Full Length Research Article

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Research Journal of Agricultural Sciences
Volume 15; Issue 01 (Jan–Feb 2024); pp 201–204

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Understanding the Imidacloprid Biodegradation Network: Bacterial Isolation and Identification in Farming Soils

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Received: 29 Nov 2023; Revised accepted: 15 Jan 2024; Published online: 03 Feb 2024

Abstract

Chemicals used as pesticides have the intention of killing pests. Pesticides undoubtedly enhance agricultural yields and protect crops from pest infestations, but their toxicity poses serious risks to human health, the environment, and the larger ecosystem. An effective chloronicotinyl insecticide for controlling sucking and biting insects is imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), which is highly persistent in soil. Because of its long-term durability and toxicity, this pollution represents a serious hazard to the ecosystem. Microbiological degradation presents a viable approach for the restoration of habitats contaminated with imidacloprid. Our goals were to find, separate, and describe microorganisms in soil that might break down imidacloprid. In order to better understand natural remediation processes and maybe use these bacteria for environmentally friendly and sustainable agricultural operations, we isolated and identified Imidacloprid-degrading bacteria from agricultural soil in this work. Enrichment cultures were used to isolate bacteria from soil samples taken from agricultural fields that had previously been treated with Imidacloprid. Our results show that the soil analyzed included a variety of bacterial communities that were able to break down imidacloprid. This work has identified several bacterial genera. It was discovered that Mycobacterium, Pseudomonas, and Bacillus are powerful agents that degrade imidacloprid. Due to its inherent eco-friendliness, affordability, and effectiveness in detoxifying pesticide-contaminated ecosystems, bioremediation appears as a tempting method.

***Key words*:** Imidacloprid, Biodegradation, Mycobacterium, Pseudomonas, Bacillus

Since pests destroy 30% of agricultural products, the use of pesticides in agriculture has grown significant. India is the largest consumer of pesticide [12]. Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imi-dazolidinimine) is a neonicotinoid insecticide that is frequently used as systemic pesticides to manage many species of sucking insects, termites, and other eating pests [5], [7-8], [13]. Imidacloprid, a popular insecticide from the neonicotinoid class, is well-known for its ability to effectively control a variety of pests. Since its initial introduction in the 1990s, its systemic nature and efficacy against a wide range of insects have made it a popular choice in horticulture, household pest management, and agricultural activities. By attacking particular receptors in insects' nervous systems, this pesticide disrupts their nervous systems and eventually causes paralysis and death. It functions as a neurotoxic. Because of its systemic nature, which enables it to be absorbed by plant tissues, it provides prolonged defence against pests that feed on treated plants, including termites, beetles, and aphids. Imidacloprid has been under investigation because of possible environmental effects, especially as it has been linked to a decrease in bee numbers and other non-target organisms, despite its efficacy. Imidacloprid is a polar molecule having a half-life of roughly 156 days. It is highly soluble in water, comparatively non-volatile, and persistent in soil [4]. Imidacloprid is frequently sprayed over fields to protect crops, which releases the pesticide into the environment. It has gained attention as an effective pesticide due to its physical characteristics and high insecticidal action at low application rates. Public concerns are raised about the fate of imidacloprid and it's by products in the environment. Research has indicated that imidacloprid functions similarly to nicotine, which paralyses and kills insects by acting as an agonist on postsynaptic nicotinic acetylcholine receptors (nAChR) [3], [5-6]. Biodegradation is the most widely employed process for turning synthetic chemicals into inorganic compounds. Generally speaking, pesticide degradation in soil is aided by both biotic and abiotic elements, such as chemical, UV, and microbial agents [1-2]. Due to the problems of pesticide contamination, for clean-up of pesticide contaminated soil, develop technologies that guarantee their elimination. Currently bioremediation is one of the most environmentally safe and cost-effective method for decontamination and detoxification of pesticide contaminated environment. The isolation of indigenous bacteria capable of metabolizing certain pesticides has received a lot of attention and is seen as an efficient method for pesticide bioremediation [10]. The present investigation was aimed to isolate and identify Imidacloprid degrading bacteria from soil samples in Samastipur district, Bihar (India).

MATERIALS AND METHODS

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Citation: Kumari A, Deokant. 2024. Understanding the imidacloprid biodegradation network: bacterial isolation and identification in farming soils. *Res. Jr. Agril. Sci.* 15(1): 201-204.

Collection of soil sample

The agricultural areas located in Bihar's Samastipur district, with coordinates of 25.712219 latitude and 85.804864 longitude, were the site of the soil sampling. The fact that local farmers frequently use imidacloprid as a pesticide on their farmed crops makes these fields especially interesting. The procedure for gathering soil samples was executed with great care, guaranteeing the precision and dependability of the findings by collecting each sample twice. Following the collection of soil samples from different parts of the district, they were carefully put into sealed containers usually jars to protect their integrity while being transported to the lab for further testing and analysis.

Preparation of chemical and media

Imidacloprid insecticide was dissolved in acetonitrile to create a stock solution. To guarantee the purity of this solution, all contaminants were carefully filtered out and sterilized. After sterilization, the Imidacloprid stock solution was carefully refrigerated to maintain its integrity for later usage. Bushnell agar, a specialized growing medium, was used in the isolation and culture of bacterial strains that could break down Imidacloprid. In order to provide the best circumstances for bacterial development, this medium was brought to a neutral pH of 7.0. The method of carefully and precisely isolating and growing Imidacloprid-degrading bacterial strains was done using this pH-adjusted Bushnell agar.

Analysis of physico-chemical parameter

Samples of the initial soil that were typical were taken, dried by air, and crushed to a thickness of one millimetre, as per chemical analysis. The gravimetric soil moisture content was ascertained by drying a 10g sample of soil at 105 °C for a whole night [11]. To display the moisture content, a percentage (%) was employed. The sample was tested for pH (soil: deionized water=1:2.5 w/v) using a glass electrode pH metre and an electrical conductivity metre (pH HI1131). To evaluate the phosphate, magnesium, and chloride, standard protocols were followed [9].

Isolation of imidacloprid degrading bacteria

Imidacloprid-degrading bacteria were isolated using minimal media containing pesticide as a carbon source. Using the serial dilution approach, the bacterial species was isolated from soil contaminated with imidacloprid (Fig 1). In Bushnell Haas agar media, many bacterial colonies were seen. Biochemical testing and Gram's staining were used to identify these colonies. As a selective enrichment agent for bacterial isolation, pesticides were added to Bushnell Haas Medium at varying concentrations of 0.1%, 0.5%, and 1%.

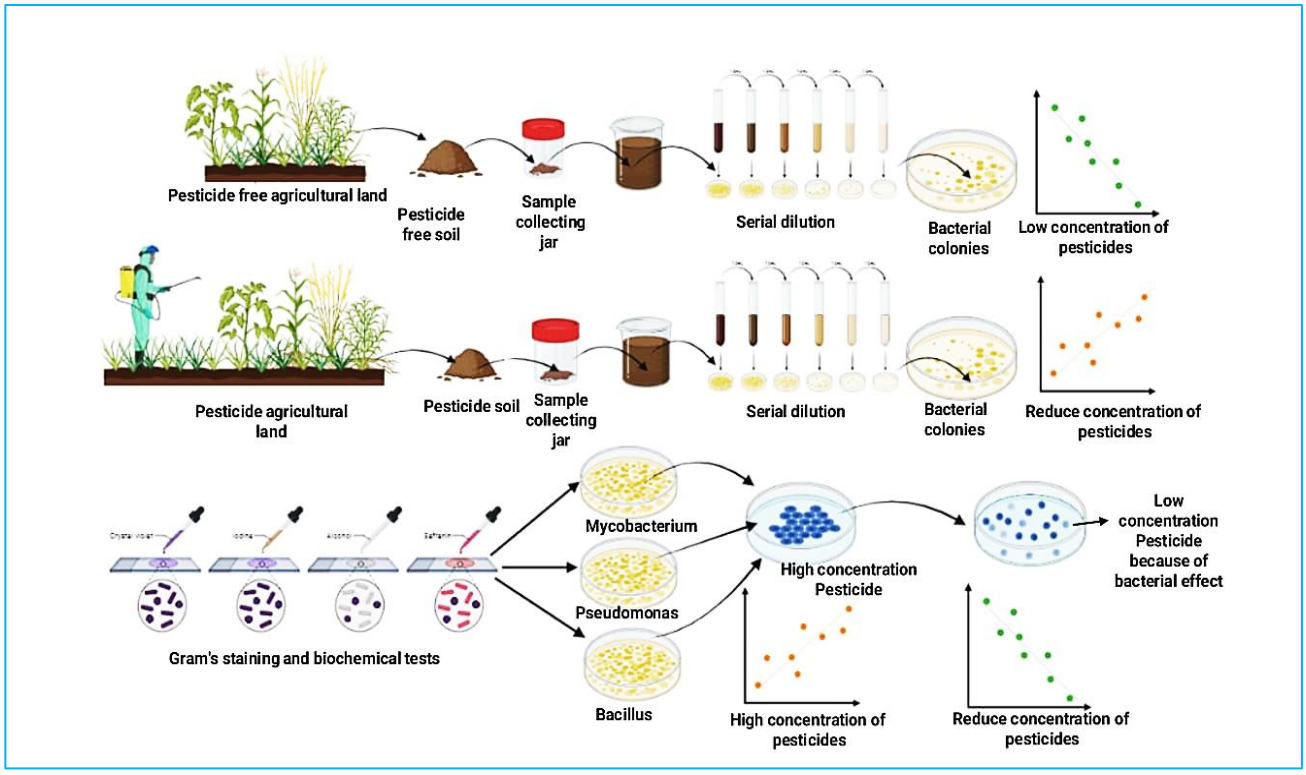

Fig 1 Experimental workflow: Isolation and degradation of imidacloprid by soil bacteria

RESULTS AND DISCUSSION

Physico-chemical characteristics of soil sample analysis

The soil sample that is being studied has a number of physicochemical characteristics that have been carefully measured and documented. These traits offer insightful information on the makeup and state of the soil. The soil sample's pH value is recorded as 7.5. This pH value, which represents the acidity or alkalinity of the soil, is a critical component affecting the availability of nutrients and microbial activity in the soil. The soil's temperature is measured to be 28 degrees Celsius (28°C). The soil ecosystem's biological activity, nutrient cycling, and plant growth are all significantly impacted by soil temperature. The soil has a comparatively high moisture content of 85%. Water is essential for supporting plant life and other soil organisms, and this measurement shows how much of it is in the soil. At 31.5 milligrams per unit of measurement, the phosphate content of the soil sample is high. A measure of the fertility of the soil, phosphate is a nutrient that is necessary for plant growth. It is discovered that there are 12.0 milligrams of magnesium per unit in the soil. Magnesium is another essential mineral for plants that is involved in a number of soil metabolism activities. A slight measurement uncertainty of ±0.6 milligrams is associated with the 11.5 milligrams of chloride per unit found in the soil. Chloride concentrations are important for determining the salinity of the soil and can affect

plant health. (Table 1) provides an extensive summary of the qualities of the soil sample by summarizing these physicochemical features. Making judgements about soil management and improvement techniques and determining if the soil is suitable for certain agricultural or environmental uses require knowledge of this information.

Table 1 Physicochemical characterization of soil sample

Parameters	Values of soil samples
pH	7.5
Temperature °C	28 °C
Moisture content	85%
Phosphate(mg)	31.5±0.0mg
Magnesium(mg)	12.0±0.5mg
Chloride(mg)	11.5±0.6mg

*Values are Mean ± standard deviation

Isolation of imidacloprid degrading bacteria

Soil samples taken from agricultural area contaminated with pesticides were used to identify bacterial species. A serial dilution procedure was used to isolate these bacteria, which entailed dilution of the soil sample several times to acquire individual bacterial colonies. The isolated bacterial colonies were then cultured on Bushnell Haas agar medium, where they became separate colonies. Then, in order to identify them, these colonies underwent a number of scientific tests. The first step involved applying Gram's staining, a method that divides bacteria into two groups: Gram-positive and Gram-negative, according to the properties of their cell walls. Different species of bacteria were made easier to distinguish using this staining method. The bacteria underwent a battery of biochemical tests after being stained with Gram's solution. Several experiments were conducted as part of these testing in order to identify particular metabolic traits and chemical processes that the bacteria displayed. Using particular nutrients, producing particular enzymes, or fermenting sugars are a few examples of these reactions. Our accurate identification and classification of the bacterial species found in the pesticide-contaminated soil was achieved by the analysis of the test findings.

Identification and growth conditions of isolates

In this work, we investigated in detail the unique characteristics linked to the development of isolated microorganisms. We used a technique that measured light absorbance at a wavelength of 600 nanometers (nm) to achieve this. In microbiology, this specific wavelength is frequently used to evaluate the density and development of bacterial cultures. In this investigation, we concentrated on the bacteria Bacillus, Pseudomonas, and Mycobacterium. We measured the absorbance values at 600 nm for each of these organisms to evaluate their growth characteristics. The results revealed unique absorbance values corresponding to every microbe, as shown in (Table 2). At 600 nm, the absorbance value for Mycobacterium was found to be 0.54, demonstrating the extent of its proliferation in the specified conditions. By comparison, Bacillus has the highest absorbance value (1.26 at 600 nm) out of the three bacteria. This much higher absorbance value suggests that, out of all the isolated organisms, Bacillus grew the most prominently, indicating a healthy and flourishing population in the experimental setting. We were able to discern between the several growth stages that the isolated microorganisms in our investigation displayed thanks to the absorbance measurements at 600 nm, which gave us important insights into the growth features of Mycobacterium, Pseudomonas, and Bacillus.

Table 2 Analysis of Imidacloprid degrading organisms

Organisms	OD value at 600nm
Mycobacterium	0.54±0.35
Pseudomonas	0.61±0.30
Bacillus	1.26± 0.5

*Values are Mean ± standard deviation

Imidacloprid utilizing bacteria

The results of the degradation of Imidacloprid by Mycobacterium, Pseudomonas, and Bacillus are presented in detail. The greatest zones of accumulation for Mycobacterium were noted at various Imidacloprid concentrations. In particular, the greatest zones of accumulation were detected at 2.19±0.40, 1.95±0.3, and 0.78±0.20 at concentrations of 0.1%, 0.5%, and 1.0%, respectively (Table 3).

Table 3 Total bacterial population and degradation of Imidacloprid at concentrations of (0.01 and 0.1%) in contaminated soil

S. No.	Organisms	Dilution	Total viable counts (CFU/g)	Imidacloprid resistance bacterial counts (CFU/g) in different Imidacloprid concentration (%)		
				0.1%	0.5%	1.0%
1	Mycobacterium	10^{-4}	5.80±0.30	3.53±0.40	2.55±0.3	1.10±0.20
		10^{-5}	4.36±0.50	2.53±1.40	2.35±0.2	0.80±0.10
		10^{-6}	3.96±0.45	1.40±0.30	1.35±0.6	0.66±0.57
		10^{-7}	3.43±0.45	1.31±0.20	1.39±0.03	0.57±0.57
2	Pseudomonas	10^{-4}	4.36±0.28	3.45±0.35	3.50±0.8	1.09±0.15
		10^{-5}	4.36±0.40	2.43±0.35	2.48±1.9	0.60±0.03
		10^{-6}	3.82±0.35	1.20±0.20	2.39±0.9	0.58±0.002
		10^{-7}	3.25±0.25	1.30±0.30	1.20±0.02	0.40±0.05
3	Bacillus	10^{-4}	5.60±0.40	3.33±0.30	3.53±0.30	1.08±0.10
		10^{-5}	4.60±0.30	2.53±0.40	2.44±0.40	0.60±0.08
		10^{-6}	3.95±0.50	1.30±0.30	1.30±0.30	0.50±0.05
		10^{-7}	3.68±0.45	1.21±0.20	1.20±0.20	0.40±0.57

*Values are Mean ±standard deviation

These numbers represent the maximum rates at which Mycobacterium degraded imidacloprid at each concentration. Similar concentration-dependent patterns were noted for Pseudomonas. Maximum zones of accumulation were measured at 2.09±0.3, 2.14±0.8, and 0.67±0.14 concentrations of imidacloprid, and at 0.1%, 0.5%, and 1.0% concentrations too. The maximum Imidacloprid degradation levels that Pseudomonas was able to achieve under the given circumstances are indicated by these numbers. Similar to Bacillus, different Imidacloprid concentrations were found to result in the greatest accumulation zones. (Table 3) shows that Bacillus showed maximal zones of accumulation measuring

2.06±0.3, 2.05±0.30, and 1.08±0.10 at concentrations of 0.1%, 0.5%, and 1.0%. At the relevant concentrations, these statistics represent the maximum levels of imidacloprid degradation that Bacillus was able to attain. The breakdown capacities of Mycobacterium, Pseudomonas, and Bacillus in response to varying Imidacloprid concentrations are comprehensively outlined in (Table 3). Their efficacy in environmental remediation initiatives is illuminated by our results, which highlight the concentration-dependent patterns of imidacloprid breakdown displayed by these microorganisms.

Imidacloprid is a neonicotinoid pesticide that is often used and well-known for both its effectiveness in managing pests and its possible negative effects on the environment. One of the most important processes in reducing imidacloprid's toxicity and persistence is its environmental degradation. Identifying and investigating Imidacloprid-degrading bacteria has emerged as a critical part of environmental remediation in order to address this issue. Imidacloprid degradation is a complex process and microbes, particularly bacteria, play a critical role in breaking down this toxic chemical. Understanding imidacloprid-degrading bacterium identification is critical for various reasons. Identifying these bacteria (Mycobacterium, Pseudomonas and Bacillus), first and foremost, can lead to the development of bioremediation techniques. Bioremediation, which uses microorganism's metabolic ability to detoxify contaminants, provides an environmentally acceptable and cost-effective solution to imidacloprid pollution.

CONCLUSION

Mycobacterium, Pseudomonas and Bacillus were isolated from farmland soil sample collected from Samastipur district, Bihar (India). It can utilize Imidacloprid as sole source of carbon, nitrogen and energy. Results of this study showed that Imidacloprid-degrading bacteria are widely distributed across various regions of the farmlands in Samastipur district, Bihar. In conclusion, our results indicated that Mycobacterium, Pseudomonas, and Bacillus could be a good choice for the bioremediation of Imadicloprid contaminated soil.

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