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# Biodegradation of Imidacloprid by Consortium of Two Soil Isolated *Bacillus* sp.

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**Abstract** Imidacloprid degradation potential of bacterial cultures from sugarcane growing soils was studied in liquid culture and *Bacillus aerophilus* and *Bacillus alkalinitrilicus* showed maximum potential to degrade imidacloprid. Hence, into a clay loam soil imidacloprid was added at 50, 100, and 150 mg $kg^{-1}$ along with 45 × $10^7$ cells $g^{-1}$ soil of both species under autoclaved and unautoclaved conditions. Under autoclaved conditions imidacloprid residues were degraded after 56 days to 3.18, 5.83 and 10.48 mg $kg^{-1}$ and under unautoclaved conditions to 5.17, 6.23 and 10.31 mg $kg^{-1}$. 6-chloronicotinic acid, nitrosimine and imidacloprid-NTG metabolites were detected in measurable concentrations under both conditions. Dissipation pattern of imidacloprid did not follow first order kinetics under both sets of conditions. The half life value of imidacloprid ranged from 13 to 16 days after bacterial inoculation. This is first report of use of mixed culture of native soil bacterial isolates for remediation of imidacloprid contaminated soils.

**Keywords** Imidacloprid · Biodegradation · *Bacillus aerophilus* · *B. alkalinitrilicus* · Soil · Residues

Microbes play an important role in eliminating toxic substances from aqueous and soil environments and microbial bioremediation is considered to be a cost effective tool for the detoxification of xenobiotics (Li et al. 2012). Bacterial species from genera like *Flavobacterium, Arthrobacter, Xanthobacter, Brevundimonas, Stenotrophomonas* and *Pigmentiphaga* have been reported to have the ability to degrade pesticides (Gossel and Bricker 1994; Greer and Robinson 1992; Ishaq and Khan 1994; Shetti and Kaliwal 2012; Tang et al. 2012; Wang et al. 2013) and it was observed that the use of pure cultures of *Bacillus aerophilus* and *Bacillus alkalinitrilicus* were helpful for bioremediation of imidacloprid (Sharma 2012). The information on the nature and amount of metabolites of any pesticide in soil is of concern, in addition to the role of soil microbes in bioremediation. Soil samples collected from different sugarcane fields with known history of extensive pesticide usage and located in Ludhiana and Gurdaspur districts of Punjab state served as sources of pesticide degrading microbes. The present studies were undertaken to identify the bioremediation potential of mixed cultures of *B. aerophilus* and *B. alkalinitrilicus* under autoclaved and natural conditions. This paper highlights the potential use of a mixed culture of two native soil strains of *Bacillus* sp. alone and with natural soil microflora for remediation of imidacloprid contaminated soils.

## Materials and Methods

Master cultures of twelve strains belonging to five different species of *Bacillus*, three of *Pseudomonas* and two other bacterial genera that were earlier isolated by enrichment method from sugarcane field soils and known to cause active metabolization of phorate (Jariyal 2013) were acquired from microbial culture collection of Insect Molecular Biology Lab, Department of Entomology, Punjab Agricultural

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**Table 1** Degradation of imidacloprid (50 mg $kg^{-1}$) by different bacterial cultures in Dorn's media after 15 days of incubation

| Culture | Residues of imidacloprid and its metabolites (mg $kg^{-1}$)[a] | | | | | Reduction (%) |
|---|---|---|---|---|---|---|
| | Imidacloprid | 6-Chloronicotinic acid | Imidacloprid-NTG | Nitrosimine | Total | |
| *Bacillus firmus*1 | 39.55 | 0.89 | BDL | 2.35 | 42.80 | 20.80 |
| *Bacillus thuringiensis* | 33.11 | 0.78 | BDL | 0.92 | 34.81 | 33.70 |
| *Brevibacterium frigoritolerans* | 35.56 | 0.96 | BDL | 2.26 | 38.79 | 28.79 |
| *Bacillus aerophilus* (strain a) | 33.63 | 1.03 | BDL | 0.25 | 34.90 | 32.66 |
| *Bacillus frexus* | 37.26 | 0.43 | 0.01 | 0.19 | 37.88 | 25.39 |
| *Bacillus aerophilus* (strain b) | 28.54 | 0.21 | BDL | 1.46 | 30.21 | 42.85 |
| *Staphylococcus aureus* | 38.17 | 0.35 | BDL | 1.54 | 40.06 | 23.57 |
| *Bacillus alkalinitrilicus* | 31.77 | 1.05 | BDL | 1.44 | 34.26 | 36.38 |
| *Pseudomonas monteilii* | 34.96 | 0.51 | 0.01 | 2.17 | 37.59 | 30.00 |
| *Pseudomonas fulva* | 34.56 | 0.85 | 0.01 | 2.17 | 37.59 | 30.80 |
| *Pseudomonas moraviensis* | 39.95 | 0.95 | 0.01 | 2.39 | 43.31 | 20.00 |
| *Bacillus firmus* 2 | 35.8 | 0.73 | 0.03 | 0.95 | 35.80 | 28.31 |
| Control | 44.71 | 0.76 | 0.01 | 1.26 | 46.74 | – |

*BDL* below the detection limit of 0.01 mg $kg^{-1}$

[a] Mean of three replications

University, Ludhiana, Punjab, India (Table 1). All the bacterial strains were maintained by regular subculturing of respective master culture on Luria Broth (LB) agar at 37°C as given by Gerhardt et al. (1994). LB medium with 1.6 % agar was autoclaved for 15 min. Imidacloprid metabolization by different bacterial species was studied in Dorn's broth- a minimal medium which was completed by addition of trace element solution (@10 mL $L^{-1}$ with components g $L^{-1}$: $MgSO_4 \cdot 7H_2O$ 10.0, $CaCl_2 \cdot 2H_2O$ 2.0, $MnSO_4 \cdot H_2O$ 3.0, $FeSO_4 \cdot 7H_2O$ 0.2). After adjusting initial pH to 7.0 ± 0.2, the medium was autoclaved for 15 min. Standard inoculum of individual bacterial strain was derived by appropriately diluting 24 h growth of a single bacterial clone (in 10 mL of LB at 37°C, 120 rpm) with sterile distilled water to provide $10^8$ cells $mL^{-1}$. 50 mL of Dorn's medium in 250 mL Erlenmeyer flask supplemented with imidacloprid to make concentration of 50 mg $kg^{-1}$ was inoculated with 1 mL of standard inoculum of a particular strain in three replications and allowed to grow at (37 ± 1°C) on shaker at 120 rpm for estimation of residual imidacloprid and its metabolites. For this purpose, a 5 mL aliquot of growing culture broth was drawn after a 15 day interval. The residues of imidacloprid and its metabolites were extracted and quantified using methodology described below. The per cent degradation of imidacloprid by each strain was calculated.

The following physical characteristics of the soil were determined: organic matter, pH, electrical conductivity and anion exchange capacity were ascertained before initiating. Clay loam soil (organic carbon = 0.885 %, pH = 7.91, cation exchange capacity = 6.4 meq 100 g $soil^{-1}$ and electrical conductivity = 0.1163 $dsm^{-1}$) collected from Sadhugarh village (Distt Patiala) was used for experimentation. The sample was mixed well and sieved (2 mm), and then extraneous matter including stones/pebbles was removed and dried under shade. A subsample of soil was autoclaved at (120°C), 15 psi for 15 min to destroy the microbial community responsible for the degradation of pesticides before initiating the experiment. While, another subsample of soil was not autoclaved to ascertain the potential of pure culture of bacteria and the effect of indigenous soil microbial communities. Clay loam soil samples were fortified with imidacloprid @ 50, 100 and 150 mg $kg^{-1}$ and bacterial cells (45 × $10^7$ cells $g^{-1}$ soil) in three replications. From each fortified (insecticide + microbes) sample, 50 g soil sample was taken and placed in plastic cups separately for each sampling date. The cups were moistened with water at 7 day interval and kept under laboratory conditions at (25 ± 2°C). Soil samples (10 g) were removed along with control samples at 7, 14, 21, 28, 35, 42, 49 and 56 days after inoculation. High performance liquid chromatograph (HPLC) equipped with RP $C_{18}$ column and photodiode array detector, dual pump supplied by M/S Shimadzu Corporation, Kyoto, Japan was used. The other parameters used were: mobile phase acetonitrile: water (40:60 v/v), 0.30 mL $min^{-1}$ pump flow, 1,500 psi pressure and detector set at 270 nm wave length. Under these operating conditions, imidacloprid and its six metabolites were separated in a single run of 20 min with retention time of 5 min (6-chloronicotinic acid), 8 min (imidacloprid-NTG), 10 min (olefin), 11 min (nitrosimine), 11 min (urea), 13 min (5-hydroxy imidacloprid) and 17 min (imidacloprid) (Sharma et al. 2013). The extraction and estimation of residues of imidacloprid and its metabolites from soil was carried out as per Sharma et al. (2013).

The compounds in the sample were identified and quantified by comparison of the retention time and peak heights of the sample chromatograms with that of standards run under identical operating conditions. The average recovery values at 0.01, 0.10, 0.20, 0.50 and 1.00 mg $kg^{-1}$ levels were found to be more than 80 % and the results have been presented as such without applying any correction factor. The residues of imidacloprid and its metabolites were statistically analysed for mean and standard deviation. When 10 g of soil was extracted, cleaned up and final volume made to 2 mL, out of which 20 µL sample (equivalent 100 mg sample material) when injected, did not produce any background interferences. Thus, the limit of quantification (LOQ) was found to be 0.01 mg $kg^{-1}$ and limit of detection (LOD) was 0.003 mg $kg^{-1}$.

## Results and Discussion

The degradation of imidacloprid by these 12 bacterial species in broth with three replications each resulted in appearance of 6-chloronicotinic acid, imidacloprid-NTG and nitrosimine as its three metabolites. Five species degraded more than 30 % imidacloprid as compared to control (Table 1). Based upon relative imidacloprid reduction bacterial species *B. aerophilus* (42.85 %) and *B. alkaninitrilicus* (36.38 %) demonstrated to be potential degraders of imidacloprid exhibiting also the capacity for degradation of phorate and imidacloprid (Sharma 2012; Jariyal 2013). The taxonomic identification of these bacterial species was previously performed in the Insect Molecular Biology Lab based on 16s rRNA sequence homology and amplification of 16s rRNA gene region. Based on maximum homology score through Blast function (Taxonomy Report) of NCBI (National Center for Biotechnology Information) with GenBank database (Jariyal 2013), selected bacterial species were identified as *B. aerophilus* and *B. alkalinitrilicus.* The bacterial species were gram positive in reaction and formed small colonies after 24 h with shining surface and entire colony margin. Whereas the colonies of *B. aerophilus* were yellowish in colour, those of bacterial isolate *B. alkaninitricus* were white.

Under autoclaved conditions, addition of *B. aerophilus* and *B. alkaninitricus* consortium in the clay loam soil sample resulted in degradation of imidacloprid to 38.36, 86.01 and 135.59 mg $kg^{-1}$ collected at 7 days, which further degraded to 3.18, 5.83 and 10.48 mg $kg^{-1}$ at 56 days after application resulting in 93.6 %, 94.2 % and 93 % reduction in imidacloprid at respective doses (Table 2). Three metabolites were detected: 6-chloronicotinic acid, nitrosimine and imidacloprid-NTG. 5-Hydroxy metabolite was detected at 15 days but during later samplings it remained below the detection limit. Olefin and urea metabolites were not detected in any of the samples. These results demonstrate the role of these microbes as the persistence of imidacloprid was found to be less in ammended as compared to unamended soils where imidacloprid was found to persist in much higher amounts to 56 days (100.27 mg $kg^{-1}$) after addition of imidacloprid at 150 mg $kg^{-1}$, respectively (Table 2).

These results are consistent with the earlier studies in which imidacloprid metabolites were reported in soil. The metabolites might be produced due to co-metabolism by bacterial communities present in soil. The metabolites reported were imidacloprid urea, 6-chloronicotinic acid and 6-hydroxynicotinic acid (Sarkar et al. 2001). These results are also in agreement with Pandey et al. (2009) who observed that 70 % of an initial concentration of imidacloprid (50 ppm) was degraded within 14 days by three strains of *Pseudomonas* after five rounds of subculturing under microaerophilic conditions. The results are also similar to Ge et al. (2006) using a strain NJ2 identified as *S. maltophilia* from soil which transformed imidacloprid into polar metabolites and imidacloprid decreased by 5 mmol $L^{-1}$ after 10 days. In our study, imidacloprid was metabolized to nitrosimine and nitroguanidine which was not reported by Pandey et al. (2009). Guanidine and urea metabolites were recovered from metabolism of imidacloprid (37 %–58 % of 25 ppm over 4 weeks) by *Leifsonia* sp. strain PC-21 (Anhalt et al. 2007). In their study, two soil-free stable enrichment cultures in N-limited media were obtained that degraded 19 (43 %) and 11 mg $L^{-1}$ (16 %) of the applied imidacloprid, and produced about 19 mg $L^{-1}$ 6-chloronicotinic acid in 3 weeks. In an earlier study also, strain SP-01 identified as *Brevundimonas* sp. MJ 15 degraded 38 % and 69 % of imidacloprid in MSM and TSB respectively in 4 weeks (Shetti and Kaliwal 2012). In another recent study, acetamiprid-degrading bacterium AAP-1 was isolated from contaminated soil, and identified as *Pigmentiphaga* sp. which could metabolize 100 mg $L^{-1}$ acetamiprid within 2.5 h (Wang et al. 2013). In a recent study *B. aerophilus* has been reported to be effective in degradation of fipronil (Mandal 2012).

Under unsterilized conditions, the two bacterial consortia resulted in the degradation of imidacloprid to its metabolites up to 46.20, 91.57 and 139.69 mg $kg^{-1}$ in soil collected at 7 days. These residues further decreased to 5.17, 6.23 and 10.31 mg $kg^{-1}$ by day 56 resulting in 89.7 %, 93.8 % and 93.1 % reduction of imidacloprid (Table 3). These studies demonstrate the role of a bacterial inoculum in degradation of imidacloprid. Three metabolites were detected: 6-chloronicotinic acid, nitrosimine and imidacloprid-NTG. 5-Hydroxy metabolite was detected by day 7 but during later samplings it remained below the detection limit. Olefin and urea metabolites were not detected in any of the samples. These studies demonstrate

**Table 2** Residues of imidacloprid and its metabolites (mg $kg^{-1}$) in autoclaved clay loam soil amended with *B. aerophilus* and *B. alkaninitricus*

| Days | Imidacloprid | Metabolites | | | | Total residues | Reduction (%) |
|---|---|---|---|---|---|---|---|
| | | 6-Chloronicotinic acid | Imidacloprid-NTG | Nitrosimine | 5-Hydroxy derivative | | |
| *Imidacloprid @ 50 mg $kg^{-1}$* | | | | | | | |
| 7 | 37.25 ± 0.34[a] | 0.85 ± 0.02 | 0.03 ± 0.00 | 0.14 ± 0.00 | 0.09 ± 0.0 | 38.36 ± 0.08 | 23.3 |
| 14 | 25.96 ± 0.61 | 1.80 ± 0.04 | 0.18 ± 0.00 | 0.26 ± 0.01 | 0.99 ± 0.04 | 29.18 ± 0.14 | 41.6 |
| 21 | 20.73 ± 0.16 | 1.91 ± 0.43 | BDL | 0.71 ± 0.18 | BDL | 23.35 ± 0.22 | 53.3 |
| 28 | 18.95 ± 0.37 | 1.04 ± 0.14 | BDL | 1.75 ± 0.15 | BDL | 21.74 ± 0.16 | 56.5 |
| 35 | 15.27 ± 1.80 | 0.22 ± 0.10 | BDL | 3.14 ± 0.24 | BDL | 18.63 ± 0.53 | 62.7 |
| 42 | 10.61 ± 0.64 | 0.15 ± 0.00 | BDL | 2.58 ± 0.54 | BDL | 13.35 ± 0.30 | 73.3 |
| 49 | 7.30 ± 0.58 | 0.25 ± 0.03 | 0.02 ± 0.00 | 0.15 ± 0.01 | BDL | 7.72 ± 0.13 | 84.6 |
| 56 | 2.71 ± 0.03 | 0.22 ± 0.08 | 0.05 ± 0.00 | 0.20 ± 0.04 | BDL | 3.18 ± 0.04 | 93.6 |
| *Imidacloprid @ 100 mg $kg^{-1}$* | | | | | | | |
| 7 | 85.35 ± 0.86[a] | 0.26 ± 0.09 | 0.01 ± 0.00 | 0.29 ± 0.09 | 0.09 ± 0.02 | 86.01 ± 0.21 | 13.9 |
| 14 | 66.43 ± 0.43 | 0.71 ± 0.13 | 0.18 ± 0.02 | 0.26 ± 0.05 | 0.89 ± 0.09 | 68.47 ± 0.14 | 31.5 |
| 21 | 39.83 ± 1.80 | 2.70 ± 0.15 | BDL | 1.52 ± 0.15 | BDL | 44.05 ± 0.53 | 55.9 |
| 28 | 35.40 ± 0.66 | 0.48 ± 0.04 | BDL | 0.71 ± 0.14 | BDL | 36.59 ± 0.21 | 63.4 |
| 35 | 20.16 ± 0.64 | 0.12 ± 0.01 | BDL | 2.40 ± 0.06 | BDL | 22.68 ± 0.18 | 77.3 |
| 42 | 15.95 ± 0.29 | 0.26 ± 0.05 | BDL | 1.79 ± 0.19 | BDL | 17.99 ± 0.13 | 82.0 |
| 49 | 12.94 ± 0.35 | 0.26 ± 0.07 | 0.06 ± 0.02 | 0.25 ± 0.02 | BDL | 13.89 ± 0.10 | 86.1 |
| 56 | 5.58 ± 0.14 | 0.14 ± 0.06 | 0.02 ± 0.00 | 0.08 ± 0.02 | BDL | 5.83 ± 0.05 | 94.2 |
| *Imidacloprid @ 150 mg $kg^{-1}$* | | | | | | | |
| 7 | 132.12 ± 0.57[a] | 2.58 ± 0.08 | 0.01 ± 0.00 | 0.54 ± 0.02 | 0.35 ± 0.02 | 135.59 ± 0.14 | 9.6 |
| 14 | 104.07 ± 0.27 | 0.30 ± 0.06 | 0.17 ± 0.03 | 0.28 ± 0.07 | 0.59 ± 0.03 | 105.41 ± 0.09 | 29.7 |
| 21 | 55.84 ± 1.17 | 1.29 ± 0.17 | 0.79 ± 0.13 | 0.95 ± 0.15 | BDL | 58.87 ± 0.41 | 60.8 |
| 28 | 45.24 ± 0.50 | 0.41 ± 0.05 | BDL | 1.33 ± 0.13 | BDL | 46.99 ± 0.17 | 68.7 |
| 35 | 35.39 ± 1.53 | 0.76 ± 0.12 | BDL | 2.68 ± 0.49 | BDL | 38.83 ± 0.56 | 74.1 |
| 42 | 25.42 ± 0.79 | 0.84 ± 0.01 | BDL | 2.87 ± 0.23 | BDL | 29.12 ± 0.26 | 80.6 |
| 49 | 17.90 ± 0.41 | 0.08 ± 0.01 | 0.02 ± 0.00 | 0.20 ± 0.06 | BDL | 18.25 ± 0.11 | 87.8 |
| 56 | 10.02 ± 0.22 | 0.09 ± 0.00 | 0.02 ± 0.00 | 0.35 ± 0.00 | BDL | 10.48 ± 0.06 | 93.0 |
| *Unamended control @ 150 mg $kg^{-1}$* | | | | | | | |
| 7 | 142.20 ± 3.11[a] | 0.72 ± 0.05 | 0.01 ± 0.00 | 0.28 ± 0.09 | 0.11 ± 0.05 | 143.31 ± 3.18 | 4.5 |
| 14 | 134.37 ± 1.35 | 0.40 ± 0.05 | 0.04 ± 0.00 | 0.64 ± 0.11 | 0.14 ± 0.08 | 135.59 ± 2.46 | 9.6 |
| 21 | 129.45 ± 2.39 | 0.49 ± 0.04 | BDL | 1.08 ± 0.05 | BDL | 130.54 ± 1.15 | 13.0 |
| 28 | 124.43 ± 2.19 | BDL | 0.06 ± 0.02 | 2.76 ± 0.15 | BDL | 127.25 ± 2.25 | 15.2 |
| 35 | 117.39 ± 2.94 | 0.40 ± 0.04 | BDL | 3.35 ± 1.02 | BDL | 121.14 ± 1.07 | 19.2 |
| 42 | 107.92 ± 1.75 | 0.72 ± 0.18 | BDL | 4.71 ± 1.83 | BDL | 113.35 ± 2.64 | 24.4 |
| 49 | 106.26 ± 1.29 | 0.24 ± 0.06 | 0.18 ± 0.05 | 0.83 ± 0.25 | BDL | 107.51 ± 1.21 | 28.3 |
| 56 | 99.46 ± 1.88 | 0.36 ± 0.04 | 0.03 ± 0.01 | 0.42 ± 0.15 | BDL | 100.27 ± 1.59 | 33.2 |

Residues of olefin and imidacloprid urea metabolite were BDL at all the days of sampling

[a] Mean ± standard deviation of three replications

the role of these microbes as the persistence of imidacloprid was found to be less compared to unamended soils where imidacloprid was found to persist in much higher concentrations (107.71 mg $kg^{-1}$) to day 56 (Table 3). Under unautoclaved conditions, the bacterial inoculum seems to cause reduction of imidacloprid to nitrosimine and imidacloprid-NTG metabolites. Also, imidacloprid residues were oxidized to 6-chloronicotinic acid which was finally converted to carbon-di-oxide as the end product of degradation. But in contrast to the autoclaved soil lesser amount of imidacloprid-NTG metabolite was formed. Comparable to autoclaved conditions, a lesser amount of 5-hydroxy metabolite was detected and indicates that the hydroxylation of imidacloprid occurred initially but the pathway was not a preferred one as the metabolite was not detected after 7 days.

**Table 3** Residues of imidacloprid and its metabolites (mg $kg^{-1}$) in unautoclaved clay loam soil amended with *B. aerophilus* and *B. alkaninitricus*

| Days | Imidacloprid | Metabolites | | | | Total residues | Reduction (%) |
|---|---|---|---|---|---|---|---|
| | | 6-Chloronicotinic acid | Imidacloprid-NTG | Nitrosimine | 5-Hydroxy derivative | | |
| *Imidacloprid @ 50 mg $kg^{-1}$* | | | | | | | |
| 7 | 43.79 ± 0.63[a] | 0.68 ± 0.09 | 0.24 ± 0.08 | 1.11 ± 0.45 | 0.38 ± 0.03 | 46.20 ± 0.26 | 7.6 |
| 14 | 37.11 ± 2.04 | 1.41 ± 0.29 | BDL | 3.08 ± 0.93 | BDL | 41.59 ± 0.82 | 16.8 |
| 21 | 26.31 ± 0.94 | 0.70 ± 0.01 | 0.03 ± 0.01 | 2.14 ± 0.03 | BDL | 29.18 ± 0.25 | 41.6 |
| 28 | 19.41 ± 0.45 | 0.61 ± 0.17 | 0.13 ± 0.02 | 1.36 ± 0.20 | BDL | 21.51 ± 0.21 | 57.0 |
| 35 | 11.85 ± 0.68 | 2.29 ± 0.39 | 0.03 ± 0.01 | 2.05 ± 0.58 | BDL | 16.22 ± 0.41 | 67.6 |
| 42 | 7.54 ± 0.16 | 0.26 ± 0.03 | 0.01 ± 0.00 | 1.04 ± 0.03 | BDL | 8.85 ± 0.05 | 82.3 |
| 49 | 5.26 ± 0.26 | 0.52 ± 0.08 | 0.04 ± 0.02 | 0.69 ± 0.03 | BDL | 6.54 ± 0.10 | 86.9 |
| 56 | 3.32 ± 0.37 | 0.35 ± 0.01 | 0.01 ± 0.00 | 1.49 ± 0.98 | BDL | 5.17 ± 0.34 | 89.7 |
| *Imidacloprid @ 100 mg $kg^{-1}$* | | | | | | | |
| 7 | 85.96 ± 1.31[a] | 4.42 ± 0.06 | 0.06 ± 0.00 | 0.47 ± 0.01 | 0.66 ± 0.01 | 91.57 ± 0.28 | 8.4 |
| 14 | 73.47 ± 0.58 | 2.44 ± 0.17 | BDL | 3.50 ± 0.14 | BDL | 79.40 ± 0.22 | 20.6 |
| 21 | 57.08 ± 1.75 | 0.83 ± 0.21 | 0.10 ± 0.05 | 2.57 ± 0.21 | BDL | 60.58 ± 0.55 | 39.4 |
| 28 | 29.68 ± 0.07 | 0.92 ± 0.01 | 0.16 ± 0.01 | 1.64 ± 0.27 | BDL | 32.40 ± 0.09 | 67.6 |
| 35 | 19.57 ± 0.32 | 2.13 ± 0.59 | 0.07 ± 0.00 | 1.21 ± 0.14 | BDL | 22.98 ± 0.26 | 77.0 |
| 42 | 11.72 ± 0.23 | 0.34 ± 0.03 | 0.12 ± 0.01 | 2.22 ± 0.43 | BDL | 14.40 ± 0.17 | 85.6 |
| 49 | 8.89 ± 0.27 | 0.62 ± 0.09 | 0.05 ± 0.01 | 1.24 ± 0.05 | BDL | 10.80 ± 0.11 | 89.2 |
| 56 | 5.60 ± 0.49 | 0.25 ± 0.03 | 0.01 ± 0.00 | 0.37 ± 0.06 | BDL | 6.23 ± 0.15 | 93.8 |
| *Imidacloprid @ 150 mg $kg^{-1}$* | | | | | | | |
| 7 | 135.05 ± 2.02[a] | 2.14 ± 0.70 | 0.18 ± 0.00 | 1.33 ± 0.65 | 0.99 ± 0.19 | 139.69 ± 0.71 | 6.9 |
| 14 | 112.76 ± 0.99 | 2.11 ± 0.03 | BDL | 2.32 ± 0.11 | BDL | 117.18 ± 0.28 | 21.9 |
| 21 | 88.78 ± 1.02 | 0.87 ± 0.07 | 0.16 ± 0.00 | 2.73 ± 0.04 | BDL | 92.55 ± 0.28 | 38.3 |
| 28 | 57.28 ± 2.97 | 1.20 ± 0.06 | 0.08 ± 0.02 | 2.06 ± 0.42 | BDL | 60.62 ± 0.87 | 59.6 |
| 35 | 33.50 ± 0.69 | 1.53 ± 0.39 | 0.06 ± 0.00 | 1.61 ± 0.48 | BDL | 36.70 ± 0.39 | 75.5 |
| 42 | 24.45 ± 0.39 | 0.62 ± 0.00 | 0.02 ± 0.00 | 2.21 ± 0.94 | BDL | 27.30 ± 0.34 | 81.8 |
| 49 | 13.39 ± 0.83 | 0.53 ± 0.02 | 0.08 ± 0.00 | 0.62 ± 0.05 | BDL | 14.62 ± 0.23 | 90.3 |
| 56 | 6.85 ± 0.67 | 0.09 ± 0.02 | 0.04 ± 0.01 | 3.33 ± 0.24 | BDL | 10.31 ± 0.24 | 93.1 |
| *Unamended control @ 150 mg $kg^{-1}$* | | | | | | | |
| 7 | 139.14 ± 3.42[a] | 0.52 ± 0.18 | BDL | 0.39 ± 0.10 | 0.55 ± 0.01 | 140.60 ± 3.18 | 6.3 |
| 14 | 130.78 ± 1.35 | 1.14 ± 0.64 | BDL | 1.92 ± 0.78 | BDL | 133.84 ± 2.46 | 10.8 |
| 21 | 120.92 ± 2.39 | 2.10 ± 0.25 | 0.66 ± 0.09 | 7.56 ± 2.95 | BDL | 131.24 ± 2.37 | 12.5 |
| 28 | 117.46 ± 2.94 | 0.87 ± 0.10 | 0.20 ± 0.05 | 7.72 ± 2.39 | BDL | 126.25 ± 3.04 | 15.8 |
| 35 | 112.23 ± 2.75 | 3.09 ± 1.68 | 0.12 ± 0.02 | 1.85 ± 0.98 | BDL | 117.29 ± 1.85 | 21.8 |
| 42 | 109.72 ± 1.54 | 0.51 ± 0.20 | 0.02 ± 0.00 | 2.47 ± 0.85 | BDL | 112.72 ± 1.45 | 24.9 |
| 49 | 107.81 ± 1.42 | 0.35 ± 0.18 | 0.05 ± 0.02 | 1.84 ± 0.09 | BDL | 110.05 ± 2.51 | 26.6 |
| 56 | 105.62 ± 2.19 | 0.32 ± 0.11 | 0.10 ± 0.03 | 1.67 ± 0.72 | BDL | 107.71 ± 1.37 | 28.2 |

Residues of olefin and imidacloprid urea metabolite were BDL at all the days of sampling

[a] Mean ± standard deviation of three replications

The results are similar to the study conducted by Liu et al. (2011) who compared the degradation of different neonicotinoids in unsterilized and sterilized soils. Under unsterilized conditions, 94 % of acetamiprid and 98.8 % of thiacloprid were degraded within 15 days, while in sterilized soils, the degradation rates of acetamiprid and thiacloprid were respectively only 21.4 % and 27.6 %.

The bacterial consortium degraded 91.71 %, 93.22 % and 92.27 % and 88.81 %, 93.20 % and 92.62 % of imidacloprid under autoclaved and unautoclaved conditions, till day 56 at the respective doses of 50, 100 and 150 mg $kg^{-1}$. On the other hand, in unammended soil samples, imidacloprid degraded up to 30.03 and 23.39 under autoclaved and unautoclaved conditions indicating

the role of these two bacterial species in degradation of imidacloprid.

The degradation kinetics of total residues of imidacloprid was determined by plotting residue concentration against time, and the maximum squares of correlation coefficients were used to determine the equations of best fit curves. Confirmation of the kinetics order was further made graphically from the linearity of the plots of log*C* against time. Total imidacloprid residues did not follow the first order kinetics with $R^2$ value of 0.877, 0.968 and 0.979 and regression equation of $y = -0.019x + 3.795$, $y = -0.022x + 4.134$ and $y = -0.021x + 4.291$ under autoclaved conditions for respective doses of 50, 100 and 150 mg $kg^{-1}$. Similarly, in unautoclaved conditions also, the $R^2$ value was 0.982, 0.986 and 0.981 and regression equation of $y = -0.021x + 3.882$, $y = -0.024x + 4.215$ and $y = -0.024x + 4.406$ for total imidacloprid residues at 50, 100 and 150 mg $kg^{-1}$ respectively. The half life ($T_{1/2}$) of imidacloprid was calculated as per Hoskins formula (1961) i.e. rate of degradation (k) = (2.303 × slope) and half-life ($T_{1/2}$) = 0.693/k. The calculated half-life was 16, 14 and 14 days under autoclaved conditions and 14, 13 and 13 days under unautoclaved conditions for treatment of imidacloprid @ 50, 100 and 150 mg $kg^{-1}$, respectively.

It can be concluded that consortium of these two *Bacillus* species could degrade nearly 90 % imidacloprid in clay loam soil under autoclaved and unautoclaved conditions in a time span of 56 days. Imidacloprid was metabolized to 6-chloronicotinic acid, nitrosimine and imidacloprid-NTG unaffected by the sterilization condition. Dissipation pattern of imidacloprid after inoculation did not follow first order kinetics under both sets of conditions with the half life value ranging from 13 to 16 days with bacteria. These results showed that these bacteria have the potential for bioremediation of imidacloprid contaminated soils.

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