

Keywords: Biodegradation; Imidacloprid; Mycoremediation; Amendments

Introduction

A pesticide is a chemical substance, biological agent (such as a virus or bacteria), antimicrobial, disinfectant or device to control any pest [1]. Pesticides are generally used against insect and nematodes infestation in plants for the improvement of food production, in spite of their benefits, the continuous use of pesticides cause severe effect on the health of human, animal and environment [2]. Different types of insecticides (organochlorine, organophosphorus, carbamates, pyrethroids and neonicotinoids) were synthesized and put into application, have gained increasing interest in the agricultural sector. Imidacloprid, a member of chloronicotinylneonicotinoid compounds is most commonly used on rice, cereal, maize, sunflower, potatoes and vegetables [3]. Imidacloprid interferes with the transmission of stimuli in the insect's nervous system. Half-life of imidacloprid ranges from 42 to 129 days. Depending on soil type, fertilizer use and presence or absence of ground cover [4].

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 [5]. Bioremediation where fungi are employed is called Mycoremediation [6]. Fungi are reported to utilize hazardous chemical for their own

which 1 ml of 4 day old fungal culture was also added. Degradation of imidacloprid was analyzed in the presence of individual fungal isolate and in the presence of their consortium (FII+FIII). Inoculated flasks were incubated at 30°C at 150 rpm. One ml aliquot of the broth was taken from all flasks separately on 0, 10, and 15 day for extraction of the imidacloprid. Uninoculated flasks spiked with imidacloprid acted as control. Quantification of imidacloprid was done by High performance liquid chromatography (HPLC).

Biodegradation of imidacloprid by immobilized fungal cultures in sodium alginate beads and agar discs

Biodegradation of imidacloprid was tested using immobilized fungal isolates in sodium alginate beads and agar discs. Sodium alginate beads were prepared by using 4% sodium alginate and mixed with homogenized fungal culture(s). Mixture was poured drop by drop into pre chilled 0.4M CaCl₂ (autoclaved) through syringe to make beads in the laminar air flow. Beads were stored at 4°C overnight. To prepare agar disc, agar solution (4%) was autoclaved and allowed to cool up to 44°C. Homogenized fungal cultures were mixed with molten agar and poured in plate aseptically in a laminar air flow. Agar discs of equal size were cut by using cork borer.

For biodegradation experiment, 50 ml of Czapek-dox medium was taken in a 100 ml flask in two sets, for sodium alginate beads and for agar discs each. Imidacloprid (20ppm) was added to all the sets with 10 sodium alginate beads and agar discs separately (having equal fresh weight of fungal culture). One ml aliquot of the broth was taken from all the flasks separately on 5, 10, and 15 day for extraction and analysis. Uninoculated flasks spiked with imidacloprid acted as control. Quantification of pesticide was done by HPLC using FID detector.

Biodegradation of pesticides in soil amended with different waste material

For this experiment, soil was collected from Breeder Seed Production Centre (BSPC) of GBPUAT Pantnagar and autoclaved 3 times. Amendments used for biodegradation study were Farm Yard Manure, Hen Manure and Bagasse which were collected from Kichha, Pantnagar and Nagla respectively. Biodegradation experiment was conducted with soil in the presence of three amendments in different sets. Fifty gm soil was taken in 16 sets of the flasks with control (Table-1). Each flask was supplemented with one type of amendment (1.25 gm). Imidacloprid (20 ppm) was mixed properly with soil and amendment in each flask. After mixing, 2 ml of 48 hr old homogenized culture of each fungal isolate was inoculated in each flask except control. In one set, mixture of both the fungi FII and FIII (1 ml of each culture) was added. Ingredients of the flask were mixed properly and autoclaved water (13%) was added in each flask. Flasks were plugged with cotton plugs for aseptic condition and aeration. Samples were extracted after 3, 6 and 15th day of incubation and residual pesticide was quantified by HPLC. (Table 1)

Extraction and cleanup of imidacloprid from soil

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[13] used organic amendments to enhance herbicide degradation and removal of contaminants but even among them fungus is most efficient contaminated soils. The use of available organic amendments, such as compost, plant residues, green manure and fertilizers, may be an effective, non-chemical way of improving the pesticidal efficacy of solarization. Combining solarization with organic amendments has a significant potential for improving pathogen control and crop production, especially when solarization alone cannot provide adequate control of the target pathogens [14].

Interest in the microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to clean up contaminated environments and the present study is significant in relation to reduce environmental pollution. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the

removal of contaminants but even among them fungus is most efficient as they take advantage of the astonishing catabolic versatility of microorganisms to degrade/convert such compounds. The recovered fungal strains and their consortium appear to be suitable candidates for use in the bioremediation of pesticides contaminated sites along with plant growth promotion. The present study can be further extended to functional genomics and metabolomics which can provide the clues about the metabolic pathways and regulatory networks.

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