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IMIDACLOPRID INDUCED INTOXICATION IN SOIL ISOLATE *BREVUNDIMONAS SP. MJ 15*

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ABSTRACT

Imidacloprid (I-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), is a chloronicotinyl insecticide and it is used to control biting and sucking insects. In India it is used for variety of crops. Present investigation was carried out to study the effect of imidacloprid on bacterial populations in soil and further to elucidate its toxic effect on growth and biochemical parameters of soil isolate *Brevundimonas sp. MJ15.* The laboratory and field studies were conducted to determine the effects of imidacloprid on soil bacterial populations. For Laboratory studies the concentrations 125, 250, 500, and 1000 ppm of imidacloprid was applied and field studies involved application of imidacloprid at recommended rates and at 1.5x rate. Toxic effect on bacteria was evaluated at 0, 7, 14, 21 and 28 days after treatment with imidacloprid. The bacterial population was estimated using the standard dilution plate technique. Results obtained from both studies revealed that imidacloprid caused significant ($P<0.05$) reduction in bacterial population in the soil. However, in the field studies toxic effect disappeared after 28 days of application. The study involving soil isolate *Brevundimonas sp. MJ15* with molar concentrations of 10^{-3} to 10^{-7} of insecticide showed there was significant increase in percent inhibition of DNA, RNA, protein and glucose. The inhibitory effect increased with the concentration of insecticide proving that the inhibitory effect is dose dependent. There was significant ($P<0.05$) decrease in the growth on treatment with various molar concentrations of insecticide when compared to that of control and effect was dose dependent. The present investigation proves that imidacloprid has toxic effect on bacterial populations in soil and this effect is dose dependent. Study also showed that imidacloprid affects the biochemical contents and intern growth of soil isolate *Brevundimonas sp.* MJ15.

Keywords: Imidacloprid, *Brevundimonas sp.* MJ15, Growth, Biochemical Parameters.

INTRODUCTION

During the last century advances in synthetic chemistry have given chemists the ability to make numerous novel compounds, some of which are xenobiotic (Xu L et al. 1999). The release of xenobiotic compounds into the environment and the problem of toxic waste disposal have become enormous due to the proliferation of these xenobiotic compounds for use as pesticides, solvents, explosives, refrigerants and dyes in industrial, urban and agricultural applications (Doung M et al. 1997). Many xenobiotic compounds, particularly those used as insecticides, are toxic (Xu L et al., 1999).Insecticides have been reported to affect the microbial populations by controlling the survival and reproduction of species (Ekundayo EO. 2006). 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 (Topp E. 1993; Benimeli CS et al . 2006). Azadirachtin had very high biocidal effects on the soil microorganisms and their activities (Gopal MA et al. 2007). Several other reports on the negative effects of xenobiotics on the soil microorganisms abounds.

Imidacloprid is the first synthetic neonicotinoid insecticide used against sucking pests, such as rice hoppers, aphids, thrips and whiteflies. Imidacloprid has been used widely for foliar and seed treatment, soil drench as well as stem application (Nauen R et al. 2003). Today imidacloprid is used in over 120 countries to treat more than 140 different crops (Krohn J and Hellpointner E, 2002). It is most commonly used on cotton, rice, cereal, maize, sunflowers, potatoes and vegetables. The active chemical in imidacloprid works by interfering with the transmission of stimuli in the insect's nervous system. Specifically, it causes a blockage in the nicotinergic neuronal pathway that is more abundant in insects than in warm-blooded animals, making the chemical much more toxic to insects than to warm-blooded animals. This binding on the nicotinic acetylcholine receptor (nAChR) leads to the accumulation of the acetylcholine neurotransmitter, resulting in the paralysis and death of the insect (Okazawa A et al. 1998). Imidacloprid is very persistent in soil with half- life often greater than 100 days (Scholz K and Spiteller M, 1992). Therefore the present investigation was carried out to study the effect of imidacloprid on bacterial populations in soil and its toxic effect on growth and biochemical parameters of soil isolate *Brevundimonas sp.* MJ15.

MATERIALS AND METHODS

1. Chemicals

The imidacloprid used was purchased from a local agricultural dealership store in Hubli. Analytical Grade Biochemical's, culture media consisting nutrient broth and minimal salt medium were used. The glass wares used in the experiments were from Borosil Company.

2. Laboratory experiment

The experiment was carried out during summer of 2011 at laboratory of department of Biotechnology and Microbiology, Karanatak University, Dharwad Karnataka. The soil samples were collected from cotton fields around Hubli city. These fields did not have a history of imidacloprid applications for last five years. Soil was collected at a depth of 15cm and samples were passed through a sieve of 2 mm to remove stones and plant debris. One gram of soil was mixed with 9 ml of sterilized water and mixed by shaking for even distribution of soil in water. 1 ml of solution from this test tube was then added to another test tube with 9 ml sterilized water. This gives a dilution of 10^{-2} and in the same pattern dilutions up to 10^{-7} were prepared. 100 µl of solution from 10^{-6} dilution was spread on nutrient plates containing different concentration (125, 250, 500, and 1000 ppm) of imidacloprid. These plates were incubated at 37^0C for 48 hr. After incubation bacterial colonies were counted using colony counter and results were expressed as the number of bacteria in 1g of soil.

3. Field experiment

Imidacloprid was applied to experimental field at recommended rates and at 1.5x rates on two plots on same field in replicates, the plot without application served as control. Soil samples were taken on 7, 14, 21, and 28^{th} day of application. 1g of sample was suspended in 9 ml of sterilized water. Serial dilutions were done as mentioned earlier. 100 µl of solution from 10^{-6} dilution was spread plated on nutrient agar plates. These plates were incubated at 37^0 C for 48 h. After incubation colonies of bacteria were counted using colony counter and results were expressed as the number of bacteria in per gram of soil.

4. Identification of bacterial isolate

Imidacloprid tolerant colonies from 1000 ppm imidacloprid plates were isolated. The pure culture was grown on nutrient agar medium. Colonies were

characterized by morphological characters, staining, and 16s RNA identification.

5. Preparation of stock solution of imidacloprid

The stock solution of 1 Molar of imidacloprid was prepared and further diluted to give 10^{-3}, 10^{-4} 10^{-5} 10^{-6} and 10^{-7} molar concentrations (Kulkarni AG and Kaliwal BB, 2009). The organism *Brevundimonas sp. MJ15*was isolated from soil and maintained at 4°C on nutrient agar (Lapage SP and Shelton JE, 1970) and sub cultured very fortnight.

6. Medium used for the study

The medium used for toxicity testing was a optimized medium (dextrose - 0.65 g l^{-1}, Yeast extract - 1.05 g /l^{1}, K HPO - 0.30 g/l, and NaCl - 0.25 g /l^{1}).

7. 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 Minimal salts medium and incubated for 18-24 hours at 37°C under static conditions depending on the exponential phases of bacteria under test.

8. Experimental procedures

Five ml of the pre-inoculum was inoculated to 250 ml Erlenmeyer's flask containing 100 ml of sterilized Minimal salts 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 (REMI – CIS-24). At regular intervals sample was taken out from each flask aseptically for analysis.

8.1 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 method and orcinol method respectively (Brown TA. 1946).

8.2 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 the centrifuged at 3000 rpm for 5 min and the supernatant was used for the estimation of protein (Lowry OH et al, 1951).

8.3 Estimation of glucose utilization

The glucose content was estimated by Anthrone method (Hedge and Hofreiter, 1962).

8.4 Growth

The concentration of cells was measured every 24 hrs using optical density (OD) at 600 nm (Kosmachevskaya OV et al. 2007).

Statistical analysis

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

RESULTS

Effect of imidacloprid on soil bacterial populations in Laboratory studies

Bacterial counts in imidacloprid treated Petri plates in the laboratory are given in Table 1. Addition of imidacloprid at 125, 250, 500, and 1000 ppm brought significant ($P<0.05$) reduction in bacterial numbers when compared with that of control. The bacterial numbers decreased significantly ($P<0.05$) from$11.05x10^{-6}$ in control plates to $9.80\ x10^{-6}$, $08.40\ x10^{-6}$, $6.73\ x10^{-6}$, and $5.60\ x10^{-6}$ at concentrations of 125, 250, 500, and 1000 ppm of imidacloprid respectively.

Table 1. ***Effect of Imidacloprid on bacterial populations in the soil under laboratory conditions***

Group	Concentration of Imidacloprid (ppm)	Bacterial Count at (X 10^{-6}cfu/g)
I	Control	11.05 ± 0.086
II	125	9.80 ± 0.11
III	250	8.40 ± 0.11*
IV	500	6.73 ± 0.06*
V	1000	5.60 ±0.08*

***Significant at P<0.05**

Effect of imidacloprid on soil bacterial populations in field studies

Results obtained from microbial enumeration of imidacloprid treated soil at both recommended and x1.5 rates are shown in Table 2. Soil from imidacloprid treated fields at recommended rates showed significant (P<0.05) decrease in bacterial populations at different post-application intervals over pre-treatment counts of 10.80 x10^{-6}. The bacterial count was 6.40 x10^{-6}, 7.60 x10^{-}6, 9.2x10^{-6} and 10.20 x10^{-6} after 7th, 14th, 21st and 28th day of application respectively.

Table 2. ***Effect of imidacloprid on bacterial populations in soil under field conditions***

Treatment	Days after treatment(at X 10^{-6}cfu/g)				
	Pre-treatment	7	14	21	28
Control	11.20 ± 0.055	11.65 ± 0.11	11.00 ± 0.10	10.93 ± 0.15	11.13 ± 0.20
Recommended rate(500ml/ha)	10.80± 0.057	6.40 ± 0.57*	7.6 ± 0.088*	9.2 ± 0.088	10.2± 0.06
1.5X rate(750ml/ha)	11.00 ± 0.054	5.3 ± 0.057*	5.90 ± 0.066*	8.10 ± 0.033*	9.5 ± 0.057

***Significant at P<0.05**

Soil from imidacloprid treated fields at 1.5x rates showed significant decrease in bacterial populations at different post-application intervals over pre-treatment counts of 11.00 x10^{-}. The bacterial count was 5.30 x10^{-6}, 5.90 x10^{-6}, 8.1 x10^{-6} and 9.5 x10^{-6} after 7, 14, 21 and 28th day of application respectively.

Isolation and Identification of imidacloprid tolerant bacteria

The colonies grown in 1000 ppm plates were isolated and further grown in nutrient broth containing 1000 ppm imidacloprid and incubated for seven days and plated on medium containing imidacloprid two colonies were isolated and named as SP-01 and SP-02. These strains were a rod-shaped, gram negative, bacterium. By sequencing the 16S rRNA gene of and comparing them with previously published 16S rRNA gene sequences, the strains were classified as a member of the genus *Brevundimonas* and *Escherichia* respectively. The sequence of strain SP-01 displayed the highest identity (99%) with the 16S rRNA gene of an *Brevundimonas sp. MJ15* (GQ250440.2). and strain SP-02 showed (99%) similarities with *Escherichia coli* strains SCDC-1 (HM576813.1).

The isolate *Brevundimonas sp. MJ15* was further used to study the effect of imidacloprid on growth and biochemical parameters by using broth medium containing 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} molar concentrations of imidacloprid.

The effect of imidacloprid on DNA content in *Brevundimonas sp. MJ15* on exposure to various molar concentrations is given in Table 3. DNA Concentration in bacterial cell in control was 105.10, 134.10, 158.20 and 169.20 µg/ml at 24, 48, 72, and 96 h of incubation respectively. The concentration of DNA in bacterial cell at 10^{-7} molar concentration of

imidacloprid was 97.30, 100.20, 106.30 and 114.30 µg/ml after 24, 48, 72, and 96h of incubation. The concentration of DNA in bacterial cell at 10^{-6} molar concentration of imidacloprid was 89.50, 91.20, 98.20 and 99.20 µg/ml after 24 h, 48 h, 72 h, and 96h of incubation. The concentration of DNA in bacterial cell at 10^{-5} molar concentration of imidacloprid was 69.50 74.20 81.24 and 84.0 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of DNA in bacterial cell at 10^{-4} molar concentration of imidacloprid was 46.40, 56.00, 64.56 and 69.20 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of DNA in bacterial cell at 10^{-3} molar concentration of imidacloprid was 29.10, 42.20, 46.20 and 51.20 µg/ml after 24, 48, 72, and 96 h of incubation.

The effect of imidacloprid on RNA content in *Brevundimonas sp. MJ15* on exposure to various molar concentrations is given in Table 4. The RNA concentration in control was 32.44, 44.20, 62.34 and 71.24 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of RNA in bacterial cell at 10^{-7} molar concentration of imidacloprid was 23.20, 28.30, 26.66 and 38.60 µg/ml after 24 h, 48 h, 72 h, and 96 h of incubation. The concentration of RNA in bacterial cell at 10^{-6} molar concentration of imidacloprid was 18.30, 23.40, 30.86 and 32.50 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of RNA in bacterial cell at 10^{-5} molar concentration of imidacloprid was 14.45, 20.44, 26.50 and 28.98 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of RNA in bacterial cell at 10^{-4} molar concentration of imidacloprid was 10.10, 16.44, 17.46, and 22.10 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of RNA in bacterial cell at 10^{-3} molar concentration of imidacloprid was 8.30, 9.75, 10.40 and 12.34 µg/ml after 24, 48, 72, and 96 h of incubation.

The effect of imidacloprid on protein content in *Brevundimonas sp. MJ15* on exposure to various molar concentrations is given in Table 5. The protein concentration in control was 82.46, 134.00, 161.50 and 171.50 µg/ml after 24, 48, 72, and 96 of incubation. The concentration of protein in bacterial cell at 10^{-7} molar concentration of imidacloprid was 64.60, 95.80, 123.40 and 126.44 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of protein in bacterial cell at 10^{-6} molar concentration of imidacloprid was 53.40, 85.45, 102.15 and 105.56 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of protein in bacterial cell at 10^{-5} molar concentration of imidacloprid was 87.23, 70.20, 85.20 and 82.52 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of protein in bacterial cell at 10^{-4} molar concentration of imidacloprid was 41.40, 61.20, 71.40 and 71.24 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of protein in bacterial cell at 10^{-3} molar concentration of imidacloprid was 34.60, 47.56, 50.80 and 58.38 µg/ml after 24, 48, 72, and 96 h of incubation.

The effect of imidacloprid on glucose content in *Brevundimonas sp. MJ15* on exposure to various molar concentrations is given in Table 6. The glucose concentration in control was 40.50, 52.74, 68.10 and 79.25 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of glucose in bacterial cell at 10^{-7} molar concentration of imidacloprid was 34.40, 35.84, 51.44 and 51.86 µg/ml after 24, 48, 72 h, and 96 h of incubation. The concentration of glucose in bacterial cell at 10^{-6} molar concentration of imidacloprid was 31.86, 30.88, 46.24 and 43.20 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of glucose in bacterial cell at 10^{-5} molar concentration of imidacloprid was 26.44, 25.88, 36.55 and 39.30 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of glucose in bacterial cell at 10^{-4} molar concentration of imidacloprid was 22.66, 18.90, 28.20 and 31.44 µg/ml after 24, 48, 72, and 96 h of incubation. The concentration of glucose in bacterial cell at 10^{-3} molar concentration of imidacloprid was 16.78, 18.20, 21.20 and 24.52 µg/ml after 24, 48, 72, and 96 h of incubation.

Table 6. ***Effect of imidacloprid on Glucose content in Brevundimonas sp. MJ-15***

Group	Treatment concentration (Molar)	Glucose Content (µg/ml) Duration (hrs)							
		24	% inhibition	48	%inhibition	72	% inhibition	96	% inhibition
I	Control	40.50±0.078		52.74±0.021		68.10±0.089		79.25±0.078	
II	10^{-7}	34.40±0.087*	15.17	35.84 ±0.035*	32.15	51.44±0.057*	24.57	51.86±0.087*	34.67
III	10^{-6}	31.86±0.034*	21.44	30.88±0.68*	37.45	46.24±0.078*	32.10	43.20±0.094*	45.50
IV	10^{-5}	26.44±0.045*	36.82	25.88±0.065*	51.00	36.55±0.065*	46.43	39.30±0.057*	50.52
V	10^{-4}	22.66±0.057*	45.00	18.90±0.023*	64.27	28.20±0.054*	58.60	31.44±0.067*	60.43
VI	10^{-3}	16.78±0.067*	58.67	18.20±0.042*	67.49	20.20±0.045*	70.44	24.52±0.024*	69.16

***Significant at P<0.05**

The effect of imidacloprid on growth in *Brevundimonas sp. MJ15* on exposure to various molar concentrations is given in Table 7. Optical density at 600 nm of growth in control was 0.320, 0.410, 0.480 and 0.520 after 24, 48, 72, and 96 h of incubation. The growth in 10^{-7} molar concentration of imidacloprid was 0.254, 0.360, 0.370 and 0.440 after 24, 48, 72 h, and 96 h of incubation. The growth in 10^{-6} molar Optical density of imidacloprid was 0.210, 0.289, 0.310 and 0.360 after 24, 48, 72, and 96 h of incubation. The growth in 10^{-5} molar concentration of imidacloprid was 0.187, 0.205, 0.265 and 0.280 after 24, 48, 72, and 96 h of incubation. The growth in 10^{-4} molar concentration of imidacloprid was 0.132, 0.182, 0.205 and 0.246 after 24, 48, 72, and 96 h of incubation. The growth in 10^{-3} molar concentration of imidacloprid was 0.094, 0.124, 0.132 and 0.146 after 24, 48, 72, and 96 h of incubation.

Table 3. Effect of imidacloprid on DNA content in *Brevundimonas sp.* MJ-15

Group	Treatment concentration (Molar)	DNA Content(µg/ml)							
		Duration(hrs)							
		24	%inhibition	48	%inhibition	72	%inhibition	96	%inhibition
I	control	105.10± 0.011		134.10±0.089		158.20±0.045		169.20±0.056	
II	10^{-7}	97.30 ±0.057*	7.53	100.20 ±0.12*	25.38	106.30±0.067*	32.80	114.30±0.045*	32.55
III	10^{-6}	89.50±0.013*	14.85	91.200±0.10*	32.00	98.20±0.034*	39.84	99.20±0.024*	41.48
IV	10^{-5}	69.60±0.34*	33.88	74.20±0.11*	44.77	81.24±0.045*	48.65	84.20±0.068*	50.34
V	10^{-4}	46.40±0.024*	55.86	56.00±0.86*	58.25	64.56±0.056*	59.20	69.20±0.028*	59.20
VI	10^{-3}	29.10±0.056*	72.42	42.20±0.77*	68.64	46.20±0.043*	70.80	51.20±0.056*	69.84

***Significant at P<0.05**

Table 4. *Effect of imidacloprid on RNA content in Brevundimonas sp. MJ-15*

Group	Treatment concentration (Molar)	RNA Content(µg/ml)							
		Duration(hrs)							
		24	% inhibition	48	% inhibition	72	% inhibition	96	% inhibition
I	control	32.44±0.011		44.20±0.024		62.34±0.086		71.24±0.034	
II	10^{-7}	23.20±0.024*	29.49	28.30±0.034*	36.0	26.66±0.12*	57.34	38.60±0.044*	45.92
III	10^{-6}	18.30±0.11*	43.69	23.40±0.054*	47.06	30.86±0.11*	50.50	32.50±0.072*	55.48
IV	10^{-5}	14.45±0.084*	55.46	20.44±0.067*	53.86	26.50±0.094*	57.34	28.98±0.082*	59.43
V	10^{-4}	10.10±0.057*	68.87	16.44±0.084*	62.97	17.46±0.074*	72.00	22.10±0.024*	69.40
VI	10^{-3}	8.30±0.065*	74.42	9.75±0.058*	78.0	10.40±0.096*	83.42	12.34±0.054*	86.78

***Significant at P<0.05**

Table 5. Effect of imidacloprid on protein content in *Brevundimonas sp.* MJ-15

Group	Treatment concentration (Molar)	Protein Content(µg/ml)							
		Duration(hrs)							
		24	%inhibition	48	%inhibition	72	%inhibition	96	%inhibition
I	control	82.46±0.056		134.00±0.057		161.50±0.11		171.50±0.024	
II	10^{-7}	64.60±0.074*	21.76	95.80±0.082*	29.5	123.40±0.13*	25.60	126.44±0.031*	26.38
III	10^{-6}	53.40±0.085*	35.35	85.45±0.032*	36.34	102.15±0.89*	36.85	105.56±0.041*	38.73
IV	10^{-5}	57.23±0.045*	38.60	70.20±0.076*	47.72	85.20±0.94*	47.25	82.52±0.057*	51.99
V	10^{-4}	41.40±0.048*	49.80	61.20±0.024*	54.43	71.40±0.87*	54.80	71.24±0.043*	58.47
VI	10^{-3}	34.60±0.038*	58.00	47.56±0.012*	64.50	50.80±0.10*	68.55	58.38±0.056*	65.00

***Significant at P<0.05**

Table 7. *Effect of imidacloprid on growth* of *Brevundimonas sp. MJ-15*

Group	Treatment concentration (Molar)	Optical density at 600nm							
		Duration(hrs)							
		24	% inhibition	48	% inhibition	72	% inhibition	96	% inhibition
I	control	0.320±0.034		0.410±0.012		0.480±0.098		0.520±0.045	
II	10^{-7}	0.254±0.076*	30.73	0.360±0.076*	12.20	0.370±0.089*	23.00	0.440±0.034*	15.40
III	10^{-6}	0.210±0.036*	41.67	0.289±0.034*	29.62	0.310±0.056*	35.52	0.360±0.056*	30.87
IV	10^{-5}	0.187±0.024*	49.48	0.205±0.045*	50.00	0.26 5±0.076*	44.80	0.280±0.023*	56.26
V	10^{-4}	0.132±0.056*	58.85	0.182±0.056*	55.71	0.205±0.067*	57.30	0.246±0.048*	52.70
VI	10^{-3}	0.094±0.089*	70.73	0.124±0.034*	69.86	0.134±0.073*	72.12	0.146±0.032*	72.00

***Significant at P<0.05**

DISCUSSION

Results obtained in laboratory studies showed significant ($P< 0.05$) decrease in bacterial count when compared to that of control. A gradual decrease in bacterial count is observed with increase in concentration of imidacloprid, with minimal count reported at 1000 ppm. The results obtained were similar to results reported earlier in a study involving five other pesticides (Ahmed S and Ahmad MS, 2006).The results indicate toxic effect of imidacloprid on bacterial populations.

Results obtained from bacterial enumeration of imidacloprid treated soils at recommended rate showed significant ($P< 0.05$) decrease in bacterial numbers, proving negative effect of imidacloprid on bacteria. This negative effect reduced after 14 days of treatment. The negative effect of imidacloprid was vanished by 28th day of application, indicated by bacterial count which was almost similar to pre treatment count. Similar results were reported in a study involving imidacloprid and five other pesticides in the study the toxic effect was vanished by 21 day of imidacloprid application (Ahmed S and Ahmad MS, 2006).

In general, the impact of pesticides on soil microflora is variable and results not only from the reaction of microorganisms to an active substances and formulation additives but also from the development of specific group of microorganisms (Nowak A et al. 1999). Some microbial groups are able to use an applied pesticide as a source of energy and nutrients to multiply (Johansen K et al. 2001), while there are some agrochemicals which are not utilizable by single microorganism and might be degraded in soil by microorganisms through co- metabolism (Bollag JM and Liu SY, 1990).

The initial decrease in bacterial count is expected as pesticides are known to affect the microbial populations by controlling the survival and reproduction of individual species (Ekundayo EO, 2006). Initial reduction in microbial count is also reported in studies involving different pesticides endosulphan, cypermirithin thiodan etc. (Ekundayo EO, 2006; Ahmed S and Ahmad MS, 2006; Adebayo TA, 2007) and herbicides like glyphosate, atrazine, simazin and alachlor (Weaver MA et al. 2007; Ayansina ADV, 2006; Fantroussi S, 1999; Ismail BS, 2005) when applied at recommended rates. It has been observed in many studies that pesticides stimulated the mineralization rate of organic carbon in comparison with control samples (Bhuyan S et al. 1993; Das AC and Mukherjee D, 1994). Pesticides are toxic to many soil microorganisms because they can penetrate the cell, disturb the bacterial metabolism and often cause the death of sensitive part of microbial populations. Changes in the abundance of one group of microorganisms may lead to reduction in soil microbial biodiversity. Any disturbance in microbial activity may result in a change of the availability and cycling of nitrogen and others important nutrients. Consequently, changes in the structure and function of microbial communities caused by agrochemicals may have the negative impact on ecosystem activity.

The increase in bacterial numbers after 14th day may be due to the ability of bacteria to degrade toxic compounds like pesticides (Cremlyn RJ. 2006). It has been postulated that pesticides killes the sensitive part of bacterial communities and the organic compounds released from dead bacteria increased the content of available nutrients, such as nitrogen and phosphorus (Jana TK et al. 1998). The ability of some microorganisms to grow in the presence of pesticides may result in the compensation of an adverse effect by the increased activity of remaining part of soil community. Microorganisms not sensitive to the pesticides utilize released nutrients what may result in increase in their number in soil (Das AC and Mukherjee D, 2000). Moreover, the effect of pesticides on the microbial activity is not necessarily correlated with changes in the microbial populations that responsible for the activity (Bollen GJ. 1979). Additionally, after fungicide treatment soil bacteria are released from competition with fungi or antagonistic inhibition *via* substances synthesized by fungi (Chen SK et al. 2001). Bacteria's are known to become resistant to toxic compound with production of specific degrading enzymes (Kulkarni AG and Kaliwal BB 2009).

The results obtained on treatment with 1.5x of imidacloprid showed significant ($P<0.05$)

decrease in bacterial number and results were similar as in recommended rates but bacterial recovery was slower. This indicates that the toxic effect of imidacloprid is dose dependent, as reported in other pesticides like metoalchlor, atrazine, dimethoate and Endosulfan (Ismail BS and Shamsuddin N, 2005*;* Ayansina ADV and Oso BA, *2006 ;* Mandal MD, 2005; Digrak M and Kazanici F,1999).

Further, we attempted to study the effect of imidacloprid on growth and other biochemical parameters in soil isolate *Brevundimonas sp.* MJ15 cells that were exposed to different concentrations of imidacloprid ranging from 10^{-7} M to 10^{-3} M for a period of 96 hrs. The percent inhibition of these parameters namely DNA, RNA, proteins and glucose concentration were compared with the dose and duration of exposure of imidacloprid in *Brevundimonas sp.* MJ15 and the results revealed that the percent inhibition of all the parameters increased significantly ($P< 0.05$) with an increase in the dose and duration of exposure of imidacloprid indicating that the imidacloprid has inhibitory effect on parameters studied. There was significant ($P<0.05$) increase in growth inhibition in treated groups when compared with that of control. Similar results were reported involving stress proteins of *E.coli* induced in response to the pesticides cypermethrin, zeta-cypermethrin, carbofuran, bifenthrin and methomyl (Asghar MN et al. 2006; Kulkarni AG and Kaliwal BB, 2008).

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. Genetic responses to oxidative stress are known to occur in bacteria, yeast, mammalian cell lines and in general in all aerobic organisms (Farr SB and Kogoma T, 1991; Hidalgo E and Demple B, 1995). It is reported that biological targets for the reactive oxygen species due to oxidative stress are RNA, DNA, proteins and lipids (Cabiscol E et al. 2000) or due to inhibitory action of enzymes and induction of apoptosis which in turn cause damage to DNA and RNA or may have possibly caused the disturbance in the cell division machinery (Awasthi M et al. 1984).

Further, an increase in the percent inhibition in protein and glucose 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, flourescence, fragmentation, formation of protein-protein crosslink's, s-s bridges and increased susceptibility to proteolysis (Stadtman ER. 1992). The secretion of extra cellular proteins, including toxins and cellular effectors, is one of the key contributing factors in a bacterium's ability to thrive in diverse environments. 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 or due to the disturbance in the cell division machinery (Cabiscol E et al. 2000).

The study of growth kinetics provides an evidence of mineralization potential of organism therefore such studies were carried out by several researchers (Kulkarni AG and Kaliwal BB, 2008). 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 (Ekundayo EO, 2006). 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.(Tormo et al, 1990). 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

Brevundimonas sp. MJ15 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.

CONCLUSION

The results of the present investigation show that imidacloprid has a negative effect on bacterial populations in soil. The laboratory and field studies on effects of imidacloprid on soil bacterial populations were dose dependent and in field studies the negative effect reduced after 14 days of application. This may be due to increase in imidacloprid tolerant bacterial population in soil. The studies on DNA, RNA, protein and glucose concentrations in soil isolate Brevundimonas *sp.* MJ15 revealed that imidacloprid affected all the above biochemical parameters and the inhibitory effect was dose and duration dependent. The study on growth of the Brevundimonas sp. MJ15 showed inhibitory effect of imidacloprid on growth of the bacterial cell and was dose dependent. Finally, we can conclude that imidacloprid has toxic effect on bacterial populations in soil, the toxic effect may be due to inhibition of synthesis of biochemical contents like DNA, RNA, protein and glucose which are required for growth of a bacterial cell. However, in order to judge the overall long- term effects of imidacloprid application on soil bacteria and other microorganisms, extensive work should be done in different cropping systems and soil varieties with a specific agriculturally important group of microorganisms for achieving a comprehensive understanding.

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