IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 3, Issue 4 (Jul. – Aug. 2017), PP 53-56
www.iosrjournals.org

Effect of Imidacloprid Intoxication on growth and phosphatase Activity in Soil Isolate *Bacillus weihenstephanensis* strain

A. Shetti[1], B. B. Kaliwal[2]*

[2]*P. G. Department of Studies and Research in Biotechnology, Davangere University, Davangere 577 002, Karnataka, India.*

[1]*Post-Graduate Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad - 580003, India.*

Corresponding Author: B. B. Kaliwal

Abstract: *Imidacloprid, , is a neonicotinoid, a class of neuro-active insecticides modeled after nicotine. The present investigation was carried out to evaluate the effect of imidacloprid treatment on growth and phosphatase activity in soil isolate Bacillus weihenstephanensis (SP-03). The soil isolate was isolated after enrichment cultures, as imidacloprid tolerant bacteria. Cells of Bacillus weihenstephanensis were exposed to imidacloprid of concentrations ranging from 10^{-7} to 10^{-3} M for a period of 96 hrs. Treatment with higher dose (10^{-4} and 10^{-3} M) of imidacloprid caused no significant increase in the phosphatase activity, whereas, significant increase was observed in the lower dose (10-7, 10-6 and 10-5 M) of imidacloprid. The growth of the isolate was dose dependent and increase in concentration of imidacloprid lead to decreased growth. The results of present investigation revealed that imidacloprid intoxication effects the growth and development of bacteria by inhibiting metabolic enzymes and other proteins necessary for the growth.*

Keywords: *Bacillus weihenstephanensis, phosphatase, Growth , Imidacloprid.*

Date of Submission: 10-07-2017 Date of acceptance: 22-07-2017

I. Introduction

Various insecticides to protect crops against insects are been used world wide. Most insecticides are applied by spraying in large quantities, thus inducing pollution of air, soils and waters (Pimentel, 1983). The residual pesticides may become the contamination sources and pose a serious threat to the soil and groundwater environment through the rainfall infiltration process. Some pesticides act on biochemical processes that are common to many animals, plants and microorganisms, and thus are a greater hazard to non-target organisms. Many pesticides are applied directly to soil, while of those applied to crop foliage, a large percentage will also enter the soil. For example, in a field experiment where the fungicide propiconazole was sprayed onto winter wheat, 15 - 45 % of the chemical was directly deposited on the soil surface, depending on the time of application. It has been estimated that, often, less than 0.1% of pesticides applied to crops actually reaches the target organisms (Pimental 1983).

The effect of pesticides on microbial populations is well studied, the populations from the pesticide treated plot (Aldicarb, Chlorfenvinphos, Benomyl, Glyphosate and Chlorotoluron) showed a higher rate of substrate utilization indicating grater metabolic diversity in the population. In a study the soil sample treated with Decis the total viable bacterial number was found to be higher than control and Bivistin and SDMA pesticides had no inhibitory effect on the number of total microorganisms. It is reported that Thiodan and Karate incicticides significantly reduced the fungi, actinomycetes population in the soil but bacterial numbers increased significantly (Adebayo 2007). Atrazine and atrazine and Metolachor applied in company recommended rates resulted in decrease in microbial count. Higher rate resulted in much lower microbial count. *Psuedomonas Sp.* and *Bacillus Sp*. were isolated frequently from treated samples (Ayansina and Oso, 2006). Ismail and Shamusuddin (2005) observed that after several years of application the faster break down of pesticides take place this is due to microbial adaptation to pesticides and its degradation in group or single.

Imidacloprid, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, is a neonicotinoid, a class of neuro-active insecticides modeled after nicotine. Today imidacloprid is used in over 120 countries to treat more than 140 different crops (Krohn and Hellpointner 2002; Liu et al. 2006). The high water solubility and low Koc, indicate a low tendency to be adsorbed to soil particles. Field studies show that imidacloprid can persist in soil, with a half-life ranging from 27 to 229 days. Increasing use of imidacloprid and potential toxicity among humans warrants a heightened awareness about this compound (David et al. 2007). Therefore the present investigation was carried out to evaluate the effect of imidacloprid treatment on growth and phosphatase activity in soil isolate *Bacillus weihenstephanensis*.

II. 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 and identified from soil as described in our previous publication (Shetti and Kaliwal, 2012). 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 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 (Shetti and Kaliwal, 2012). The pure culture was grown on nutrient agar medium.

Experimental Procedures

Five ml of the pre-inoculum was inoculated to 250ml Erlenmeyer's flask containing 100ml 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.

Growth

The concentration of cells was measured every 24 hrs using spectrophotometer taking optical density (OD) at 600 nm described by Kosmachevskaya et al., (2007).

Phosphatase activity

Alkaline phosphatase was estimated as per the procedure of Vastraete et al., (1976). Two ml of the sample or an aliquot diluted to 2ml was incubated with 2 ml of tris buffer pH 8.4and 1ml of 0.1mM p-nitrophenol phosphate incubated for 4 hours and 2 ml of the reaction mixture was added to 2ml of 0.5 N NaOH and 2 ml of 0.05 M EDTA. OD was measured at 420nm against a blank treated in the same way. The amount of p-nitrophenol released was calculated by referring to the standard graph of p-nitrophenol. The unit of phosphatase activity is the micro grams of p-nitrophenol 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 ($P \pm < 0.05$).

III. Results

Effect of imidacloprid on growth of Bacillus weihenstephanensis

The optical density in *Bacillus weihenstephanensis* at 24, 48, 72 and 96 hrs was 0.268, 0.445, 0.532 and 0.626 respectively in the control groups. On treatment with the lowest concentration of 10-7 M of imidacloprid optical density was 0.261, 0.390 0.487 and 0.571 and the inhibition observed was 2.62, 12.36, 28.21 and 8.79 % treated for 24, 48, 72 and 96 hrs respectively. On treatment with 10-6 M of imidacloprid the optical density was 0.224, 0.322, 0.436 and 0.495 and their corresponding inhibition obtained was 16.42, 27.65, 38.46 and 20.93 % for 24, 48, 72 and 96 hrs respectively. Treatment with 10-5 M concentration of imidacloprid showed the optical density of 0.189, 0.285, 0.397 and 0.431and the corresponding inhibition observed was 29.48, 35.90, 45.15 and 32.16 % at a given duration of 24, 48, 72 and 96 hrs respectively. At 10-4 M concentration of imidacloprid the optical density was 0.162, 0.257, 0.331 and 0.390 with their corresponding inhibition of 39.56, 42.25, 43.97 and 37.70% at a given duration of 24, 48, 72 and 96 hrs respectively. On treatment with 10-3 M of imidacloprid the optical density was 0.134, 0.235, 0.285 and 0.343 and their corresponding inhibition was 50.00 47.20, 46.66 and 45.21% at a given duration of 24, 48, 72 and 96 hrs respectively.

Effect of imidacloprid on phosphatase in *Bacillus weihenstephanensis*

The activity of phosphatase observed in the control groups of *Bacillus weihenstephanensis* was 0.358, 0. 442, 0.496 and 0.578 U at duration of 24, 48, 72 and 96 hrs respectively. On treatment with 10-7 M of imidacloprid the phosphatase activity was0.404, 0.480, 0.572 and 0.592 U at duration of 24, 48, 72 and 96 hrs

respectively. Treatment with 10-6 M of imidacloprid showed an activity of 0.460, 0.512, 0.589 and 0.628 U at the duration of 24, 48, 72 and 96 hrs respectively. On treatment with 10-5 M of imidacloprid the phosphatase activity was 0.524, 0.593, 0.672 and 0.703 U at duration of 24, 48, 72 and 96 hrs respectively. Treatment with 10-4 M of imidacloprid showed an activity of 0.620, 0.652, 0.704 and 0.771 U at the duration of 24, 48, 72 and 96 hrs respectively. On treatment with 10-3 M of imidacloprid the phosphatase activity was 0.646, 0.716, 0.772 and 0.853 U at duration of 24, 48, 72 and 96 hrs respectively.

IV. Discussion

Phosphatases are enzymes, which hydrolyze esters and anhydrides of phosphoric acid contributing to increase the amount of phosphorous available; these activities have been detected in bacteria, zooplankton and phytoplankton (Petersson, 1980). Phosphatase is a nonspecific phosphomonoesterase, which removes inorganic phosphate from phosphoric esters or transfers the phosphoryl group to an acceptor hydroxyl group (Fernley, 1971). They are widely found in various organisms, indicating their important role in metabolism of different phosphorus containing organic compounds (McComb *et al*., 1979) and *Escherichia coli* phosphatase has been studied in most detail by Yerchenko *et al*., (2003). Phosphatases are lysosomal enzymes which catalyze the splitting of phosphoric acid from certain phosphoric esters. They help in autolysis of the degenerated cells and mediate membrane transport (De Duve *et al*., 1955). In a study it is reported that the presence of organochlorinated insecticides (aldrin and lindane), organophosphorous insecticides (dimetoate, methidation and methyl-parathion), atrazine (herbicide) and captan (fungicide) significantly increased phosphatase activities after 28 days of incubation. Heterotrophic mesophilic and psychrophilic aquatic bacteria counts as well as culturable phosphate solubilizing microorganisms, increased when the pesticides were added to lake water samples (Lo *et al.,* 2006).

In the present graded dose and durational exposure study of imidacloprid it was found that the phosphatase activity in the treated groups increased significantly in higher dose (10^{-5}, 10^{-4} and 10^{-3} M) of exposure to imidacloprid, where as there was no significant increase observed in the lower dose (10^{-7} and 10^{-6} M) of imidacloprid in *Bacillus weihenstephanensis.* Similar results were obtained by Perisamy and Raman, (1995), who reported that phosphatase, is produced under stress conditions on exposure to toxic chemicals like heavy metals to degrade the phosphate groups of nucleic acid and this enzyme occurs in all living cells. It has been reported that elevated levels of phosphatase was observed on exposure to carbamates which may be indicative of an adaptive rise in enzyme activity in response to persistent stress. Ponmurugan and Gopi (2006) have suggested that for phosphate solubilizations, the bacteria produce the phosphatase enzyme. It has been reported that the synthesis of phosphatase takes place as an adaptive mechanism to chemical stress. It is suggested that the contaminants like metal ions, can inhibit apatite formation and growth, cell proliferation, extra cellular mineralization and specific cellular functions, such as phosphatase activity (Park *et al*., 2006). It has been demonstrated that, a positive correlation exists between phosphate solubilizing capacity and phosphatase enzyme activity, which may be due to the availability of higher amount of phosphorus and/`or soluble phosphates in the medium.

Growth

The term growth as commonly applied to bacteria and other microorganisms usually refers to changes in the total population rather than an increase in the size or mass of an individual organism, growth denotes the increase in number beyond that present in the original inoculum (Pelczar *et al*., 1993). In the present graded dose and durational exposure study, there was a significant decrease in the growth in all the groups treated with imidacloprid in *Escherichia coli*, *Brevundimonas Sp. MJ 15* and *Bacillus weihenstephanensis* with an increase in the % inhibition. Present investigation also revealed that *Bacillus weihenstephanensis* showed higher growth on treatment with imidacloprid than other two soil isolates. The study on growth kinetics provides an evidence of mineralization potential of organism (Lee *et al.,* 1998). Cypermethrin and monocrotophs had adverse effect on the total number of soil bacteria (Rangaswamy and Venkateshwarlu, 1992). In a study Endosulfan inhibited the growth of *B. subtilis* in 32-80 µ/l concentrations. In the study there was no growth observed above 64 µ/l concentration of endosulfan (Tolan and Ensari, 2006). The imidacloprid might have affected the bacterial growth via possible attack to the membrane components and inhibited activity of DNA polymerase I. 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 (Johansen *et al.,* 2001). 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).

V. Conclusion

The present investigation was carried out to evaluate the effect of a neonicotinoid, class of neuro-active insecticides imidacloprid treatment on growth and phosphatase activity in soil isolate *Bacillus weihenstephanensis* (SP-03). The soil isolate was isolated after enrichment cultures, as imidacloprid tolerant bacteria. Treatment with higher dose (10^{-4} and 10^{-3} M) of imidacloprid caused no significant increase in the phosphatase activity, whereas, significant increase was observed in the lower dose (10-7, 10-6 and 10-5 M) of imidacloprid. The growth of the isolate was dose dependent and increase in concentration of imidacloprid lead to decreased growth. The results of present investigation revealed that imidacloprid intoxication effects the growth and development of bacteria by inhibiting metabolic enzymes and other proteins necessary for the growth. The mechanism of imidacloprid intoxication in prokaryotes should be studied in- detail.

References

[1] Adebayo, T. A., Ojo, O. A. and Olaniran, O. A. (2007). Effect of two insecticides Karate and Thiodan on Population Dynamics of Four Different Soil Microorganism. *R.J.B.S.*, **2(5),** 557-560.

[2] Ayansina, A. D. V. and Oso, B. A. (2006) Effect of two commonly used herbicides on soil microflora at two different concentrations. *A.J.B.*, **5(2),** 129-132.

[3] David, D., George, I. A. and Peter, J. V. (2007) Toxicology of the newer neonicotinoid insecticides: imidacloprid poisoning in a human. *Clin. Toxicol*., **45**, 485–486.

[4] De Duve, C., Presman, B. G., Gianetto, G., Wattiau, R. and Appelmans, F. (1955). Intra cellular distribution pattern of enzymes in rat liver tissue. *Biochem J.*, 60**,** 604-617.

[5] Fernley, H.N. (1971). Mammalian alkaline phosphatases. In: *Enzymes*. Boyer P.D. Ed. N.Y.: Acad. Press, **4**, 417–447.

[6] Ismail, B. S. and Shamsuddin, N. (2005). Effects of Alachlor and Metolachor on microbial populations in the soil. *M.J.M.*,**(1),** 36-41.

[7] Johansen, K., Jacobsen, C. S. and Torsvik, V. (2001). Pesticide effects on bacterial diversity in agricultural soils – a review. *Biol. Fertil. Soils*., **33**, 443–453.

[8] Kosmachevskaya, O. V., Shumaev, K. B., Arredondo-Peter, R. and Topunov, A. F**. (2007).** Influence of Tert-Butyl Hydroperoxide and nitrosoglutathione on *Escherichia coli* cells expressing leg-hemoglobin. *Journal of Stress Physiology & Biochemistry*., **3**, 18-24.

[9] Krohn, J. and Hellpointner, E.(2002) Environmental fate of imidacloprid. *Pflanzenschutz- Nachr Bayer*., **55**, 3–26 (special edition).

[10] Lee, S. G., Yoon, B. D., Park, Y. G. and Oh, H. M. (1998). Isolation of a novel pentachlorophenol degrading bacterium, *Pseudomonas sp*. Bu 34. *J. Appl. Microbiol.*, **85**, 1-8.

[11] Lo´ pez L, Pozo C, Rodelas B, Calvo C, Gonzalez-Lo´pez J. 2006 – Influence of pesticides and herbicides presence on phosphatase activity and selected bacterial microbiota of a natural lake system. Ecology and Toxicology 15, 487–493.

[12] McComb, R. B., Bowers, G. N. and Posen, S. (1979). Alkaline Phosphatase. (New York, NY: Plenum Press).

[13] Park, S. M. Kim, H. S. and Yu, T. S. (2006). Effect of Titanium Ion and Resistance Encoding Plasmid of *Pseudomonas aeruginosa* ATCC 10145. *The Journal of Microbiology*., 44**(3),** 255-261.

[14] Pelczar, M. J, Chan, E. C. S. and Krieg, N. R. (1993). In: Microbiology. Concept and Application. Tata Ed., Mc Graw- Hill Inc., New York. 281-324.

[15] Perisamy, K. and Raman, K. (1995). Effects of cu and Ni on acid phosphatase activity on ectomycorrhizal fungus, *Laccaria Laccata. Ind. J. Agri.Sci*., **72,** 249-253.

[16] Petersson, K. (1980). Alkaline phosphatase activity and algal surplus phosphorous as phosphorous deficiency indicator in Lake Erken. *Arch Hydrobiol.*, **89**,54-59.

[17] Pimentel, D. (1983). Effects of pesticides on the environment.' 10th Intl. Congress on Plant Protection. *Crydon.*, **2**, 685-691.

[18] Ponmurugan, P. and Gopi, C**.** (2006). *IN VITRO* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. *American journal of biotechnology*, **5(4),** 348-350.

[19] Rangaswamy, V. and Venkateswarlu, K. (1992). Degradation of selected insecticides, monochrotophos, quinalphos, cypermethrin and fenvalerate, by bacteria isolated from soil. *Bull. Env. Contam. Toxic*., **49(6)**, 797-804.

[20] Tolan, V. and Ensari, Y. (2006). Effect of endosulfan on growth, α-amylase activity and plasmids amplification in *Bacillus subtilis. Indian journal of Biochemistry and Biophysics.*, **43**, 123-26.

[21] Tormo, A., Almiron, M. and Kolter, R. (1990). Sur A, an *Escherichia coli* gene essential for survival in stationary phase. J. Bacteriol., 172, 4339-4347.

[22] Yerchenko, V., Khromov, I. S. and Budilov, A. V. (2003). Thermostable alkaline phosphatase of bacterium *Meiothermus rubber*: Gene cloning, expression in *Escherichia coli*, and biochemical charecterization of the recombinant protein. *Molecular Biology*., **37(6)**, 841-848.

IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

A. Shetti. "Effect of Imidacloprid Intoxication on growth and phosphatase Activity in Soil Isolate Bacillus weihenstephanensis strain." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 3.4 (2017): 53-56.