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Research Article

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Impact of Imidacloprid Intoxication on Amylase and Protease Activity in Soil Isolate *Escherichia coli*

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ABSTRACT

Imidacloprid, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, is a neonicotinoid. The present investigation was under taken to evaluate the effect of imidacloprid treatment on amylase and protease activity in soil isolate Escherichia coli (SP-02). The soil isolate Escherichia coli were isolated after enrichment cultures, as imidacloprid tolerant bacteria. The isolate was exposed to imidacloprid of varying concentrations ranging from 10^{-7} M to 10^{-3} M for a period of 96 hrs. Treatment with higher dose (10^{-4} and 10^{-3} M) of imidacloprid resulted into no significant increase in the activity of amylase and protease enzymes whereas, significant increase was observed in the lower dose (10^{-7}, 10^{-6} and 10^{-5} M) of imidacloprid. Present investigation revealed that imidacloprid intoxication suppresses amylase and protease at higher doses of imidacloprid. Reduction in dosage of imdacloprid resulted in increase in the amylase and protease activity. The result indicates that imidacloprid toxicity results in reduced activity of essential metabolic enzymes thus effecting the growth of the Escherichia coli.

Keywords: *Escherichia coli*; Imidacloprid amylase; Protease

INTRODUCTION

The excessive use of pesticides in modern agriculture has leads to an accumulation of a large amount of pesticide residues in the environment. The accumulation of residues leads to substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds in the food chain and drinking water [1]. Assessing the side effects of pesticides on microbial populations is important to maintain soil fertility and to prevent critical damage to the agricultural ecosystems [2]. Microbial parameters, such as microbial population, biomass, activity and community structure, are affected by natural stresses and fluctuate in the environment; the side-effects caused by the pesticides should be evaluated by comparing them with those caused by natural stresses [3]. Amylase is a starch hydrolysing enzyme. It is known to be constituted by α-amylase and β-amylase. Studies have shown that α -amylases are synthesized by plants, animals and micro-organisms, whereas, β-amylase is mainly synthesized by plants. The bacterial amylase expressed in *Escherichia coli* is secreted in the periplasmic space by a cold osmotic shock or stress. The main role of this enzyme is the starch metabolisms in the extra cellular medium, therefore, lot of microorganisms depend on amylases for their survival [4].

Proteases are a group of enzymes that belong to one of the four major classes of proteolytic enzymes and are generated by a variety of organisms including viruses, bacteria, protozoa, yeasts, plants, helminthes, insects and mammals. This is an extracellular enzyme secreted by soil microorganisms, including bacteria and fungi widely available, where the protein rich effluents dislodge into the soil increases proteolytic activity in the due to the presence of high organic wastes (amino acids) discharged in the effluents [5].

MATERIALS AND METHODS

Preparation of Stock Solution of Imidacloprid

The stock solution of one molar imidacloprid was prepared and further diluted to give 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} molar concentrations [6]. Soil isolate was isolated and identified from soil as described in our previous publication [7]. The bacterium was maintained at 4°C on nutrient agar [8] and sub cultured every fortnight. The medium used for toxicity testing was an optimized medium (dextrose - 0.65 g/l: Yeast extract - 1.05 g/l; K HPO - 0.30 g/l; NaCl - 0.25 g/l).

Preparation of Inoculum

Pre-inoculum was prepared by inoculating a loop full of bacteria from the overnight incubated nutrient agar slant cultures on a 100 ml sterilized optimized growth medium and incubated for 24 hours at 37°C under static conditions.

Identification of Bacterial Isolate

Imidacloprid tolerant colonies were isolated and identified morphologically, cultural and biochemical characterization and 16S rDNA identification was done as described earlier [7]. The pure culture was grown on nutrient agar medium.

Experimental Procedures

Five ml of the pre-inoculum was inoculated to 250 ml Erlenmeyer's flask containing 100 ml of sterilized optimized growth medium amended with different molar concentrations of imidacloprid. The flasks were incubated at 37°C for 96 hours under shaking conditions at 120 rpm on a rotary shaker. At regular intervals, sample was taken out from each flask aseptically for analysis.

Extraction of Enzymes

The cells were centrifuged at 8,000 rpm for 3 min and the pellet was dissolved in 0.2 ml of lysis buffer (50 mM tris-cl and 10 mM lysozyme). The tubes were incubated at 37°C and centrifuged at 10,000 rpm for 10 min, each supernatant was used as the source of enzyme.

Estimation of Amylase

Amylase enzyme activity was assayed using the standard method [6]. 2 ml of the sample or an aliquot diluted to 2 ml was incubated with 2 ml of phosphate buffer pH 7.0 and 2 ml of 1 per cent soluble starch at 37°C for 15 minutes. 2 ml of DNS reagent was added and the reaction mixture was boiled for ten minutes. The OD was measured at 540 nm against a blank. The amount of reducing disaccharide released by the enzyme was calculated by referring to a standard graph of maltose. The unit of amylase activity is µ moles per ml per min of the sample.

Estimation of Protease Activity

The protease activity of the respective samples was measured as per the procedure of [7]. Two ml of the sample was incubated with phosphate buffer pH 7.6 and 1 per cent casein for two hours at 37°C the reaction was stopped by the addition of 3 ml of 10 per cent TCA solution and filtered through Whatman No 42 filter paper. 2 ml of the filtrate was mixed with 3 ml of 0.5 N NaOH and 0.5 ml of folin phenol reagent. The optical density was estimated against an appropriate blank with a spectrophotometer at 660 nm. The unit of protease activity is calculated as micrograms of tryptophan released per minute per ml of the sample.

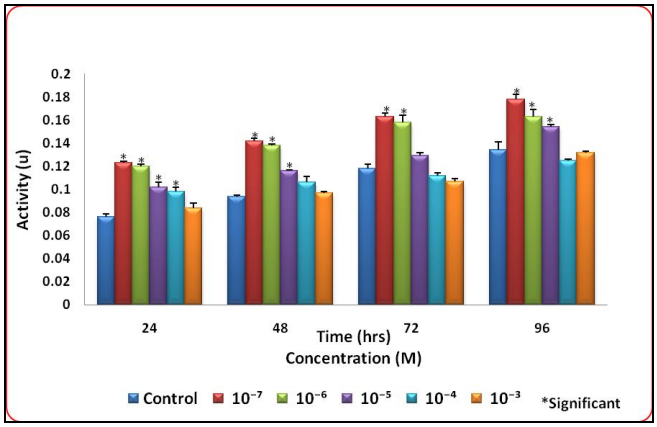
Statistical Analysis

Statistic significance between the control and experimental data were subjected to analysis of variance (ANOVA) followed by post-hoc dunnet's test ($Pd<0.05$).

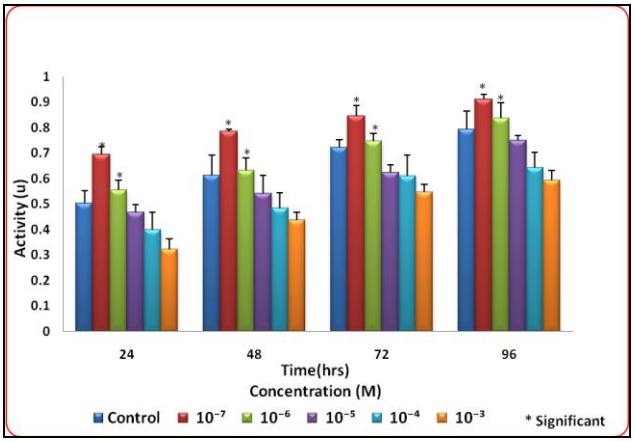
RESULTS

The imidacloprid tolerant soil isolate was grown in nutrient broth containing 10^{-3} molar imidacloprid and incubated for seven days and plated on medium containing 10^{-3} molar imidacloprid colony isolated was named as SP-02. The isolated strain was a rod-shaped, gram negative, bacterium and further it was identified as *Escherichia coli*. The toxic effect of imidacloprid on amylase and protease activity in soil *Escherichia coli* was studied using broth containing 10^{-3} to 10^{-7} Molar imidacloprid.

The present study revealed that the amylase and protease activity in the treated groups increased significantly in lower dose (10^{-6} and 10^{-7} M) of exposure to imidacloprid, whereas, no significant increase was observed in the higher dose (10^{-5}, 10^{-4} and 10^{-3}M) of imidacloprid when compared with that of the control (Graphs 1 and 2).



Graph 1: Effect of imidacloprid on amylase activity in *Escherichia coli*



Graph 2: Effect of imidacloprid on protease activity in *Escherichia coli*

DISCUSSION

Effect of Imidacloprid on Amylase Activity in Soil Isolates *Escherichia coli*

Amylase is a starch hydrolyzing enzyme It is known to be constituted by α-amylase and β-amylase. β -amylase converts starch like substrates to glucose and/or oligosaccharides and α - amylase, converts starch to maltose. Studies have shown that α -amylases are synthesized by plants, animals and micro-organisms, whereas, β -amylase is mainly synthesized by plants [4]. The present study revealed that the amylase activity in the treated groups increased significantly in lower dose (10^{-6} and 10^{-7} M) of exposure to imidacloprid, whereas, no significant increase was observed in the higher dose (10^{-3}, 10^{-4} and 10^{-5} M) of imidacloprid in *Escherichia coli,* cells. This indicates that the insecticide inhibits amylase activity at higher concentration. Increase in amylase activity was reported on exposure of cypermethrin and monocrotophus to free and immobilized *Escherichia coli* [6]. ROS are generated by many environmental pollutants leading their toxic effects. Many microorganisms depend on amylases for survival [9]. Some cells show increased catabolism to meet the energy demand under stress induced by the xenobiotics like pesticides [10]. Drastic reduction in amylase production was recorded at a concentration of 15 mg/L during the logarithmic phase where a reduction to 50% was observed. When compared with earlier observations on amylolytic microorganisms, the toxic effects on enzyme production seem to be very pronounced [11,12].

Effect of Imidacloprid on Proteases Activity in Soil Isolates *Escherichia coli*

Proteases are a group of enzymes that belong to one of the major classes of proteolytic enzymes and are generated by a variety of organisms, including viruses, bacteria, protozoa, yeasts, plants, helminthes, insects and mammals [13,14]. Proteases participating in the protein metabolism either by degradative or biosynthetic pathways release

hormones and pharmacologically active peptides from precursor proteins [15]. The present study revealed that the protease activity in the treated groups increased significantly in lower dose (10^{-6} and 10^{-7} M) of exposure to imidacloprid, whereas, no significant increase was observed in the higher dose (10^{-3}, 10^{-4} and 10^{-5} M) of imidacloprid in *Escherichia coli*, this indicates that the insecticide inhibits protease activity at higher concentration. It is also reported that protease is essential for *Escherichia coli* to survive at elevated temperatures, and degrades abnormal and misfolded proteins [16,17]. In a study protease activity in cultivated soil of Rajkot region of Gujarat pesticides viz. chlorpyrifos (an organophosphate) and endosulfan (an organochlorine cyclodiene) inhibited by 25% after one day of treatment [18].

Proteases are of vital importance to all bacteria as they are involved in bacterial resistance against xenobiotics [19]. The increase in the protease activity at lower doses could be due to the expression of intracellular proteins [20]. Proteases are a widespread group of enzymes that catalyze the hydrolysis of different proteins and degradation and turnover of intracellular proteins [5]. Extracts of *Escherichia coli* have been shown to degrade the damaged enzyme, but not the native protein, and several preliminary reports suggest that the *Escherichia coli* protease that may be responsible for selective degradation of the modified proteins.

The significant decrease in the protease activity of *Escherichia coli*, cells on dose and durational exposure of imidacloprid observed in the present study may be due to expression of intracellular proteins, which require cell lysis for purification which will result in exposure to proteases, hydrolysis of different proteins that perform a pivotal role in the degradation and turnover of intracellular proteins, homeostasis maintenance and metabolism regulation in the cell, protect the cells against effects of toxic peptides, selective degradation of the modified proteins.

CONCLUSION

In the present study the increase in the amylase activity in lower doses of imidacloprid observed may be due to the toxic effects of the pollutant, by generating ROS, compensate metabolism for their survival, increased catabolism to meet the energy demand under stress induced by the pesticide, an adaptive responses induced under oxidative stress and thereby overcome stress. The significant decrease in the protease activity of *Escherichia coli*, cells on dose and durational exposure of imidacloprid observed in the present study may be due to expression of intracellular proteins, which require cell lysis for purification which will result in exposure to proteases, hydrolysis of different proteins that perform a pivotal role in the degradation and turnover of intracellular proteins, homeostasis maintenance and metabolism regulation in the cell, protect the cells against effects of toxic peptides, selective degradation of the modified proteins. The study revealed that imidacloprid effects amylase and protease activity in bacterial isolate *E. coli* and intern it effects the growth and development of the bacteria.

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