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INFLUENCE OF IMIDACLOPRID ON BIOCHEMICAL PARAMETERS IN SOIL ISOLATE *ESCHERICHIA COLI*

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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 *Escherichia coli*, further the toxic effect of the insecticide on the growth was also studied. The study involving soil isolate *Escherichia coli* 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. Further, imidacloprid inhibited the growth of *Escherichia coli* when compared to control. The present investigation indicates that imidacloprid reduced the DNA RNA, glucose and protein content which intern effects the growth of the *Escherichia coli*.

KEYWORDS: Imidacloprid, Escherichia coli, Biochemical Parameters, Growth

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INTRODUCTION

Millions of tons of pesticides applied annually are used in modern agriculture to increase production through controlling harmful effects caused by the targets organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops. However, less than 5% of these products are estimated to reach the target organisms. One of the most important problems with the use of pesticides is their possible persistence in the environment and therefore, their possible incorporation into the food chain affects ecosystem and all human beings [1]. However, increased use of pesticides has led to environmental pollution. The microbial biomass plays an important role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition [2]. Insecticides have been reported to affect the microbial populations by controlling the survival and reproduction of species [3]. In recent years, the role of soil microorganisms in affecting the persistence of agricultural pesticides has been the subject of two areas of study. The first is the capacity for rapid elimination of highly persistent or toxic chemicals. The second is reduced pesticide efficacy attributed to enhanced biodegradation, particularly of chemicals applied under a continuous cropping program [4]. In fact, some insecticides such as gammalin, vetox and cypermethrin have been reported to exhibit differential effects on various groups of microorganisms in which a reduction or stimulatory effect is noted [5,6].

Imidacloprid (I-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), is a neonicotinoid insecticide and has been introduced as worlds' fastest growing insecticide in global market [7]. It has been considered as possible replacement for the widely used organophosphorus pesticides like diazinon [8]. Imidacloprid has high insecticidal activity at low volume and therefore widely used to control the pests of vegetables, cereals, tea and cotton throughout the world [9]. Imidacloprid is an agonist of the nicotinic acetylcholine receptor (nAChR) at the neuronal and neuromuscular junctions in insects and vertebrates. It is structurally and functionally related to nicotine. The toxicity of imidacloprid is largely due to interference of the neurotransmission in the nicotinic cholinergic nervous system. Prolonged activation of the nAChR by imidacloprid causes desensitization and blocking of the receptor, and leads to in coordination, tremors, decreased activity, reduced body temperature and death. Presently, there is no specific antidote, which acts as an antagonist to the effects of imidacloprid [10]. Excess use of this pesticide and potential toxicity to humans warrants a heightened awareness about this compound [11]. Imidacloprid is a potential ground and surface water contaminant [12]. Imidacloprid is very persistent in soil with half- life often greater than 100 days [13]. 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 *Escherichia coli.*

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 [14]. Soil isolate was isolated from soil as described in the previous publication[15]. The bacterium was maintained at 4°C on nutrient agar [16] 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

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[17].

Protein estimation

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 [18].

Estimation of glucose utilization

The glucose content was estimated by Anthrone method [19].

Growth

The concentration of cells was measured every 24 hrs using optical density (OD) at 600 nm [20].

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 negative, 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 *Escherichia*. Based on nucleotide homology and phylogenetic analysis (fig. 1), the culture SP-02 was identified as Escherichia *coli strain SCDC-1* (GenBank Accession Number: HM576813.1). Further, the toxic effect of imidacloprid on biochemical parameters (DNA, RNA, protein and glucose) and on growth in soil isolate *Escherichia coli* 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≤0.05) in the level of DNA (Table 1.), RNA (Table 2.), protein (Table 3.) and glucose (Table 4.) content in all the treated groups. However, increase in the % inhibition was observed with an increase in dose and durational exposure to imidacloprid. There was a significant decrease (P≤0.05) in the growth in all the treated groups and an increase in the % inhibition was observed with an increase in dose and durational exposure to imidacloprid (Table 5.).

DISCUSSION

The results of the present investigation revealed that the percent inhibition of all the parameters increased significantly (P≤ 0.05) with an increase in dose and duration of exposure to imidacloprid, indicating that the imidacloprid has inhibitory effect on parameters studied. Similar results were reported in study involving stress proteins of *Escherichia coli* induced in response to the pesticides cypermethrin, zeta-cypermethrin, carbofuran and bifenthrin [21]. The significant (P≤0.05) increase in the percent inhibition in DNA and RNA observed in the present study may be due to genotoxic action of imidacloprid on bacterial cells. The pesticides are known to affect growth by inhibiting the DNA and RNA synthesis by blocking the binding of polymerase to the DNA template [22]. Imidacloprid is known to induce free radical generation that leads to protein degradation and DNA damage [23].The damage of membrane lipids, proteins and DNA is the endpoint biomarkers of oxidative stress-induced effects of pesticides [24]. 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 [25].

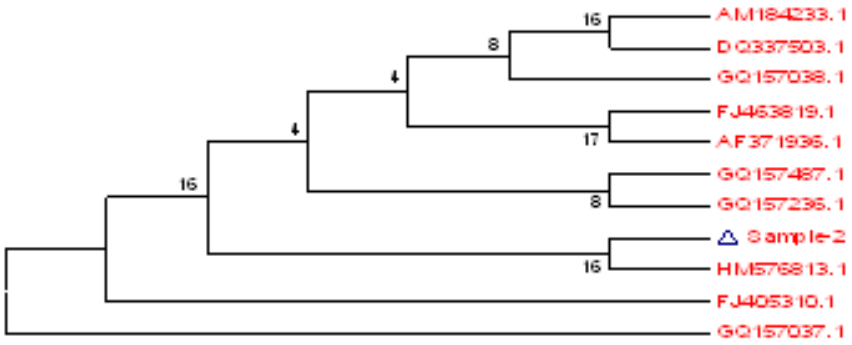

Figure 1
Phylogenetic Tree for Escherichia coli strain SCDC-1

Table 1
Effect of imidacloprid treatment on DNA content in Escherichia coli

Group	Treatment (M)	DNA Content(µg/ml) Duration(hrs)							
		24	%	48	%	72	%	96	%
I	Control	94.40±0.70		115.00±0.90		145.00±0.58		156.00±0.87	
II	10^{-7}	88.40±0.97*	6.46	97.60±0.27*	15.14	100.40±0.55*	20.76	108.30±0.77*	30.58
III	10^{-6}	82.40±0.23*	12.82	85.20±0.18*	26.00	90.20±0.39*	37.80	96.30±0.23*	38.27
IV	10^{-5}	62.30±0.53*	36.00	67.20±0.24*	41.57	71.20±0.28*	50.90	76.40±0.65*	52.00
V	10^{-4}	44.60±0.25*	52.86	42.00±0.37*	63.48	50.40±1.17*	65.25	60.20±0.64*	61.42
VI	10^{-3}	26.10±0.24*	72.36	33.50±0.48*	70.87	37.40±0.31*	74.21	42.50±0.34*	72.76

%- percent inhibition
Values are mean ± S.E.M (n=10)
*** Significant P ≤ 0.05 compared to control**
M- Molar Concentration

Table 2
Effect of imidacloprid treatment on RNA content in *Escherichia coli*

Group	Treatment (M)	RNA Content(µg/ml) Duration(hrs)							
		24	%	48	%	72	%	96	%
I	Control	23.83±0.16		36.40±0.41		52.40±0.50		64.20±0.60	
II	10^{-7}	16.40±0.47*	31.18	22.40±0.65*	31.47	28.40±0.48*	45.81	31.20±0.42*	51.31
III	10^{-6}	12.20±0.51*	48.81	21.70±0.18*	40.39	23.40±0.37*	54.35	26.40±0.41*	58.88
IV	10^{-5}	10.40±0.25*	56.36	18.40±0.16*	49.46	21.30±0.20*	59.36	24.60±0.21*	61.69
V	10^{-4}	8.80±0.10*	63.08	13.20±0.22*	63.74	15.40±0.37*	70.62	18.90±0.60*	70.57
VI	10^{-3}	6.80±0.10*	71.47	8.40±0.41*	76.97	10.60±0.26*	79.88	12.40±0.66*	80.69

%- percent inhibition
Values are mean ± S.E.M (n=10)
**** Significant P ≤ 0.05 compared to control***
M- Molar Concentration

Further a 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 [26]. It has been reported that the biological targets for the reactive oxygen species due to oxidative stress are RNA, DNA, proteins and lipids. Imidacloprid might have affected protein synthesis. The increase in percent inhibition of glucose utilization 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 [27]. Similar results were reported in *Escherichia coli* and *Pseudomonas aeruginosa* exposed to various concentration of methomyl [28].

Table 3
Effect of imidacloprid on Protein content in Escherichia coli

Group	Treatment (M)	Protein Content(µg/ml) Duration(hrs) 24	%	48	%	72	%	96	%
I	Control	80.40±0.56		128.20±1.17		154.40±1.40		173.40±0.37	
II	10^{-7}	68.40±0.40*	20.00	95.60±0.24*	25.43	122.80±0.28*	18.47	126.40±0.17*	21.44
III	10^{-6}	52.60±0.37*	34.68	84.20±0.50*	34.33	102.20±0.66*	32.81	110.20±0.52*	36.45
IV	10^{-5}	47.20±0.48*	41.30	61.80±0.23*	51.80	76.80±0.40*	50.26	80.80±0.42*	53.41
V	10^{-4}	41.40±0.16*	48.51	60.50±0.25*	56.64	70.10±0.26*	54.60	77.30±0.21*	58.31
VI	10^{-3}	34.60±0.16*	67.00	47.20±0.23*	63.19	50.40±0.17*	67.36	57.80±0.40*	66.67

%- percent inhibition
Values are mean ± S.E.M (n=10)
** Significant P ≤ 0.05 compared to control*
M- Molar Concentration

Table 4
Effect of imidacloprid on Glucose content in Escherichia coli

Group	Treatment (M)	Glucose Content(µg/ml) Duration(hrs) 24	%	48	%	72	%	96	%
I	Control	35.40±0.26		40.90±1.81		61.80±0.22		72.40±0.21	
II	10^{-7}	30.80±0.22*	13.00	34.80±0.25*	15.00	47.60±0.11*	23.00	58.50±0.19*	19.20
III	10^{-6}	25.20±0.40*	28.82	30.50±0.28*	25.43	41.20±0.19*	33.34	49.40±0.19*	31.63
IV	10^{-5}	24.50±0.13*	30.80	27.90±0.32*	31.69	32.40±0.16*	47.58	38.20±0.24*	47.24
V	10^{-4}	19.50±0.13*	45.00	21.20±0.44*	48.17	24.40±0.36*	44.34	28.20±0.15*	61.05
VI	10^{-3}	13.40±0.14*	62.15	14.20±0.16*	65.29	18.50±0.12*	70.17	20.30±0.33*	72.00

%- percent inhibition
Values are mean ± S.E.M (n=10)
** Significant P ≤ 0.05 compared to control*
M- Molar Concentration

The study on growth kinetics provides an evidence of mineralization potential of organism[29]. Endosulfan inhibited the growth of *B. subtilis* in 32-80 µ/l concentrations used for study the effect on growth. In the study there was no growth observed above 64 µ/l concentration of endosulfan [30]. The imidacloprid might have affected the bacterial growth via possible attack to the membrane components and inhibited activity of DNA polymerase-I [31]. The increase in percent inhibition in growth with increase in dose and duration of exposure of imidacloprid in cells is obligatory since some microbial groups will be able to use an applied pesticide as a source of energy and nutrients, where as others may well be toxic to other organisms and as such the soil microbial community is a complex picture of interwoven relationships between organisms in different tropic levels, this will lead to many indirect effects [32]. It is widely accepted that bacterial cells in the natural environment exist in constant flux between short periods of exponential growth and much longer periods of non-growth. This has been termed the “Feast and Famine” existence of bacteria, when

nutrient are available, bacteria can attain rapid growth rates, but when nutrients are depleted, they must be able to endure prolonged periods of starvation [33].

Table 5
Effect of imidacloprid on growth of Escherichia coli

Group	Treatment (M)	Optical density at (600nm)							
		Duration(hrs)							
		24	%	48	%	72	%	96	%
I	Control	0.250±0.0		0.380±0.03		0.442±0.03		0.510±0.05	
II	10^{-7}	0.192±0.03*	24.20	0.260±0.04*	31.58	0.290±0.04*	34.10	0.456±0.07*	21.77
III	10^{-6}	0.180±0.01*	28.00	0.240±0.02*	39.48	0.275±0.01*	38.64	0.300±0.03*	41.18
IV	10^{-5}	0.155±0.02*	40.10	0.205±0.02*	47.37	0.230±0.40*	47.73	0.275±0.03*	47.06
V	10^{-4}	0.132±0.01*	47.20	0.160±0.01*	51.90	0.184±0.01*	59.10	0.200±0.02*	60.79
VI	10^{-3}	0.120±0.01*	52.0	0.134±0.01*	65.79	0.150±0.01*	66.00	0.164±0.01*	67.65

%- percent inhibition
Values are mean ± S.E.M (n=10)
**** Significant P ≤ 0.05 compared to control***
M- Molar Concentration

This fact supports the idea that, transitional metabolic states are characteristic of natural microbial populations affected by changes in environmental conditions and stress factors. Bacteria display complex adaptive reactions in response to adverse environmental conditions in order to survive various combinations of stress factors. Since the *Escherichia coli* cells possess antioxidant enzymes, which are induced in response to stress and are directly exposed to the pesticide. Although the proteins and nucleic acids play a major role in the cellular defense mechanism, they are susceptible to inactivation [34].

CONCLUSION

The present investigation indicates that the imidacloprid has a negative effect of biochemical parameters studied. Imidacloprid also inhibited the growth of soil isolate *Escherichia coli.* However, in order to judge the overall long-term effects of imidacloprid application on bacteria it is necessary to carryout extensive studies on it effect to different group of bacteria in soil and it is important to reduce Imidacloprid pollution in soil by isolating bacteria capable of degrading the insecticide.

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