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ISOLATION AND CHARACTERIZATION OF IMIDACLOPRID DEGRADING MICROORGANISMS FROM PESTICIDES CONTAMINATED SOIL

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ABSTRACT

Pesticides are the chemical substances that prevent, kill, repel any pest. Repeated use of same pesticide, bulky handling or accidental release results in accumulation of pesticides residues or its metabolites in soil or water streams. The persistent nature of pesticides carries potential hazards to man and show lethal effects on living system. Imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), is a second-generation neonicotinoid pesticide (chloronicotinyl insectile). It is used to treat a wide range of pests on rice, maize, potatoes and vegetables. Imidacloprid has been reported as a stable compound in the environment with a half-life over 100 days. There are very few repots of imidacoprid degrading soil microorganisms. In an effort to find active microbial strains over the problem of commonly used pesticides like imidacloprid, a study was carried out. The objectives of this study were to isolate and characterize the efficient imidacoprid degrading microorganisms from the contaminated agricultural soil. The minimal salt medium (MSM) was used for testing the imidacloprid degrading ability of the isolates. They were characterized based on their morphological, cultural and biochemical characteristics. Among 20 soil isolates, bacterial isolates Bacillus spp., Azotobacter spp, Azospirillum spp. and Pseudomonas spp. showed degradation of imidacloprid after 48-72 hours of incubation. Imidacloprid was degraded by Azospirillum spp up to 500 mgL^{-1} and Bacillus spp., Pseudomonas species and Azotobacter species upto 200 mgL^{-1}. Detection of presence of NO_2- in the inoculated broth was the indication of imidacloprid degradation by the microorganisms. These findings suggest that these strains may be the promising organisms for bioremediation of imidacloprid contaminated soils.

KEY WORDS

Biodegradation, bioremediation, Imidacloprid and insecticide.

1. INTRODUCTION:

The use of pesticide has become important in agriculture as pests destroy 30% of the agricultural product. India is the largest consumer of pesticide [1]. Pesticides are large and varied group of substances that are specifically designed to kill biological organisms including weeds, insects and rodents. Pesticides include insecticides, fungicides, herbicides, rodenticide, molluscicides, nematicide and plant growth regulators [2]. Classification of pesticides can be done on the basis of chemical structure, physical state and target organism. The indiscriminate use of pesticides leads to the potential hazards to man and show lethal effects on living system [3]. Chronic pesticide poisoning is attributed to increasing incidences of cancer, chronic kidney, suppression of immune system, sterility among males and females, endocrine disorders, neurological & behavioral disorders specially among childrens [4].

Insecticides like organochlorine and thyphus have been banned or restricted after 1960s in most of advanced countries [5]. Imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine) is a second-generation neonicotinoid insecticide (chloronicotinyl insectide). Imidaclorntrid is commonly applied as a systemic pesticide and effective against many sucking insects, fleas, termites and other chewing pests [6]. Because of its excellent systemic properties, it can be used as seed dressing, as soil and foliar treatment in different crops including rice, maize, potatoes, cotton, cereals, sugar beets and vegetables. In industry imidacloprid is considered to be a pesticide of relatively low toxicity, it has been found to be very toxic to the non-target insects such as bees [7,8]. Sorption of imidacloprid increases with increasing soil organic carbon and pesticide concentration. The dissipation rates of imidacloprid in field are varied widely and it has been reported as a stable compound in the environment with a half-life over 100 days. In non-vegetated soil it has been reported with half live exceeding 180 days and more than three years in dry and aerobic conditions [9]. The degradation of imidacloprid in soil, is generally facilitated by chemical, sunlight and microbial agents. Biodegradation is the most commonly used method [10]. Biodegradation studies of imidacloprid in soil demonstrated that imidacloprid urea, imidacloprid guanidine and 6-chloronicotinic acid are the possible metabolic products [11,12]. Previous report have revealed that very few species of bacteria are able to degrade this compound. The imidacloprid degradation by microorganism Leifsonia strain PC-21 is the first report [13].

The aim of the present work was to isolate and characterize imidacloprid-degrading microbial strains which have the maximum ability to degrade high concentrations of imidacloprid.

2. MATERIALS AND METHODS

2.1. Chemicals

The technical grade imidacloprid was made available from the Chemistry department, Tuljaram Chaturchand College, Baramati.

2.2. Culture media

Mineral salt medium (MSM)

MSM was used for the isolation of bacteria [14]. The composition of the media in grams per litre of distilled water was Na_2HPO_4 (2.4), KH_2PO_4 (2), NH_4NO_3 (0.1), $MgSO_4.7H_2O$ (0.01), $CaCl_2$ (0.01) at pH 6.5. The medium was sterilized by autoclaving at 121 ^{0}C for 20 min.

2.3 Collection of soil sample

For the isolation of pesticide degrading microorganisms, soil sample was collected from Harni and Jogawadi, Maharashtra which was having history of repeated pesticide application. The soil sample collected in a sterile zipper polythene bags from 10 cm of depth was air dried, sieved and stored at 4^{0}C before processing for characterization.

2.4 Physico-chemical parameters of soil sample

Physico-chemical parameters were studied according to standard procedure [1].

2.4.1. Moisture content: 10 gm of collected soil sample was dried at 60^{0}C for 72h in oven. The dry weight of sample was taken till it showed constant weight. The % moisture was expressed as follows:

Moisture % = W_1-W_2 ×100/100

Where, W_1 = Weight of soil before oven drying

W_2 = Weight of soil after oven drying

2.4.2. pH of soil sample: Soil sample were dried at 60^{0}C for 72 h, powdered in pestle and mortar and filtered through 2 mm sieve and the sieved soil were dissolved in distilled water (2.5 w/v) and vortexing for 5 minutes at 120 rpm. P^H was measured by digital pH meter.

2.4.3. Percent organic Carbon/Nitrogen: One-gram soil sample was mixed with 10 ml potassium dichromate (1 N) and 20 ml concentrated H_2SO_4. Then 150 ml distilled water and 25 ml $FeSO_4$ (0.5 M) were added and the excess was titrated against potassium permanganate (0.1N) solution to pink end point.

% Organic carbon = A – B × 0.3 × 1.33 / C

Where;

i. Volume of $K_2Cr_2O_7$ X Normality of $K_2Cr_2O_7$,

ii. Volume of $KMnO_4$ X Normality of $KMnO_4$,

iii. Weight of sample

Soil organic nitrogen was calculated using following equation:

2.4.4. Organic nitrogen (%) = 0.862 × % organic carbon

2.5 Isolation and characterization of imidacloprid degrading bacteria: Primary screening:

5gm of collected soil sample was inoculated in 100ml of Mineral Salt Broth (MSB) containing 50mgL^{-1} imidacloprid for bacterial isolation. Flasks were incubated at 37^{0}C for 5-7days for bacterial growth. Loopful of culture was streaked after 5days on sterile MSM plates were incubated at 37^{0}C for 24-48h. Individual colonies were sub cultured on MSM

containing the same concentration of pesticide. Bacterial colonies were characterized on the basis of morphology, cultural and biochemical tests like capsule staining (Manvel's method), Gram staining, motility, catalase, oxidase and sugar fermentation tests, IMViC test.

2.6 Degradation of pesticide at different concentration by bacterial isolates

Soil bacterial isolates were streaked on MSM agar plates respectively at different concentrations of imidacloprid (50 mg L^{-1} to 500mg L^{-1}). Plates were incubated for 24-48h and growth was observed.

2.7 Determination of imidacloprid metabolites by Nitrite detection test

Microorganisms were grown in MSM broth containing imidacloprid for 4-5 days. Uninoculated MSM broth with pesticide and inoculated MSM broth without pesticide were taken as control. After incubation, 3-4 drops of two reagents sulfanilic acid and alpha Naphthylamine were added to 1 ml of each inoculated and uninoculated broth and mixed.

3. RESULT AND DISCUSSION

3.1 Physicochemical parameters of soil sample: The physicochemical parameters affect the growth and diversity of microorganisms. The moisture content, certain amount of organic matter like carbon and nitrogen is essential for the growth and active functioning of the microbial population in the soil. Therefore, it is necessary to study the soil factors and its relation to the microbial population. In the present study some of the important physicochemical properties of the soil were determined for the evaluation of natural microbial flora of the soil. (Table1) The analysis of physicochemical parameters of soil was done in the present study for the isolation of microorganisms. The colour of the soil sample was black with pH (7.0) and percent moisture content (0.62). The percent organic carbon and percent organic nitrogen was (0.3574) and (0.3167) respectively. These results were parallel to the findings of Rohilla SK et. al; (2012).

3.2 Isolation of imidacloprid degrading microorganisms:

Primary screening was done by using enrichment technique. With successive transferences in MSM agar medium with imidacloprid as the only source of carbon and energy, bacterial strains were obtained from the soil sample degrading this pesticide. On the basis of morphology, staining technique, biochemical characters and by referring Bergey's manual of determinative bacteriology, the isolated bacterial strains on MSM media were *Azotobacter spp, Azospirillum spp, Bacillus spp* & *Pseudomonas spp*.

3.3 Microbial degradation of imidacloprid at different concentration:

When these soil isolates were grown at different concentration of imidacloprid (50 mgL^{-1} to 1000 mgL^{-1}), *Azospirillum spp.* were able to degrade imidacloprid up to maximum concentration of 500 mgL^{-1} whereas *Bacillus*, *Pseudomonas species* and *Azotobacter species* were up to 200 mgL^{-1} (Table 2). The result shown by bacterial isolates in this study were able to degrade imidacloprid at higher concentration than the findings of Geeta Negi et.al., 2014 where they found the tolerance of imidacloprid by bacterial isolates from 100 to 200 mg L^{-1}.

3.4 Determination of imidacloprid metabolites (imidacloprid guanidine) by Nitrite (NO_2^-) detection test:

Azospirillum Spp , Azotobacter, Pseudomonas spp. & *Bacillus spp*. have shown red colour after addition of 3-4 drops of both the reagents namely Sulfanilic acid and alpha Naphthylamine. Red colour was not detected in any of the control. Thus, the formation of red colour indicates that nitrite is present in the broth inoculated with imidacloprid and microorganism. This indicates that these microbial species may be responsible for cleaving off the nitrite group on the end of the imidazolidin ring of imidacloprid to produce its metabolite imidacloprid guanidine. Research work of Jennifer C. et al (2007) have found concomitant production of nitrite as a metabolite of imidacloprid degradation by analytical procedure (cadmium reduction of nitrate to nitrite with colorimetric detection).

Table 1. Physicochemical Parameters of soils

Sample	Soil characteristics					
	Pesicide application	Colour	% Moisture	pH	Organic carbon %	Organic nitrogen %
Soil	History of 2yrs	Black	0.62	7.0	0.3574	0.3167

Table 2. Degradation of Glyphosate at different concentration by cultures

	Concentration in mg $^{L-1}$						
Organisms	50	100	125	200	250	500	1000
Azospirillum spp.	+	+	+	+	+	+	-
Azotobacter spp.	+	+	+	+	-	-	-
Pseudomonas spp.	+	+	+	+	-	-	-
Bacillus spp.	+	+	+	+	-	-	-

+ = Growth - = No growth

4. CONCLUSION

Microorganisms isolated from contaminated soil were able to grow on media containing imidacloprid & degrade it by using imidacloprid as a sole source of carbon and energy. The highest pesticide degradation was found by *Azospirillum spp* up to 500 mgL^{-1} and *Bacillus spp.*, *Pseudomonas species* and *Azotobacter species* were upto 200 $mgL^{-1.}$ Presence of NO_2^- was the indication of the imidacloprid degradation into its metabolite imidacloprid guanidine. The application of these microorganisms or microbial consortium can improvise the bioremediation over conventional technique such as land rolling or incineration. Thus, these microorganisms can be used to remediate soil contaminated with pesticides.

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