ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 09, pp.19901-19907, September, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF IMIDACLOPRID ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN *ESCHERICHIA COLI AND BREVUNDIMONAS* SP

Shetti, A.A. and *Kaliwal, B.B.

Department of Biotechnology and Microbiology Karnatak University, Dharwad 580003, Karnataka, India

ARTICLE INFO

Article History:
Received 10th June, 2015
Received in revised form
21st July, 2015
Accepted 15th August, 2015
Published online 16th September, 2015

Key words:

Imidacloprid, *Escherichia coli, Brevundimonas Sp.* MJ 15, Antioxidant enzymes

ABSTRACT

Imidacloprid is a neonicotinoid compound, which is a neuro-active insecticide. The over use of this pesticide requires higher awareness about this pesticide. Present investigation was carried out to analyze the effect of imidacloprid on metabolic enzymes amylase, protease and phosphatase in soil isolates *Escherichia coli* and *Brevundimonas Sp.* MJ 15 Study on effect of exposure of 10^{-7} to 10^{-3} Molar concentrations of imidacloprid for a period of 24, 48, 72 and 96 hrs on three antioxidant enzymes Catalase Peroxidase and superoxide dismutase showed that there was an increase in the activity of all the three enzymes significant increase was observed for doses (10^{-3}-10^{-7}) of imidacloprid. The enzyme activity increased with an increase in the concentration of insecticide at lower doses proving that the inhibitory effect is dose dependent. The present investigation indicates that imidacloprid induces the significant increase in activity of antioxidant enzymes in the soil isolates. This proves that imidacloprid induces oxidative stress in bacterial isolates.

***Citation*:** Shetti, A.A. and Kaliwal, B.B, 2015. "Effect of Imidacloprid on the activity of antioxidant enzymes in *Escherichia coli and Brevundimonas* sp", *International Journal of Current Research*, 7, (9), 19901-19907.

INTRODUCTION

Insecticides are agents of chemical or biological origin that control insects. Control may result from killing the insect or otherwise preventing it from engaging in behaviors deemed destructive. Insecticides may be natural or manmade and are applied to target pests in a myriad of formulations and delivery systems (sprays, baits, slow-release diffusion, etc.). The science of biotechnology has, in recent years, even incorporated bacterial genes coding for insecticidal proteins into various crop plants that deal death to unsuspecting pests that feed on them. Some 10,000 species of more than 1 million species of insects are crop-eating, and of these, approximately 700 species worldwide cause most of the insect damage to man's crops, in the field and in storage (Ware and Whitacre, 2004). Oxidative stress is a misbalance between reactive oxygen species (ROS) generation and detoxification resulting in the increased levels of enzyme activity. ROS are of increasing interest in environmental toxicity as they may provide insights to toxicity mechanisms and may identify novel biomarkers. ROS can modify and inactivate proteins in a variety of ways. It is commonly recognized that *Escherichia coli* is the most suitable model system for the investigation of the cell response to oxidative stress (Semchyshyn *et al*., 2004).

Corresponding author: Kaliwal, B.B.
Department of Biotechnology and Microbiology Karnatak University, Dharwad 580003, Karnataka, India

When organisms or cells are exposed to low levels of certain harmful physical or chemical agents, the organisms acquire an induced tolerance against the adverse effects (Flahaut *et al*. 1996). The effect of hydrogen peroxide on the activity of Sox RS and Oxy R regulon enzymes in different strains of *Escherichia coli* (Semchyshyn *et al*., 2004). The soil isolates *Escherichia coli*, *Pseudomonas* and *Bacillus subtilis* on exposure to acetemaprid resulted in stress enzymes synthesis (Yao *et al*. 2006). Super oxide dismutase (SOD) are one of the important enzymes of the defense systems against oxidative damage in aerobic organisms (Gerlach *et al*., 1998), catalyzes superoxide ion (O_2^-) to O_2 and H_2O_2, which is then reduced to H_2O by H_2O_2 scavenging enzyme catalase (Jung, 2003). Catalase (kat) and superoxide dismutase (SOD) are among the most potent antioxidants known in the nature. Catalase is among the most efficient antioxidants known so far. It is present in the peroxisomes of nearly all aerobic cells and serves to protect the cell from the toxic hydrogen peroxide effects by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. Catalase acts on the toxic compounds by per oxidative reaction (Stefano *et al*., 2004).

Peroxidase (POX) and superoxide dismutase (SOD) form the first line of defense against reactive oxygen species (Lushchak, 2001). Catalase and/or peroxidase is one of the radical scavenging enzymes in cells produced in response to oxidative stress and has been observed in *Escherichia coli,*

Pseudomonas putida, Streptomyces ceolicolor and *Arcobacter nitrofrigilis* (Muley, 1990; Park, 1997). Oxygenation is the most frequent step in the bio-transformation of pesticides and other organic xenobiotics and many of these reactions are mediated by oxidative enzymes such as peroxidase.

The use of biological methods in environmental monitoring is essential in order to complement chemical analysis with information about actual toxicity or genotoxicity of environmental samples. Microorganisms are widely applied test-species in different bioassays because of the ease and low costs of their culturing as well as the lack of ethical issues often accompanying the use of higher organisms. With the development of toxicogenomic approaches, the use of microorganisms for environmental monitoring purposes is expected to become even more extensive because of better knowledge about potential analogies in toxicity mechanisms between higher organisms and microbes (Logar and Vodovnik, 2007). 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 *et al.,* 2003). Today imidacloprid is used in over 120 countries to treat more than 140 different crops (Krohn and Hellpointner, 2002).

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 *et al*. 1998). Imidacloprid is very persistent in soil with half- life often greater than 100 days (Scholz and Spiteller, 1992). It is very much clear from the above findings that pesticides interact with different components and enzymes of the target and non-target organisms, thereby impairing the physiological and metabolic activities of the cell. The enzymes and other biochemical indices are providing sensitive index to the changes due to pesticide toxicity. These parameters are widely used for rapid detection and to predict early warming of pesticide toxicity. Therefore, imidacloprid, a chloronicotinyl pesticide was evaluated for its toxicity to soil isolates *Escherichia coli, Brevundimonas Sp.* MJ 15 and *Bacillus weihenstephanensis* with emphasis on its effect on antioxidant enzymes.

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. 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 isolated and identified morphological, cultural and biochemical characters and 16S rDNA identification as described in our previous publication (Shetti and Kaliwal, 2012). 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 37^0C for 10 min and centrifuged at 10,000 rpm for 10 min. Supernatant was used as the source of enzyme.

Estimation of antioxidant enzyme activity

The activity of SOD was assayed using the supernatant from centrifuged (15000 rpm) 12 min at 4^0 C homogenate in 50mM sodium phosphate buffer of (Beauchamp and Fridovich, 1971). The assay method put forth by was followed. Catalase enzyme was assayed using and peroxidase were carried out according to (Sadashivam and Manickam 1996).

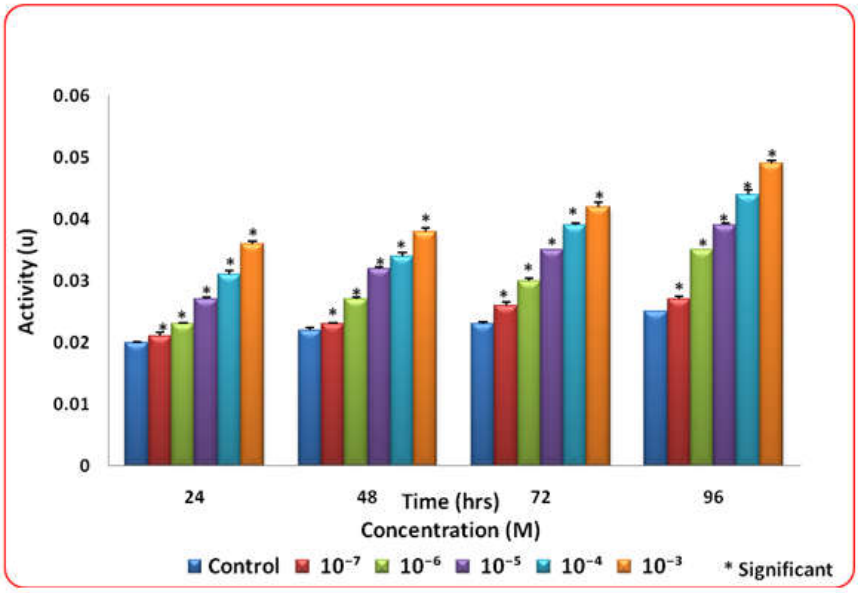
RESULTS

Effect of imidacloprid on super oxide dismutase activity of *Escherichia coli* and *Brevundimonas Sp.* MJ 15

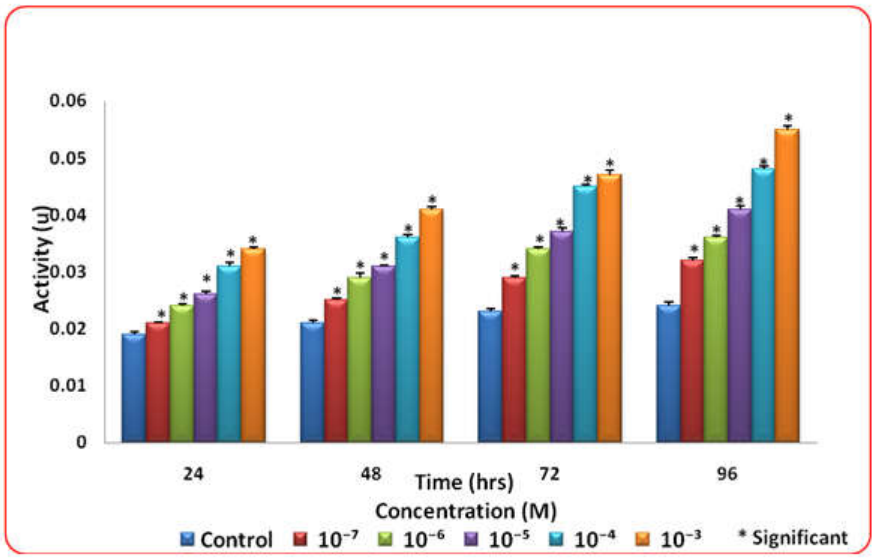
In the present study it was observed that the super oxide dismutase activity in all the treated groups increased significantly on imidacloprid exposure when compared with that of the corresponding controls. Super oxide dismutase activity in the treated groups increased significantly in higher dose ($10^{-5,}$ 10^{-4} and 10^{-3} M) and lower dose (10^{-7} and 10^{-6} M) of imidacloprid exposure in *Escherichia coli* and *Brevundimonas Sp.* MJ 15 (Graph 1 and 2).

Effect of imidacloprid on catalase activity in *Escherichia coli* and *Brevundimonas Sp.* MJ 15

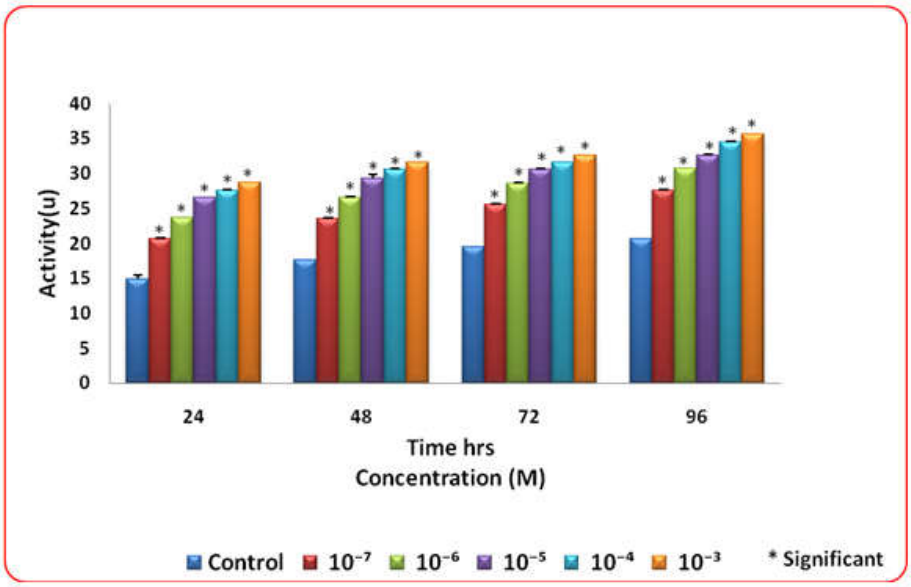
The present study revealed that the catalase activity was significantly increased in all the groups with increase in the dose and durational exposure of imidacloprid when compared with that of the control.



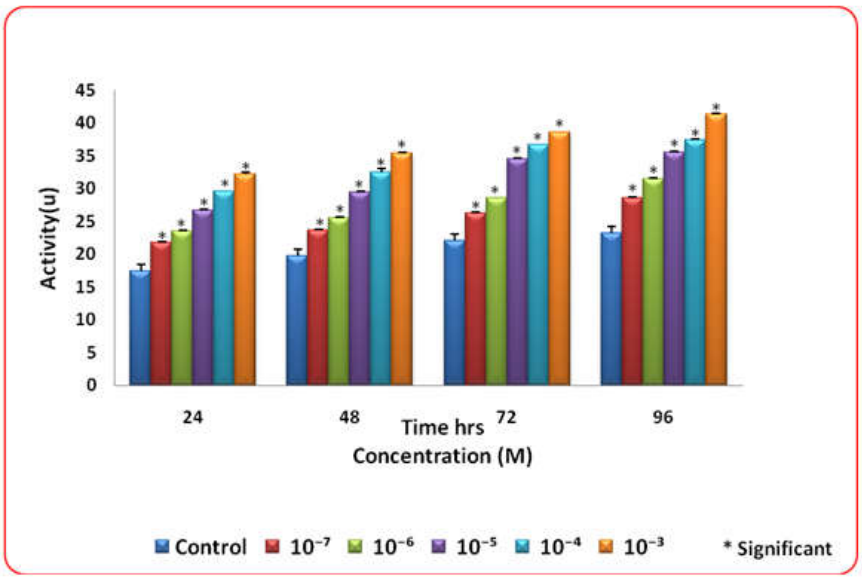
Graph 1. Effect of imidacloprid on superoxide dismutase activity in *Escherichia coli*



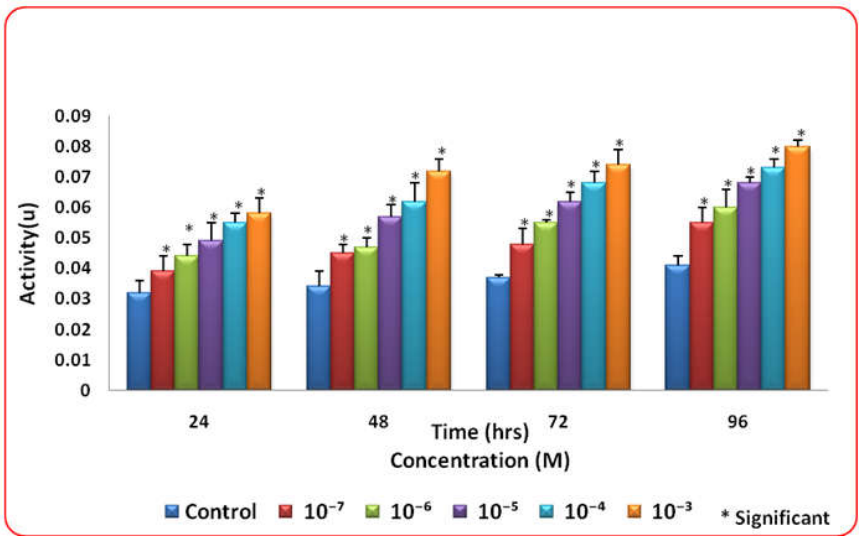
Graph 2. Effect of imidacloprid on superoxide dismutase activity in *Brevundimonas Sp.* MJ 15



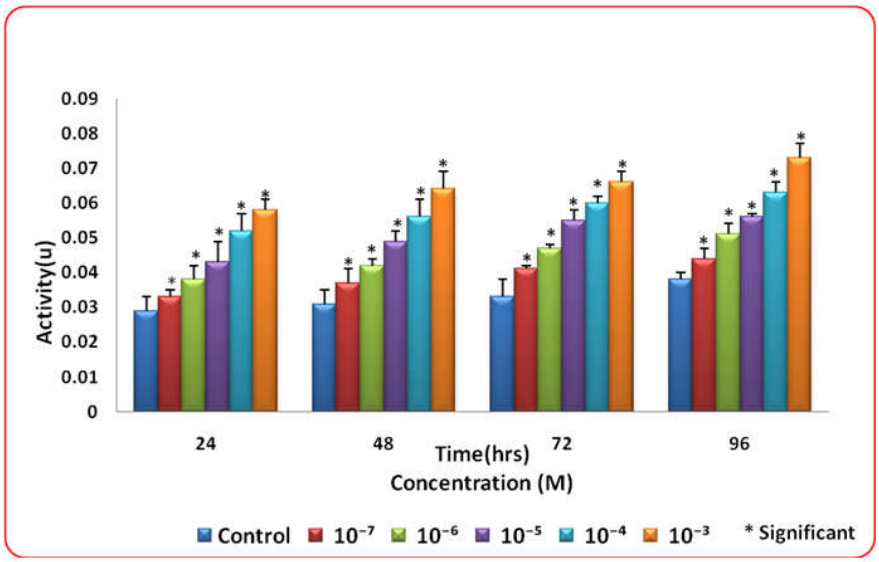
Graph 3. Effect of imidacloprid on catalase activity in *Escherichia coli*



Graph 4. Effect of imidacloprid on catalase activity in *Brevundimonas Sp.* MJ 15



Graph 5. Effect of imidacloprid on peroxidase activity in *Escherichia coli*



Graph 6. Effect of imidacloprid on peroxidase activity in *Brevundimonas Sp.* MJ 15

Treatment with graded dose and durational exposure of imidacloprid caused a significant increase in the catalase activity in all the groups of *Escherichia coli* and *Brevundimonas Sp.* MJ 15 when compared with that of control (Graph 4 and 5).

Effect of imidacloprid on peroxidase activity in *Escherichia coli* and *Brevundimonas Sp.* MJ 15

The present study revealed that the peroxidase 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 when compared to their corresponding controls in *Escherichia coli* and *Brevundimonas Sp.* MJ 15 (Graph 7 and 8).

DISCUSSION

Effect of imidacloprid on the activity of antioxidant enzymes in soil isolates *Escherichia coli* and *Brevundimonas Sp.* MJ 15

Partial reduction of oxygen to water during microbial respiration gives rise to reactive oxygen intermediates, e.g. superoxide radicals, hydrogen peroxide and hydroxyl radicals (Fridovich, 1978; Gregory and Dapper, 1983). Microorganisms have developed efficient enzymatic and nonenzymatic mechanisms to eliminate these toxic and mutagenic reactive oxygen species (Storz *et al*. 1990). Superoxide is eliminated by dismutation to H_2O_2 catalyzed by superoxide dismutase (Fridovich, 1978) and accumulation of H_2O_2 is prevented by the action of catalases and peroxidases (Hassan and Fridovich, 1978). SODs are essential for aerobic survival and are ubiquitous among aerobic and aerotolerant organisms (Hassan, 1989) and even some anaerobic organisms. The SODs are metalloenzymes and exhibit strict metal cofactor specificity (Ose and Fridovich, 1976; Kirby *et al*. 1980). Substitution of a wrong metal in *E. coli* renders the enzyme inactive (Ose and Fridovich, 1976). Bacteria in natural environment experience oxidative stress from different sources. The reactive oxygen species (ROS) are produced in cells, not only during microbial aerobic growth as by-products of normal cellular metabolism, but also under stress situations induced by pesticides causes generation of free radicals and alters antioxidants (Sunters, 2002; Lu, 2004).

Numerous pesticides such as paraquat, DDT, PCB, Arochlor etc have been used as model factors inducing oxidative stress both *In-vivo* and *In vitro* (Bus and Gibson, 1984; Soderlund *et al*., 2002). The conversion of pesticides to free radicals or via the formation of superoxide radical is a by-product of their metabolism and this is regarded as one of the basic mechanisms of tissue damage. Several studies have pointed out the risk of carcinogenic effect, neurological actions and brain damage in living organisms exposed to various concentrations of xenobiotics in the environment (Palmeira, 1999). In other to ameliorate these damages, organisms have evolved mechanisms to control the amount of hydroxyl and superoxide radicals generated. These fragments are quickly scavenged by natural protective molecules in the cells called antioxidants (Guven and Kaya, 2005). Antioxidants are intimately involved in the prevention of cellular damage – the common pathway for cancer, aging and a variety of diseases (Palmeira, 1999). They safely interact with free radicals and terminate the chain reactions before vital molecules are damaged, antioxidants could be enzymatic or nonenzymatic, the latter include glutathione reductase, glutathion-S-transferase, glutathione peroxidase, superoxide dismutase (SOD) and catalase (Al-Omar *et al*. 2004). The studies with *E. coli* showed that paraquat induces about 40 proteins in this bacterium, most of which have not been identified, including antioxidant and repair enzymes. Some of these are positively or negatively regulated at the transcriptional level (Tsaneva and Weiss, 1990). SOD is an important enzyme family in living cells for maintaining normal physiological conditions and for coping with stress. The action of SOD therefore, is to protect the biological integrity of the cells and tissues against harmful effects of superoxide free radical (Bowler, 1992). Humans and other non-target organisms are sensitive to a great number of pollutants in the environment such as pesticides and heavy metal. Bioaccumulation of these pollutants in the biological system may pose a serious challenge to public health. SOD dismutates O_2 - to H_2O_2, POD subsequently scavenge the H_2O_2. The enzymatic status of SOD indicates the level of generations of free radicals or reactive oxygen species (ROS) in any plant, exposed to biotic and abiotic stress conditions (Xiong and Zhu, 2002). They further explained that the scavenging of ROS is associated with antioxidative processes of the cell. The harmful influence of ROS on macromolecules in cell is alleviated by the activities of SOD (Zheng *et al*., 2001; Xiong and Zhu, 2002).

Acetamiprid caused oxidative stress on the three bacteria *B. subtilis, E. coli* K12 and *Pseudomonas sp*. FH2 which resulted in elevated SOD and CAT activities but also generated new SOD isozymes to antagonize oxidative stress. In the present study it was observed that the super oxide dismutase activity in the treated groups increased significantly in higher dose ($10^{-5,}$ 10^{-4} and 10^{-3} M) of exposure to imidacloprid, whereas, no significant increase was observed in the lower dose (10^{-7} and 10^{-6} M) of imidacloprid exposure in *Escherichia coli* and *Brevundimonas Sp*. MJ 15. *Escherichia coli* possess antioxidant enzymes which are induced in response to oxidative stress (Smith *et al*. 2003). In a study the genetic responses to superoxide stress in *Pseudomonas putida* differ from those seen in *Escherichia coli* and *Salmonella* (Park *et al*. 2006). The significant increase in the superoxide dismutase activity observed in *Escherichia coli* and *Brevundimonas Sp. MJ 15* in the present study may be due to the oxidative stress induced by the toxicant (Sunters, 2002; Lu, 2004), induction of antioxidant proteins (Kim *et al*. 2002) or specific enzymes in response to oxidative stress (Scott and Easton, 1997), as a compensatory response to oxidative stress induced by this xenobiotic (Cristina *et al*. 2005). Catalase is one of the most efficient antioxidants known so far. It is present in peroxisomes of nearly all aerobic cells and protects the cells from the toxic hydrogen peroxide effects by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. In addition catalase is known to act on toxic compounds by per oxidative reactions (Kirkman and Geatani, 1984). It is demonstrated that acetamiprid induced oxidative stress on *Escherichia coli*, *Pseudomonas* sp and *Bacillus subtilis* resulted in elevated superoxide dismutase and

catalase activities to antagonize oxidative stress (Yao *et al*., 2006). The present study revealed that the catalase activity was significantly increased in all the groups with increase in the dose and durational exposure of imidacloprid to *Escherichia coli* and *Brevundimonas* Sp. *MJ* 15. Similarly, it has been reported that induction of major antioxidant enzymes, such as superoxide dismutase and catalase, were observed after their exposure to a single oxygen generating system in *Escherichia coli* (Kim *et al*., 2002). It is suggested in response to low concentrations of hydrogen peroxide induces catalase in *Escherichia coli* during logarithmic growth (Zaid *et al*., 2003). The increase in the activity of oxidative stress enzymes may be due to synthesis of these enzymes as an adaptive mechanism to chemical stress or due to inhibition of the membrane bound enzymes by affecting the enzyme complex (Elisa *et al*., 2000). Reactive oxygen species inactivate antioxidant enzymes. The oxidative processes lead to loss of key antioxidant enzymes (Tabatabaie and Floyd, 1994), which may increase cytotoxicity due to oxidative stress. Enhanced expression of SOD, catalase, and other antioxidant enzymes in prokaryotes and eukaryotes is to compensate for the inactivation of antioxidant enzymes by the toxicants. Superoxide dismutases and catalases are the first line of defense against oxygen-derived free radicals includes in *Escherichia coli*, and almost all other aerobic cells, (Zaid *et al*., 2003). Peroxidase is found among animals, plants and micro-organisms, where they perform essential roles in the metabolism (Spatafora *et al*., 2002). To prevent the lethal effects of such metal-ion-catalyzed oxidation (MCO), bacterial cells have evolved protective mechanisms to neutralize the formation of toxic oxygen radicals. For instance, small molecule antioxidants, such as catalases and peroxidases, have been reported to play protective roles in the enteric bacteria in *Pseudomonas* sp. and in *Bacteroides* sp. (Cha *et al*., 1995; Kim *et al*., 1996; Hassett *et al*., 1996; Rocha *et al*., 1996).

The enzyme peroxidase is an important antioxidant enzyme, which plays a pivotal role in plant growth and development. A close correlation exists between the enhanced activity of peroxidase (POD) and the concentration of phenolic substances. The increase in POD has been linked with resistance to stress and self defense. Under stress conditions the rate of respiration increases with stimulation in peroxidase activity (Weimberg, 1972). The increase in the peroxidase and catalase activity with an increase in the dose and duration of exposure observed in *Escherichia coli, Pseudomonas putida, Streptomyces coelicolor* and *Arcobacter nitrofigilis* (Zaid *et al*., 2003). Oxidative stress is experienced by Pollutant-degrading bacteria as a direct effect of the pollutants themselves, and from intermediates generated during biodegradation processes (Schewigert, 2001; Park *et al*., 2004). The present study revealed that the peroxidase activity in the treated groups increased significantly in higher dose (10^{-5}, 10^{-4} and 10^{-3} M) of exposure and there was no significant increase observed in the lower dose (10^{-7} and 10^{-6} M) of imidacloprid in *Escherichia coli* and *Brevundimonas Sp. MJ 15*. Similar results were reported in other organisms who suggested that a gradual increase of catalase or peroxidase production in aging cultures is not surprising since catalase and/or CP is one of the radical scavenging enzymes in cells in response to oxidative stress (Yun and Lee, 2000). On the other hand, several organisms produce two or more catalase peroxidase, whereby one enzyme was expressed at the end of exponential growth and during the stationary phase. This behavior was observed in *Escherichia coli, Pseudomonas putida, Streptomyces coelicolor* and *Arcobacter nitrofigilis* (Muley *et al*., 1990; Kim *et al*., 1992; Park and Hah, 1997). It is also reported that superoxide dismutase and peroxidase form the first line of defense against reactive oxygen species (McCord and Fridovich, 1969; Keele *et al*., 1970).

REFERENCES

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276.

Bowler, C., Montagu, M.V., Irize, D., 1992. Superoxide Dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 43: 83 – 116.

Bus, J.S., Gibson J.E., 1984. Paraquat model for antioxidant – initiated toxicity. Environ. *Hlth. Persp*., 55: 37-46.

Cha, M.K., Kim, H.K. Kim, I.H., 1995. Thioredoxin-linked ` thiol peroxidase' from periplasmic space of Escherichia coli. *J Biol Chem*., 270: 28635-28641.

Cristina, M., Andreea, C.S., Eugen, S., Lotus, E.M., Elena, I., Marieta, C., Anca, D., 2005. Liver, kidney and gill biochemical changes in carassius auratus gibelio during lindane acute intoxication, proceedings of the balkan scientific conference of biology in plovdiv (bulgaria). gruev, m. nikolova and a. donev., 682–692.

Elisa, Cabiscol., Jordi, Tamarit., Joaquim, Ros., 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Internatl Microbial.,* 3: 3–8.

Flahaut S., Hartke A., Giard J.C., Auffray Y., 1996. Alkaline stress response in *enterococcus feacalis* : adaptation, cross-protection , amd ghanges in protein synthesis. *Appl Environ Microbiol*., 62: 2416-2420.

Gerlach, D., Reichardt, W., Vettermann, S., 1998. Extracellular superoxide dismutase from Streptococcus pyogenes type 12 strain is manganese-dependent. FEMS Microbiol Lett.,160:217–24.

Gregory, E.M., Dapper, C.H., 1983. Isolation of iron-containing superoxide dismutase from Bacteroides fragilis: reconstitution as a Mn-containing enzyme. *Arch. Biochem. Biophys.,* 220: 293–300.

Guven, A., Kaya, N., 2005. Determination of reduced glutathion, glutathione-S-transferase and selenium levels in Goose liver cells with damage induced by carbon tetrachloride and ethanol. *Turk. J. Vet.* Anim. Sci., 29: 1233- 1238.

Hassan, H.M., Fridovich, I., 1978. Regulation of the synthesis of catalase and peroxidase in *Escherichia coli.* J. Biol. Chem., 253: 6445–6450.

Hassett, D.J., Sokol, P.A., Howell, M.L., Ma, J. F., Schweizer, H.T., Ochsner, U., Vasil, M.L., 1996. Ferric uptake regulator (Fur) mutants of Pseudomonas aeruginosa demonstrate defective siderophore-mediated iron uptake, altered aerobic growth, and decreased superoxide dismutase and catalase activities. *J. Bacteriol*., 178:3996-4003.

Kim, S.Y., Kim, E.J., Park, J.W., 2002. Control of singlet oxygen-induced oxidative damage in *Escherichia coli*. *Journal of Biochemistry and Molecular Biology.,* 35(4): 353-357.

Kirby, T., Blum, J., Kahane, I., Fridovich, I., 1980. Distinguishing between Mn-containing and Fe-containing superoxide dismutases in crude extracts of cells. *Arch. Biochem. Biophys*., 201: 551–555.

Kirkman, H., Gaetanit, G. F., 1984 .Catalase: A tetrameric enzyme with four tightly bound molecules of NADPH. *Proc. Natl. Acad. Sci. USA.,* Vol. 81: 4343-4347.

Keele, B.B., McCord, Jr., J.M., Fridovich, I., 1970. Superoxide dismutase from Escherichia coli B. A new manganesecontaining enzyme. J. Biol. Chem., 245:6176-6181.

Krohn, J., Hellpointner, E., 2002. Environmental fate of imidacloprid. Pflanzenschutz- Nachr Bayer*.,* 55: 3–26 .

Li XQ, Tan, A., Voegtline, M., Bekele, S., Chen, C.S. and Aroian, R.V. 2008. Expression of Cry5B protein from Bacillus thuringiensis in plant roots confers resistance to root-knot nematode. *Biol. Control*., 47: 97-102.

Logar R.M., Vodovnik, M., 2007. The applications of microbes in environmental monitoring Communicating Current Research and Educational Topics and Trends in Applied Microbiology Méndez-Vilas (Ed.).

Lu, Z.M., Min, H., Ye, Y.F., 2004. Short-term influence of herbicide quinclorac on enzyme activities in flooded paddy soils. Pedosphere., 14(1):71-76.

Lushchak, 2001. Oxidative stress and mechanisms of protection against it in bacteria. Biochemistry (Mosc)., 66: 476-89.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymatic function for erythrocuprein (Hemocuprein). *J. Biol. Chem.,* 244: 6049-6055.

Muley, M.R., Switala, J., Borys, A., Loewen, P.C., 1990. *J. Bacteriol.,* 172: 6713-15.

Nauen, R., Ebbinghaus-Kintscher, U., Schmuck, R., 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Managem Sci., 57: 577–586.

Okazawa, A., Akamatsu, M., Ohoka, A., Nishiwaki, H., Cho, W., Nakagawa, N., Ueno, T., 1998. Prediction of the Binding Mode Of Imidacloprid And Related Compounds To House-Fly Head Acetylcholine Receptorsusing Three-Dimensional Qsar Analysis. Pestic Sci., 54: 134–144.

Ose, D.E., Fridovich, I., 1976. Superoxide dismutase: reversible removal of manganase and its substitution by cobalt, nicel, or zinc. *J. Biol. Chem*., 251: 1217–1218.

Palmeira, C.M., 1999. Herbicide – induced mitochondrial and Cellular liver toxicity: A review of paraquat, Dinoseb and 2 4-D effects. J. Tox. Substs. Mech., 18: 187 – 204.

Park, S.M., Kim, H.S., 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.

Park, W., Jeon, C.O., Cadillo, H., DeRito, C., Madsen, E.L., 2004. Survival of naphthalene-degrading *Pseudomonas putida* NCIB 9816-4 in naphthalene-amended soils: toxicity of naphthalene and its metabolites. *Appl. Microbiol. Biotechnol.,* 64: 429–435.

Park, Y.B., Han, Y.H., 1997. Increased activities of SOD and catalases on aerobic growth in *Arcobacter nitrofigilis*. *J. Microbiol.,* 35: 239-240.

Rocha, E.R., Selby, T., Coleman, J.P. Smith, C.J., 1996. Oxidative stress response in an anaerobe, Bacteroides fragilis: a role for catalase in protection against hydrogen peroxide. *J. Bacteriol*., 178: 6895-6903.

Schewigert, N.A., Zehnder, J.B., Eggen, R.I.L., 2001. Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals., Environ. Microbiol., 3: 81–91.

Scholz, K., Spiteller, M., 1992. Influence of groundcover on the degradation of 14C-imidacloprid in soil. Brighton Crop Protection Confer-ence - Pests and Diseases., 883-888.

Scott, M.D, Eaton, J.W., 1997. Free radical toxicology. ed. by Wallace KB., Taylor&Francis. 401–420.

Smith, A.H., Imlay, J.A., Mackie, R.T., 2003. Increasing the oxidative stress response allows Escherichia coli to overcome inhibitory effects of condensed tannins. Appl. Environ. Microbiol., 69: 3406-3411.

Spatafora, G., Hoeven, N.V., Katherine, W., Taylor, P., 2002. Evidence that ORF3 at the *Streptococcus parasanguis fimA* locus encodes a thiol-specific antioxidant., Microbiology., 148: 755–762.

Semchyshyn, H., Bagnyukova, T., Storey, K. and Lushchak, V., 2005. Hydrogenperoxide increases the activities of soxRS regulon enzymes and the levels of oxidizedproteins and lipids in Escherichia coli. Cell Biol Int., 29:898-902

Shetti, A.A., Kaliwal B.B., 2012. Imidacloprid Induced Intoxication In Soil Isolate Brevundimonas Sp. Mj 15. Ijlpr. 2(3) :L-105-17.

Storz, G., Tartaglia L.A., Farr, S.B., Ames, B.N., 1990. Bacterial defenses against oxidative stress. Trends Genet., 6: 363–368.

Suntres, Z.S., 2002. Role of anti oxidants in paraquat toxicity. Toxicology., 180 (1): 65-77.

Tabatabaie, T., Floyd, R.A., 1994. Susceptibility of glutathione peroxidase and glutathione reductase to oxidativedamage and the protective effect of spin trapping agents. Arch.Biochem. Biophys., 314: 112-119.

Tsaneva, I., Weiss, B., 1990. SOXR, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *J. Bacteriol.,* 172: 4197-4205.

Ware, G.W., Whitacre, D.M., 2004. An Introduction to Insecticides (4th edition). MeisterPro Information Resources, A division of Meister Media Worldwide, Willoughby, Ohio., 90: 900.

Weimberg, R., 1970. Enzyme level in pea seedlings grown on highly salinized media., *Plant Physiol*., 46: 466-470.

Xiong, L., Zhu, J.K., 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant, Cell & Environment., 25(2): 131-9.

Yao, X.H., Min, H., Zhen-Mel, L.V., 2006. *Biomedical and Environmental Sciences.,* 19: 309-314.

Yun, E.J., Lee, Y.N., 2000. Production of two different catalase-peroxidases by *Deinococcus radiophilus*. FEMS Microbiol. Lett., 184: 155-159.

Zaid, T., Sukumaran, T., Srikumar, and Benov, L., 2003. Growth of *Escherichia coli* in Iron-enriched Medium Increases HPI Catalase Activity. *Journal of Biochemistry and Molecular Biology*., 36(6): 608-610.

Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A., Storz, G., 2001. DNA microarray-mediated transcriptional profiling of the Escherichia coli response to hydrogen peroxide., J. Bacteriol., 183, 4562–4570.

****