 80th day after treatment (Figure 3, Table 2). In short, the imidacloprid degradation in non-autoclaved soil was more than 60% for 320 and 640 mg/kg imidacloprid concentrations, while it was more than 50% for 1280 ppm imidacloprid concentration. This degradation percentage was more than the ones seen in MSM broth (Figure 1).

From all the above observations it would be appropriate to say that *B. safensis* was able to degrade more imidacloprid from non-autoclaved soils with the help of indigenous soil microbes compared to autoclaved soils and the broth, where *B. safensis* was the only predominant organism.

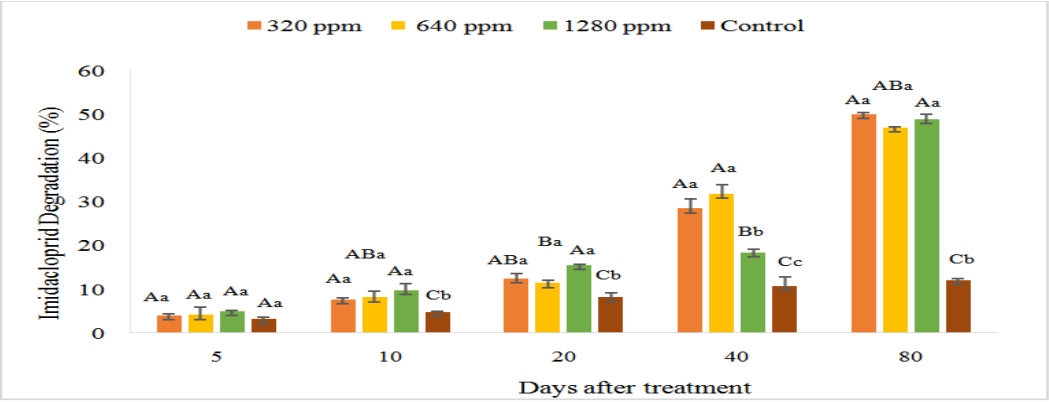

Figure 2. Imidacloprid degradation (%) by *B. safensis* in autoclaved soil spiked with three imidacloprid concentrations at different time intervals (Each bar is the mean value of triplicates and the error bar shows the SD. Capital alphabets denote significant difference among the imidacloprid concentrations on the same day after the treatments and small letters denote significant difference among the same imidacloprid concentration on different days at 0.05% significance level as per DMRT)

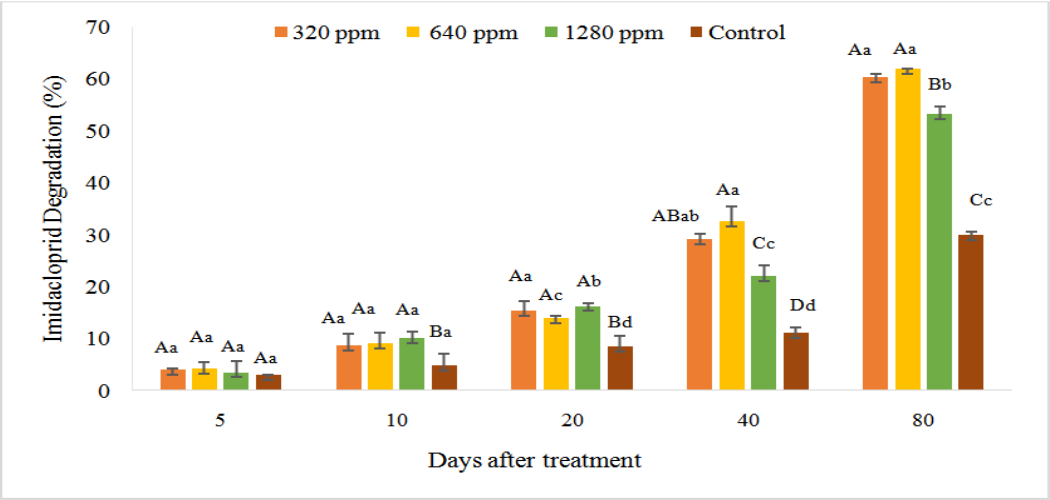

Figure 3. Imidacloprid degradation (%) by *B. safensis* in non-autoclaved soil spiked with three imidacloprid concentrations at different time intervals (Each bar is the mean value of triplicates and the error bar shows the SD. Capital alphabets denote significant difference among the imidacloprid concentrations on the same day after the treatments and small letters denote significant difference among the same imidacloprid concentration on different days at 0.05% significance level as per DMRT)

Table 2. Imidacloprid Degradation (%) from Black Cotton Soil Amended with *B. safensis*

Days after treatment	Degradation of imidacloprid (%) from soils fortified at 320, 640 and 1280 mg/kg imidacloprid*							
	Autoclaved soil				Non-autoclaved soil			
	Control	320	640	1280	Control	320	640	1280
5	3.12	3.98	4.09	4.98	2.87	4.01	4.23	3.45
10	4.76	7.65	8.11	9.81	4.76	8.54	9.08	10.01
20	8.11	12.45	11.35	15.44	8.45	15.23	13.87	16.22
40	10.65	28.45	31.8	18.41	11.13	29.14	32.62	21.98
80	12.06	50.05	46.9	48.87	29.81	60.31	61.9	53.13

(*Mean of three replications, Percent degradation at different time intervals after the imidacloprid treatment).

3.4. Degradation Kinetics of Imidacloprid Residues in Autoclaved and Non-autoclaved Soils

To determine the degradation kinetics of imidacloprid residues from sterile and non-sterile soils, a graph of log of imidacloprid residues against time was plotted. The maximum squares of correlation coefficients were useful in determining equations of the best fit curves.

The imidacloprid residues in autoclaved soils followed Pseudo first-order kinetics with R^2 values of 0.9487 and 0.9692 with regression equations of -0.0035x + 5.1054, and -0.0035x + 4.7971 for treatment of soil with imidacloprid at 320 and 640 mg/kg respectively. The R^2 value for 1280 mg/kg imidacloprid treated soil was 0.9969 which was seen to follow first order kinetics with an equation of 0.0038x + 4.5067 (Figure 4).

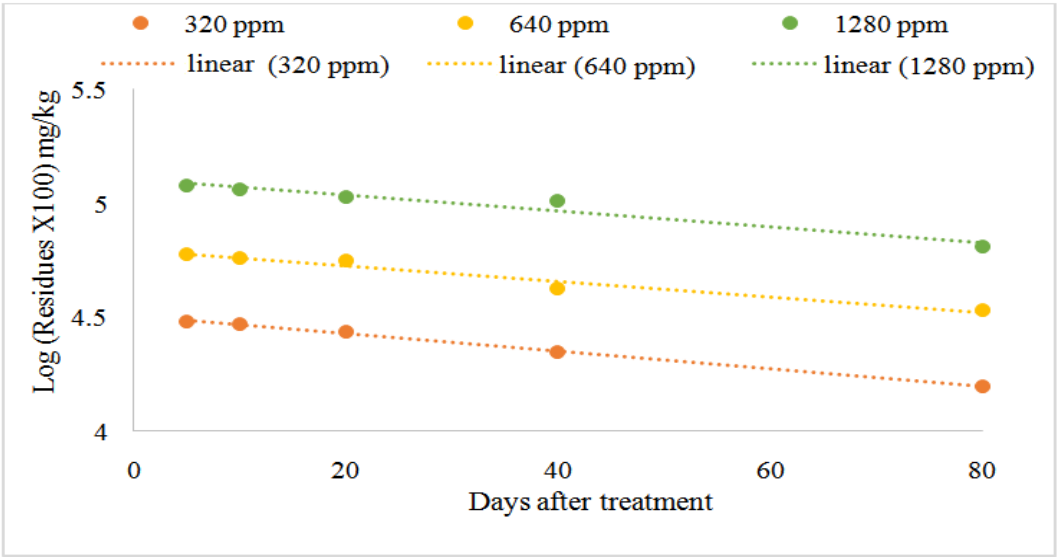

Figure 4. Persistence of imidacloprid residues in autoclaved black cotton soil fortified with *B. safensis*

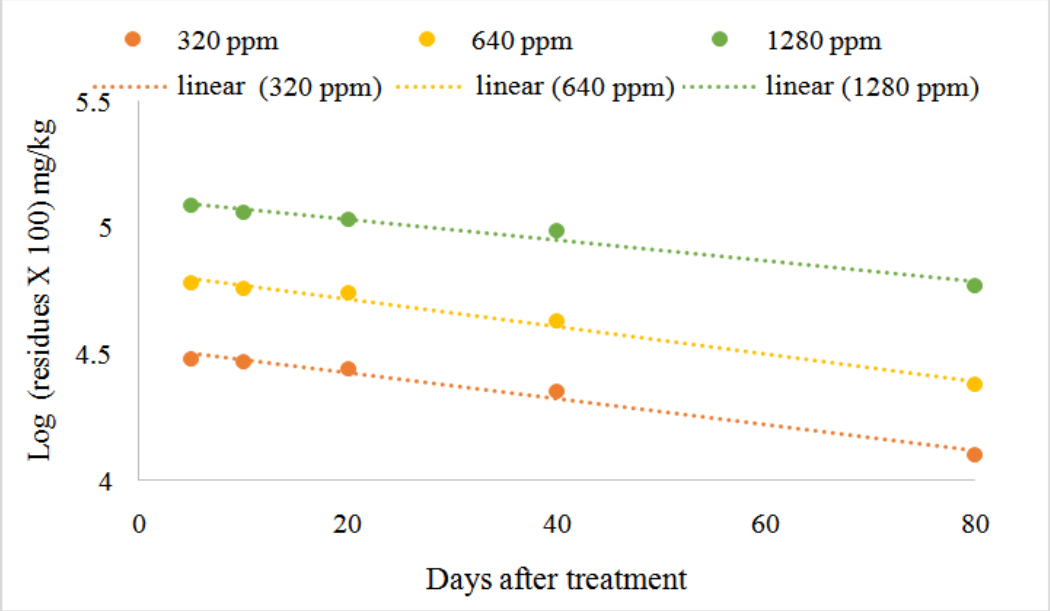

Figure 5. Persistence of imidacloprid residues in non-autoclaved black cotton soil fortified with *B. safensis*

In non-autoclaved soils, the R^2 value for 320, 640 and 1280 mg/kg imidacloprid treated soil was 0.9856, 0.9686 and 0.982 with the regression equation of -0.0054x + 4.8254, -0.0041x + 5.1154, and -0.0051x + 4.5275 respectively which followed the Pseudo first-order kinetics (Figure 5). The Pseudo-first order nature of the reaction signified that for both the soils, the rate of reaction was mainly dependent upon the pesticide concentration added to the soil rather than the microbial load present in the soil.

4. Discussion

The biodegradation of pesticides like imidacloprid from nature using microorganisms is a safe, efficient, cheap, quick, and eco-friendly method to clean the environment. Assessment of imidacloprid degradation by microorganisms like bacteria and fungi from broth and soil is a widely studied topic of research. In a study, out of 12 isolated microorganisms, *B. alkalinitrilicus* and *B. aerophilus* could degrade 36.38% and 42.85% imidacloprid respectively from medium spiked with 50 mg/kg imidacloprid [14]. *Leifsonia* sp., was able to degrade imidacloprid by 37-58% in Tryptic Soy Broth full strength media spiked with 25 mg/kg imidacloprid into six different metabolites [19]. *B. subtilis, P. putida, Rhizobium* sp., and *Brevibacterium* sp. were able to degrade 25-46% of imidacloprid from a medium spiked with 25 mg/kg imidacloprid [20]. *Aspergillus terreus*, and *Trichoderma* sp. isolated from agricultural wastewater were able to degrade 96.23% imidacloprid from Czapek Dox medium amended with 25 and 50 mg/L imidacloprid on 20th day of inoculation. However, at 400 mg/L, the fungal strains did not grow in the medium exhibiting the inhibitory effect of excessive imidacloprid [21]. In a recent study, *Tepidibacillus decaturensis* strain ST1, was reported to degrade imidacloprid from broth and soil community. The soil microcosm studies using the culture resulted in the degradation of about 77.5 and 85% of imidacloprid (200 ppm) in sterile and unsterile soils within 45 days. The imidacloprid degradation in soil followed first-order kinetics [22].

The potential of many bacterial species to degrade imidacloprid is due to their enzymatic machinery acting against xenobiotic compounds [23,24]. The nature of enzymes differs from one organism to another hence a continuous study to find out the enzymes and its mode of action is necessary. For this, detailed microbial and biochemical studies of the identified microorganism must be done. One work reports cloning of an enzyme involved in 6-Chloronicotinic acid mineralization from *Bradyrhizobiaceae* strain SG-6C [25]. *Pseudomonas* sp. RPT52 was degraded 0.5 mM aqueous solution of imidacloprid to 46.5% within 40 h [26]. The highest imidacloprid degradation of 99.7% by *Mycobacterium* sp. strain MK6 for 150 µg/mL imidacloprid in 12-15 days along with production of 6-chloronicotinic acid as a metabolite has been reported [27]. It is reported that two bacterial isolates, in consortium, Achromobacteria sp. and Paracoccus sp. were successful in degrading ~ 100% of imidacloprid from soil within 15 days [28]. *Gordonia alkanivorans* CGMCC 21704, a novel actinomycete, could degrade 95.7% imidacloprid with a concentration of 200 mg/L in soil into its nitroso metabolite within 4 days [29]. Some workers have reported the metabolites like imidacloprid guanidine, urea, guanidine-olefin formed during biodegradation of imidacloprid in sterile and unsterile soils [30,31,32].

In vitro degradation of imidacloprid by consortia of microorganisms from sterile and unsterile soil spiked with 5 mg/L and 10 mg/L over a period of 50 days was studied. It was seen that the consortia could degrade about 72-74% imidacloprid from sterile soil while only 51-53% from non-sterile soil [33]. In a study, *B. safensis* isolated from marine mangrove sediments was reported to absorb and reduce heavy metal Cadmium from the environment. Hence, this bacterium is known to have remediation capabilities to clean up contaminated environmental sites [34].

The degradation of imidacloprid was more rapid in soils which are under some crop-cover as compared to bare soils [35]. It is reported that the dissipation of imidacloprid in soil followed first-order kinetics after the application of two pesticides, Gaucho WS 700 g/kg and Confidor SL 200 g/L [36]. The dissipation kinetics of imidacloprid in Confidor 200 SL spiked soil under tea cultivation at 240 ga.i./ha was seen to follow first-order reaction [37].

The observations of the above two reports are different then the observations of the present work as the soil that is analysed is without any crop cultivated on it. As a result, the degradation studies of present work failed to show the first order kinetics reaction.

From the published available data, it is observed that the bacterial species used by earlier workers are effective only at low concentrations of imidacloprid. The results of the present study greatly differ from the above reports as the imidacloprid concentrations used in this study are very high (320, 640 and 1280 mg/kg) as compared to concentrations of 150 µg/mL, 5 mg/L, 10 mg/L, 25 and 50 mg/kg reported earlier. The isolated organism, *B. safensis*, showed an exceptional ability to efficiently degrade imidacloprid from contaminated soils. The organism took more time to degrade the pesticide as compared to other organisms in the previous reports, but degradation achieved by *B. safensis* was more than 50% in 80 days from highly contaminated autoclaved soils while it was more than 60% in contaminated non-autoclaved soils. This research work is novel as such high imidacloprid concentrations have not been used earlier in any research work.

5. Conclusion

Soil is considered as an ultimate sink for pesticides and a reservoir of rich biodiversity of microbes including the plant's rhizosphere microflora. *Bacillus* genus of bacteria is a common inhabitant of rhizosphere soil of many plants which can thrive in contaminated soils. In the present study, *Bacillus safensis* was reported to degrade imidacloprid into its metabolite, 6-Chloronicotinic acid from broth, sterile and unsterile soils spiked with 320, 640 and 1280 mg/kg imidacloprid. An increase in the imidacloprid degradation was seen with an increase in the number of days after the treatment. It is difficult to predict the exact fate of pesticides in soil as many complex, uncontrolled natural processes are taking place in nature. *In vitro* pesticide degradation studies in growth medium on the other hand have controlled conditions, making pesticide removal studies easy. Although the degradation rate of the isolated organism was slow as compared to other organisms, it can be considered worth if it can make the contaminated soil pesticide free and healthy. This is the first-time report of degradation of imidacloprid by *B. safensis* from broth and soils which are spiked with very high imidacloprid concentrations. The removal was approximately 58%, 50% and 60% in liquid medium, sterile and non-sterile soils respectively. Hence, *B. safensis* can be used as a bioremediating agent that can remove imidacloprid from polluted soils. The imidacloprid degradation shown by this organism in highly polluted soil is appreciable. The isolated organism can be used as a biofertilizer in contaminated soils to remove extra imidacloprid contents and make it fertile for the growth of plants.

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Statement of Competing Interests

The authors declare that they have no competing interests.

List of Abbreviations

6CNA, 6-Chloronicotinic acid, MSM, Mineral Salt medium, gai/ha, gram active ingredient per hectare, ~ approximately.

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