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RESEARCH ARTICLE

INFLUENCE OF IMIDACLOPRID ON BIOCHEMICAL PARAMETERS IN SOIL ISOLATE *BACILLUS WEIHENSTEPHANENSIS*

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ABSTRACT

Imidacloprid (I-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), a chloronicotinyl insecticide is used widely to control biting and sucking insects. Present investigation was carried out to analyse the effect of imidacloprid on biochemical parameters like DNA, RNA, protein and glucose in soil isolate *Bacillus weihenstephanensis.* The study involving soil isolate *Bacillus weihenstephanensis* with molar concentrations of 10^{-3} to 10^{-7} of imidacloprid insecticide showed that there was an increase in the percent inhibition of DNA, RNA, protein and glucose, the inhibitory effect increased with an increase in the concentration of insecticide proving that the inhibitory effect is dose dependent. The present investigation indicates that imidacloprid reduced the DNA RNA, glucose and protein content which intern effects the growth of the *Bacillus weihenstephanensis*.

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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 (Mohammed, 2009). Assessing the side effects of pesticides on microbial populations is important to maintain soil fertility and to prevent critical damage to the agricultural ecosystems (Francis *et al*., 1987). 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 (Domsch *et al*., 1983; Itoh *et al*., 2003). Pesticides are known to affect the metabolism of an organism by imbalancing the homeostasis and causing impairment in the levels of biochemical contents (Ksheersagar and Kaliwal, 2003). DNA is the genetic material and carries necessary biological information's. It controls the regulation of cell metabolism and expression of the characters within the organism. RNA is a nucleic acid synthesized in the nucleus and mainly found in the cytoplasm to carry out the protein synthesis. RNA plays a vital role in protein synthesis. The reactive oxygen species targets the DNA, RNA and proteins. The DNA binding proteins produced during the nutritional stress and oxidative stress protects the DNA from physical oxidative damage and also it maintains low level of gene expression. The micro-organisms resistance against the damaging effects of xenobiotics is due to genetic response against stress conditions (Cabiscol *et al*., 2000). Therefore, the present investigation was carried out to study the effect of imidacloprid ranging from 10-7 to 10–3 Molar concentrations for a period of 24, 48, 72 and 96 hrs on biochemical parameters and growth in soil isolate *Bacillus weihenstephanensis*.

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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. Soil isolate was isolated from soil as described in the previous publication The bacterium was maintained at 4°C on nutrient agar 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 inoculums: 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: The pure culture was grown on nutrient agar medium. Colonies were characterized by morphological, cultural and biochemical characters and 16S rRNA identification.

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.

Isolation and estimation of nucleic acids: Perchloric acid (0.5 N, 4 ml) was added to the pellet of 10 ml culture and the mixture was allowed to stand in water bath at 70°C for 15 min with occasional shaking and centrifuged at 3,000 rpm for 15 min. The extraction was repeated twice with 0.5 N Perchloric acid (3 ml) each for 15 min. and the extracts were combined and made up to 10 ml with 0.5 N Perchloric acid. From this extract DNA and RNA were determined by diphenylamine and orcinol methods respectively

Protein estimation and Estimation of glucose utilization: Cell pellet from 10 ml of the culture was mixed with 2 ml of 0.5 N NaOH and boiled over a water bath for 5 min and cooled. It was centrifuged at 3000 rpm for 5 min and the supernatant was used for the estimation of protein. The glucose content was estimated by Anthrone method

Statistical analysis: Statistic significance between the control and experimental data were subjected to analysis of variance (ANOVA) followed by post –hoc dunnet's test ($P \leq 0.05$).

RESULTS

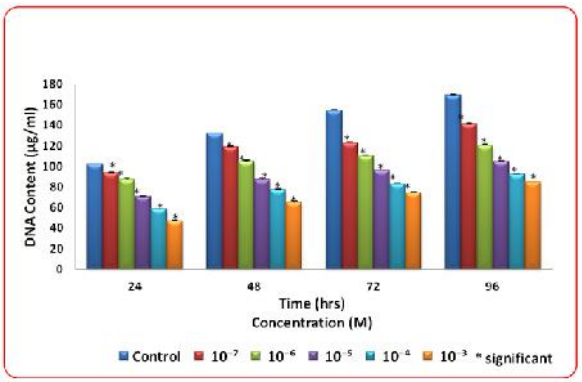
The imidacloprid tolerant soil isolate was grown in nutrient broth containing 10-3 molar imidacloprid and incubated for seven days and plated on medium containing 10-3 molar imidacloprid single colony was isolated and named as SP-02. The isolated strain was a rod-shaped, gram positive, bacterium. By sequencing the 16S rRNA gene and comparing them with previously published 16S rRNA gene sequences, the strain was classified as a member of the genus *Bacillus* Based on nucleotide homology and phylogenetic analysis, the culture SP-03was identified as *Bacillus weihenstephanensis*. Further, the toxic effect of imidacloprid on biochemical parameters (DNA, RNA, protein and glucose) and on growth in soil isolate *Bacillus weihenstephanensis* was studied using broth containing 10^{-3}, 10^{-4} 10^{-5} 10^{-6} and 10^{-7} molar imidacloprid. On exposure of *Escherichia coli* to various molar concentrations (10^{-3} to 10^{-7}) of imidacloprid for 24, 48, 72 and 96 hrs there was a significant ($P \leq 0.05$) decrease in the concentration of all the biochemical parameters studied. There was a significant decrease ($P \leq 0.05$) in the level of DNA (Graph1.), RNA (Graph 2.), protein (Graph 3.) and glucose (Graph 4.) content in all the treated groups. However, increase in the % inhibition was observed with an increase in dose and durational exposure to imidacloprid.

DISCUSSION

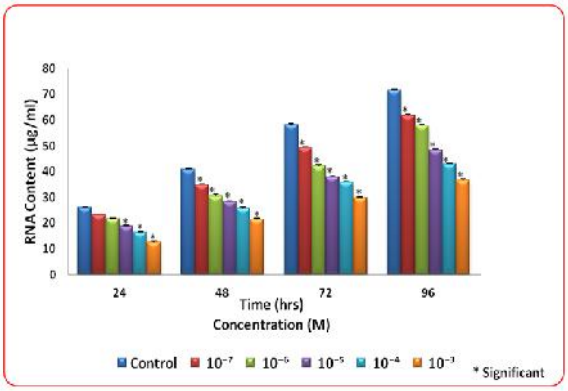
In the present study the reason for significant ($P \leq 0.05$) increase in the percent inhibition in DNA and RNA observed in soil isolates under the influence of imidacloprid might have caused genotoxic action by disturbed cell growth and division. The pesticides are known to affect growth by inhibiting the DNA and RNA synthesis by blocking the binding of polymerase to the DNA template (Sangeeta *et al.,* 1997). Imidacloprid is known to induce free radical generation that leads to protein degradation and DNA damage (Kumar *et al.,* 2010).The damage of membrane lipids, proteins and DNA is the endpoint biomarkers of oxidative stress- induced effects of pesticides (Twzmen *et al.,* 2008). ROS causes DNA damage in the form of modification of all bases, and leads to production of base-free sites, deletions, frame shifts, DNA cross-links, and chromosomal rearrangements (Gultekin *et al.,* 2000). Oxidative stress results in genetic responses in bacteria, yeast and mammalian cell line and in general in all aerobic organisms (Farr and Kogoma, 1991; Hidalgo and Demple, 1995). ROS known to induce lipoperoxidation that results in changes in membrane permeability, which leads to protein impairment, enzyme inactivation and at the end leads to DNA damage (Sreelaxmi and Kaliwal, 2007).

Further an significant ($P<0.05$) increase in the percent inhibition in protein and glucose contents in treated groups observed in the present study may be due to the fact that the major protein modification is observed due to stress and the loss of catalytic activity, amino acid modification, carbonyl group formation, increase in acidity, decrease in thermal stability, change in viscosity, fluorescence, fragmentation, formation of protein- protein crosslink's, s-s bridges and increased susceptibility to proteolysis (Stadtman, 1992). It has been reported that the biological targets for the reactive oxygen species due to oxidative stress are RNA, DNA, proteins and lipids (Cabiscol *et al*., 2000). Imidacloprid might have affected protein synthesis. The increase in percent inhibition of protein with increase in dose and duration of exposure of imidacloprid in cells may be due to the inhibitory action of imidacloprid on the enzymes and protein (Cabiscol *et al*., 2000). Similar results were reported in *Escherichia coli* and *Pseudomonas aeruginosa* exposed to various concentration of methomyl (Kulkarni and Kaliwal, 2010).

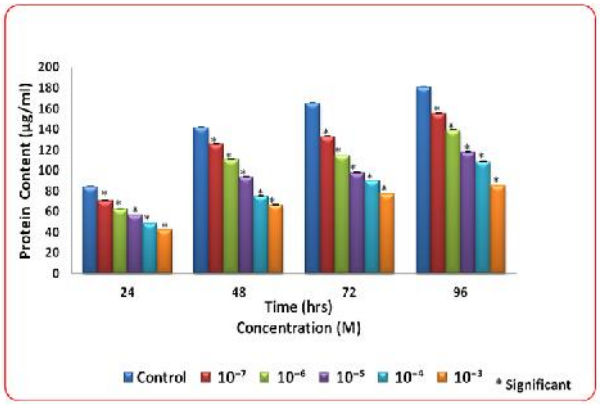
A pesticide like methomyl exposure is reported to promote the oxidative damage of cells by enhanced membrane lipid peroxidation (Benjamin and McMillan, 1996). It has also been proven that the most frequent combination of adverse factors, especially for aerobic or facultatively anaerobic microorganisms such as *Escherichia coli or Brevundimonas*, is the combination of glucose starvation and oxidative stress (Salakhetdinova, 2000). ROS are unstable free radical species in cells produced when oxidative stress occurs. At the cellular level, proteins exposure to ROS leads to modifications of amino acid side chains and, consequently, the protein structure is altered. These modifications lead to functional changes in protein that alter cellular metabolism (Stadtman, 1992).



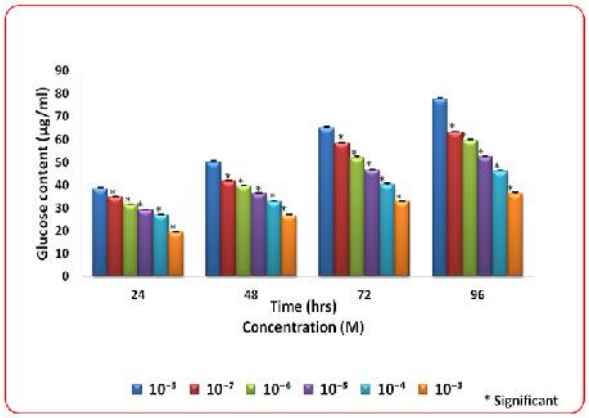
Graph 1. Effect of imidacloprid on DNA content in *Bacillus weihenstephanensis*



Graph 2. Effect of imidacloprid on RNA content in *Bacillus weihenstephanensis*



Graph 3. Effect of imidacloprid on protein content in *Bacillus weihenstephanensis*



Graph 4. Effect of imidacloprid on glucose of *Bacillus weihenstephanensis*

Reactive oxygen species also known to attack DNA, resulting in chain breaks, modification of the carbohydrate subunits and nitro bases, this may lead to point mutation (Halliwell and Gutteridge, 1989).

Conclusion

Present investigation was carried out to analyze the effect of imidacloprid on biochemical parameters like DNA, RNA, protein and glucose in soil isolate *Bacillus weihenstephanensis.* The study involving soil isolate *Bacillus weihenstephanensis* with molar concentrations of 10^{-3} to 10^{-7} of imidacloprid insecticide showed that there was an increase in the percent inhibition of DNA, RNA, protein and glucose, the inhibitory effect increased with an increase in the concentration of insecticide proving that the inhibitory effect is dose dependent. The present investigation indicates that imidacloprid reduced the DNA RNA, glucose and protein content which intern effects the growth of the *Bacillus weihenstephanensis.* The present investigation has proven that imidacloprid has toxic effect on soil isolate and the effect is dose and duration dependent.

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