Biodegradation of Imidacloprid by microorganism from contaminated soil

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Abstract

Imidacloprid (1-[(6-chloro 3 pyridinyl) methyl]- N-nitro 2 – imidazolidinimine) is a pesticide included in neonicotinoid family. The excessive use of pesticide is harmful to the environment. The microbial degradation of pesticide is cost effective and most affordable method. Mineral salt Medium (MSM) was used for the degradation. Out of 11 isolates, VG2, VG3 and VG6 isolates were selected for degradation study. These isolates were grown in Mineral Salt broth for a duration of 30 days. Biomass was checked every after 5 days on colorimeter and degradation of imidacloprid for the removal of nitrate/nitrite from its imidazole ring was determined by Nitrate Reduction Test. Biomass was increased in all the three strains by 10 and 20 days and decreased after 10 and 20 days. The nitrate reduction test showed increased in pink colour intensity by 5 and 20 days, decreased after 5 days and 30th day. These findings suggest that VG2, VG3 and VG6 were capable of degrading imidacloprid in some metabolites and can be used for bioremediation in soil.

Keywords: Imidacloprid; Neonicotinoid; Pesticide; Bioremediation.

Introduction:

Imidacloprid(1-[(6-cloro-3-pyridyl)methyl]-N-nitro 2imidazolidinimine) is an insecticide belongs to neonicotinoid class, used against various pests in agriculture[1]. imidacloprid acts on the postsynaptic nicotinic acetyl choline receptors causing the paralysis and death in insect at low concentration. It shows toxic effect on birds, bees and aquatic animal and also have low toxicity to mammals[3]. Pesticide shows the various effect on human such as carcinogenicity, mutagenicity, reproductive toxicity and other health problem. It is contaminant of underground and surface water due to less soil sorption and high leaching capability. Rate of dissipation of imidacloprid is increased due to vegetation yielding a range of half-life from 42 to 129 days[3].

6-chloronicotinic acid, two-cyclic urea,olefinic cyclic nitroguanidine, acyclic guanidine and nitroso and nitroderivative are the metabolites of imidacloprid found in soil [3].

This chemical pollutant is essential to remove from the environment. Biological method is become the choice since the xenobiotic compound such as pesticide are used by microorganism for their
growth, mineralize and detoxify them[9]. This study was carried out to check the potential of microorganisms to degrade imidacloprid.

Materials and methods

Media-

0.5 gm of Imidacloprid was added in flask containing 100 ml sterile MSM broth to check pesticide degradation by selected isolates VG2, VG3, VG6. The composition of MSM in (g/lit) K₂HPO₄ - 1.7 gm, KH₂PO₄ - 0.68 gm, MgSO₄(H₂O)₇ - 0.1 gm, NaCl - 4 gm, FeSO₄(H₂O)₇ - 0.03 gm, NH₄NO₃ - 1 gm, CaCl₂ - 2H₂O - 0.02 gm with pH 7.2 was taken.

Pesticide used –
The commercial grade pesticide that is Imidacloprid (Admire 70 WG; Bayer crop science Ltd).

Degradation of Imidacloprid by using different isolates -

MSM supplemented with pesticide used as control. Degradation of imidacloprid was checked in 3 different sterile MSM broth supplemented with 0.5 gm/100 ml of imidacloprid by inoculating one ml of each selected isolates VG2, VG3, VG6 of optical density 0.20. All the flasks were incubated at 37°C for 30 days. Sampling was done for each isolates every after 5 days and microbial density was measured calorimetrically at 600 nm wavelength.

Nitrate reduction test- Detection of free NO₃/ NO₂

Every after 5 days, nitrate reduction test was done for the detection of degradation of imidacloprid. Nitrate reduction test reagent was prepared by adding equal volume of sulfanilic acid and α-naphthalamine. Formation of pink or red colour in broth after addition of few drops of this reagent was checked and colour intensity was measured colorimetrically at 600 nm[8].

Mulliken and Barker’s Reaction - Detection of free Nitro group

Ethanol, few drops of calcium chloride and zinc dust was added to the control and treated sample and boiled for 5 min. The sample was filtered and Tollens reagent was added to the filtrate to check the formation of bright silver mirror or black precipitate.

Detection of urea/amide (-CONH₂) group: Turmeric Paper Test

Samples withdrawn from 5 days interval were heated with NaOH in 1:1 ratio. Presence of Amide/ Urea was tested by holding turmeric paper in contact with vapours generated after heating of broth and NaOH mixture.

Sample (broth) + NaOH → shake (vapours contact with turmeric paper)

Result and Discussion

Variation in growth pattern was observed in all the three isolates in 30 days of incubation. All isolates were showing maximum growth and maximum density in the MSM broth containing imidacloprid at the end of 10th day. Decreased in growth was observed by the end of 15th day and suddenly increased in growth was shown by 20th day by all the three isolates (table 1). These findings were comparable to the work of Anhalt J. 2007[4]. This variations in the growth pattern is indicative of production of metabolic products of imidacloprid during biodegradation process. The degradation of imidacloprid was detected by nitrate reduction test and turmeric paper test. (The metabolites and percent degradation will be carried out in future by using HPLC and GC-MS).

The pink colour intensity was checked by colorimeter during 30 days for the detection of NO₃/NO₂ reduction by Nitrate reduction test. The test showed maximum pink colour intensity in 5 and 20 days, while it was decreased by 20th and 30th day[4]. The isolate VG3 shows highest degradation as compared to the VG2 and VG6 (Table.2). Development of pink colour confirmed the reduction of nitrate group and hence the degradation of imidacloprid.

The bright silver mirror was formed in the respective samples confirms the presence of nitro/ urea group released during biodegradation (fig.4). Present study firstly detected the free Nitro group by Mulliken and Barker’s reaction. Turmeric paper turned to red after exposing to vapours confirmed the presence of –CONH₂ / urea.
which is obtained in one of the metabolites of imidacloprid.

![Fig.1 Effect of Imidacloprid on growth of microorganism](image1)

![Fig.2 Nitrate reduction test](image2)

growths of isolates.

![Fig.3 Nitrate reduction test](image3)

![Fig.4 Mulliken and Barker’s test](image4)

![fig.5 Amide test (turmeric paper test)](image5)

**Conclusion**

In the present study variation in growth pattern of isolates was affect due to the production of intermediate metabolites of Imidacloprid. Selected microorganisms VG2, VG3, VG6 can be further used for bioremediation of soil to clean environment. The isolate VG3 shows maximum degradation.

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References:
7] Soudamini Mohapatra, Ashok Kumar Ahuja, “Residue study of Imidacloprid in grapes (Vitisvinifera L) and soil” (2011).

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<th>10th day</th>
<th>15th day</th>
<th>20th day</th>
<th>30th day</th>
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<td>0.69</td>
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Table.1 growth pattern of tree isolates VG2, VG3 and VG6

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<th>15th day</th>
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Table.2 Nitrate reduction test

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