DEGRADATION OF IMIDACLOPRID RESIDUE IN SOIL BY EFFECTIVE MICROBES

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Abstract

The fungi *Aspergillus sp* isolated from soil samples of Agricultural College and Research Institute, Madurai, Tamil Nadu, India was found to be deplete imidacloprid in minimal media and in soil. But the depletion was more in soil when compare to minimal media.

Index terms: Imidacloprid, biodegradation, soil microbes

Introduction

Chemical control still forms the first line of defense against insect pests on vegetables, though over dependence and excessive use of insecticides resulted in development of resistance to insects and resurgence of pests, destruction of natural enemies, pollution of environment and also potentially toxic to humans. They may induce adverse health effects including cancer, effects on reproduction and immune or nervous systems. Indiscriminate pesticide usage also contaminates soil, air and water bodies (Berrada *et al*., 2010). Bioremediation can be an effective solution for reducing pollution level in the environment by reducing the concentrations and/or the toxicity of chemical compounds and restoring natural conditions (Ahemad *et al.,* 2009). With this aim the present study was undertaken to analyze the ability of soil microbes in degradation of imidacloprid.

Materials and Methods

3.1. Studies on biodegradation of imidacloprid by soil microbes

3.1.1. Screening of fungal strains for imidacloprid degradation

Microbial culture isolation was done by shaking 20 g soil in 100 ml potato dextrose broth for overnight on a rotary shaker at 28º C and 150 rev min^{-1}. After filtration the soil suspension was used to inoculate potato dextrose agar plates supplemented with 20 ppm imidacloprid. The inoculated plates were incubated for 5 to 10 days at 28^0 C. The recovered fungal isolates were purified by growing them on Czapek-Dox medium (sucrose 30 gm, sodium nitrate 2 gm, dipotassium phosphate 1gm, magnesium sulfate 0.5 gm, potassium chloride 0.5 gm). The medium was adjusted to pH 6.0 and autoclaved to sterilize at 121º C for 20 min. The fungal isolates with maximum tolerance level of the imidacloprid were grown in broth supplemented with imidacloprid (Gangola *et al.*, 2015).

3.1.2. Morphological characters of fungi

Morphological identification of imidacloprid tolerant fungus was performed using an online interactive key (Eltem, 2003) based on the colony appearance and pigmentation, growth rate at different temperature, the presence or absence of pustules on PDA, the sizes of conidia, the branching patterns of conidiophores and the presence or absence of chlamydospores.

3.1.3. Biodegradation of imidacloprid in minimal medium

Biodegradation of imidacloprid was studied using selected fungal isolates (FI) in liquid medium. Czapek-dox broth (50 ml) was taken in a 100 ml flask. To this 1ml of four day old fungal

culture was added as per treatment and 20 ppm of imidacloprid was added to fungal culture and untreated check. Uninoculated flasks spiked with imidacloprid were kept as control. Inoculated flasks were incubated at 30°C at 150 rpm (He *et al.*, 2014). One ml aliquot of the broth were taken from all flasks separately on 0, 10 and 15th day for extraction of the imidacloprid. Quantification of imidacloprid was done by HPLC.

3.1.4. Biodegradation of imidacloprid in soil

Biodegradation of imidacloprid in soil was conducted in laboratory at Agricultural College and Research Institute, Madurai, Tamil Nadu, India. Soil was treated with 20 ppm of imidacloprid and 2ml of 48 hours old fungal culture which was replicated seven times (Gangola *et al.*, 2015). Samples were extracted after 3, 6 and 15th day after treatment and residual pesticide was quantified by HPLC. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used to extract and cleanup the soil samples.

3.2. Quantification of pesticide residues

The final quantification was worked out using the following formula with the parameters from chromatogram as

$$\text{Residues (ppm)} = \frac{As}{Astd} \times \frac{Wstd}{Ws} \times \frac{Vs}{Asj}$$

As- Peak area of the sample
Astd- Peak area of the standard
Wstd- Weight of the standard in ng
Ws- Weight of the sample in g
Vs- Volume of the sample (final extract in ml)
Asj- Aliquot of the sample injected in ml

4 Result and Discussion

4.1. Screening of imidacloprid degrading fungal strains

The imidacloprid tolerant fungal strains isolated from soil were identified as *Aspergillus* sp. based on their morphological character, which showed olive green colour conidia. The conidial diameter of this isolate was 2.513 µm, biseriate and unbranched, chlamydospores were obvious, rough, globose or subglobose in shape. The earlier reports of Klich *et al*. (2002) also follow the potential method to identify *Aspergillus* sp. based on colour, shape and also the similar method to identify colony appearance of the *Aspergillus* sp. was reported by Samson *et al.* (2010).

4.2. Biodegradation of imidacloprid in minimal media and soil

The fungi isolated from soil *i.e. Aspergillus sp.* (FI) had the ability to degrade imidacloprid when applied separately as well as together both in minimal media and soil (Table 1). In minimal media imidacloprid concentration was depleted from 20 ppm to 0.0329 ppm by FI on fifteenth Days After Application (DAA) . On third DAA the imidacloprid was reduced to 0.0549 ppm by FI isolate and again the concentration was only 0.0419 ppm on sixth DAA. In soil, the reduction was superior in the treatment FI. The initial deposit of imidacloprid (20 ppm) was reduced up to a level of 0.016 ppm by FI isolate on 15 DAA. In both minimal media and soil, the untreated check recorded low reduction rate. The highest reduction in FI may be due to utilization of pesticides molecules by the isolate and the untreated check reduction may be associated with several abiotic factors which includes the soil properties also. The biodegradation of imidacloprid in soil was

observed as 85 to 81 per cent by *Aspergillus oryzae* (Gangola *et al*., 2015). The enhanced bio degradation activity of *Aspergillus* along with *Trichoderma* for endosulfan in sugarcane eco system was reported by Gangola (2014). Mohammed *et al.* (2017) observed that the 85 per cent of imidacloprid degrade in Czapek-dox broth by using *Aspergillus* sp. The comparative study on chlorpyrifos degradation activity of *A. niger* and *T. viridae* shown that per cent degradation was more in *T. viridae* than *A. niger* (Mukherjee and Gopal, 1996). Any pesticides remnants in the environment are governed by both biotic and abiotic factors. The harmful residues depletion by microorganisms is significant in the environment which is safe to the nature. The bio remediation by microbial isolates can be advanced by identification of primary cause for degradation.

Table 1. Biodegradation of imidacloprid in minimal medium and soil

Treatments	**Pesticide Residue (ppm)**					
	3 DAY (Mean± SD)		**6 DAY (Mean± SD)**		**15 DAY (Mean± SD)**	
	Minimal media	**Soil**	**Minimal media**	**Soil**	**Minimal media**	**Soil**
T1 F1 + Imidacloprid 20 ppm	0.0549 ± 0.0004	0.034 ± 0.0003	0.0419 ± 0.0003	0.025 ± 0.0009	0.0329 ± 0.0004	0.016 ± 0.0001
T2 Untreated check	0.0744 ± 0.0004	0.061 ± 0.0004	0.0730 ± 0.0004	0.052 ± 0.0005	0.0716 ± 0.0004	0.043 ± 0.0005

*FI-*Aspe*F1 *Aspergillus sp* *Mean ± SD of three determinations

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