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RESEARCH ARTICLE

EFFECT OF CARBARYL ON ENZYME ACTIVITIES IN *Escherichia coli* AND SOIL ISOLATE- *Pseudomonas aeruginosa*

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ABSTRACT

Carbamate insecticide, carbaryl (1-naphthyl-*N*methylcarbamate), is highly toxic with a wide range of activity and known to be metabolized by microorganisms belonging to a variety of bacterial genera by interacting with different components. To evaluate its toxicity, the present study was undertaken by using increasing concentrations of carbaryl (10^{-8} to 10^{-2}M) and evaluated for its toxicity to *Escherichia coli* with emphasis on enzyme assays such as such as activities of amylase, protease, phosphatase, superoxide dismutase (SOD), catalase and peroxidase assay and was further compared to the soil isolate- *Pseudomonas aeruginosa* at a given periods of 24 to 72 hrs respectively. The results indicated that carbaryl treated groups exhibited a significant ($P \leq 0.05$) increase in the enzyme activities of *Escherichia coli* and *Pseudomonas aeruginosa* with an increase in dose and duration when compared to the controls. While, in assessment with its free corresponding, the activity was less in immobilized *Escherichia coli* cells enlightening that immobilized system is less responsive to carbaryl. Present study suggested that carbaryl is a toxicant affecting the synthesis of enzyme activities in of *Escherichia coli* and *Pseudomonas aeruginosa* and alteration in these enzymes occurs as an adaptive mechanism to chemical stress or participation of these enzymes in the protection against ROS, thereby impairing the physiological and metabolic activities of the cell.

INTRODUCTION

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 (Marinšek Logar and Vodovnik, 2007). In India, pesticides are most commonly manufactured in industries and used in agriculture for preventing, controlling and mitigating the pest. The residual pesticide comes in contact with water causing surface/ground water pollution leading to toxicity to biotic environment; therefore, toxicity testing and bioremediation technique for treatment of pesticide is of paramount importance (Fulekar *et al*., 2009).

The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Aurelia, 2009). It has been documented that, the excessive use of pesticides leads to an accumulation of a huge amount of residues in the environment, thereby a posing a substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds in the food chain and drinking water (Mohammed, 2009). Assessing the side effects of pesticides on microbial ecosystems is important to maintain soil fertility and to prevent critical damage to the agricultural ecosystems (Anderson and Domsch, 1978). As microbial parameters, such as microbial population, biomass, activity and community structure, could be affected by natural stresses and fluctuate in the environment, the side-effects caused by the pesticides should be evaluated by comparing them with those caused by natural stresses (Itoh *et al*., 2003). The extensive use of carbamate pesticides in agriculture has induced the rapid evolution and dissemination of specific degradative pathways for the compound in soil bacteria (Felsot *et al*., 1981). Carbamate insecticides, such as carbaryl (1-naphthyl-*N*methylcarbamate, Fig.1), are highly toxic, have a wide range of activity, and comprise a major portion of pesticides used in the agriculture industry.

Widespread and repeated use leads to pollution of soil and groundwater (Chaudhry *et al*., 2002). The ester bond between *N*-methylcarbamic acid and 1-naphthol is responsible for carbaryl toxicity. Carbamates are competitive inhibitors of neuronal nicotinic acetylcholine receptors and acetylcholinesterase (Smulders *et al*., 2003). *N*-Nitrosocarbamates and the 1-naphthol that they generate are potent mutagens and are more toxic and recalcitrant than carbaryl itself (Obulakondaiah *et al*., 1993; Wilson *et al*., 1985). Carbaryl is known to be metabolized by microorganisms belonging to a variety of bacterial genera, such

as *Achromobacter* (Sud *et al*., 1972), *Blastobacter* (Hayatsu and Nagata, 1993), and *Pseudomonas* (Chapalmadugu and Chaudhry, 1991, 1993). It is very much clear from the above findings that pesticides interact with different components 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. Since reports regarding carbaryl effects are scanty and more studies are essential to elucidate the exact toxic potential of carbaryl. Therefore, the present investigation was undertaken and evaluated for its toxicity to *Escherichia coli* and soil isolate- *Pseudomonas aeruginosa* cells with emphasis on enzyme assays such as activities of amylase, protease, phosphatase, superoxide dismutase (SOD), catalase and peroxidase assay and was further compared to the soil isolate- *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Preparation of stock solution of Carbaryl

The sample of carbaryl (Sevin ® 80 WSP) used in the experiment was commercial insecticide supplied by Bayer Crop science, USA obtained from the local company's market containing 80% (w/w). The stock solution of 0.1 M of carbaryl was prepared in distilled water and sterilized separately. This 0.1 M stock solution was further diluted to give different required molar final concentrations.

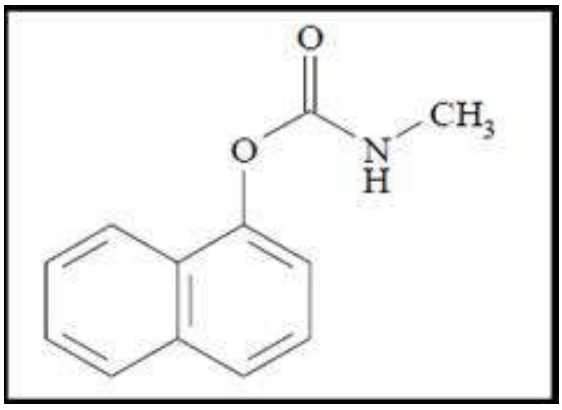

Fig. 1. Chemical structure of carbaryl ($C_{12}H_{11}NO_2$), adapted from IPCS (1992).

Maintenance and propagation of culture

The organism *Escherichia coli* procured from NCL, Pune and the isolated bacteria *Pseudomonas aeruginosa* were maintained at 4°C on nutrient agar and were subcultured very fortnight (Lapage *et al*., 1970).

Medium used for the study

Synthetic sewage medium (S-medium) formulated by Lackey and White (Babich and Stotzky, 1977) (Na_2HPO_4, 50 mg/L, NaCl 15 mg/L, KCl 7 mg/L, $MgSO_4$ 5 mg/L, peptone 100 mg/L, dextrose 1000 mg/L with distilled water and pH (7.0 - 7.2) was adjusted as the medium for toxicity texting.

Preparation of inoculum for free cells

Pre-inoculum was prepared by inoculating a loopful of bacteria from the overnight incubated nutrient agar slant cultures on a 100 ml sterilized synthetic sewage medium and incubated for 18-24 hours at 37°C under static conditions depending on the exponential phases of bacteria under test.

Preparation of immobilized cells

Immobilized cells were prepared by mixing pellet of bacteria obtained by centrifuging the 24 hours culture in nutrient broth at 3,000 rpm for 20 min with 4% sodium alginate (prepared in 0.1 N NaCl) at a final concentration of 1.5% wet weight of bacteria. The mixture was dropped into 4% $CaCl_2$ by syringe and kept at 4°C for 12 hours for hardening. The beads were washed twice with normal saline and maintained at 4°C in normal saline until use.

Experimental procedures

Free cells

Five ml of the pre-inoculum was inoculated to 250 ml Erlenmeyer's flask containing 100 ml of sterilized S-medium amended with different molar concentrations of carbaryl. The flasks were incubated at 37°C for 72 hours under shaking conditions at 120 rpm on a rotary shaker (REMI–CIS-24 BL). At regular intervals, sample was taken out from each flask aseptically for analysis.

Immobilized cells

Immobilized beads were inoculated to 250 ml Erlenmeyer's flask containing 100 ml of sterilized S-medium amended with different molar concentrations of carbaryl. The flasks were incubated at 37°C for 72 hours under shaking conditions at 120 rpm on a rotary shaker (REMI–CIS-24 BL). 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. The supernatant thus obtained was used as enzyme source.

Amylase Activity

Amylase activity was estimated according to the method of Bernfeld (1995). Briefly, 2 ml of the respective samples diluted to 2ml was incubated with 2ml of phosphate buffer (PBS), pH 7.0 and 2ml of 1% soluble starch at 37^0 C for 15 minutes. The reaction was stopped by adding 1 ml of DNS (Dinitro salycilic acid) reagent to each tube and incubated in boiling water bath for 10 min. Each reaction mixture was then diluted to 10 ml using distilled water. The amount of maltose liberated was read at 540 nm wavelength against the blank (DNS added before adding the enzyme). The concentration of maltose liberated was calculated using the standard maltose curve. The unit of amylase activity is µmoles per ml per min of the sample.

Protease Activity

Protease activity was determined using the method given by Takami *et al*. (1990) where, casein was used as substrate. Briefly, the reaction mixture in a total volume of 1 ml of 1 % casein in phosphate buffer of pH 7.5 and 0.5 ml of enzyme extract was incubated at 30^oC for 30 min. After incubation, the reaction was terminated by adding 2 ml of 10% trichloroacetic acid (TCA). After the separation of unreacted casein precipitate

by centrifugation, 1 ml of clear supernatant was taken and mixed with 5 ml of 0.4 M Na2CO3 and 0.5 ml of Folin-Ciocalteau's phenol reagent. After 30 min, the absorbance was measured at 660 nm wavelength against blank. The unit of protease activity is calculated as micrograms of tryptophan released per minute per ml of the sample.

Phosphatase activity

Alkaline phosphatase activity estimation done by the method of Vastraete *et al.* (1976). Briefly, 2 ml of the respective samples diluted to 2 ml was incubated with 2 ml of Tris buffer pH 8.4 and 1ml of 0.1mM p-nitrophenol phosphate incubated for 4 hrs 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.

Superoxide dismutase (SOD) activity

SOD was estimated according to the method of Dhindsa *et al.* (1981) which is followed by nitroblue tetrazolium chloride monohydrate (NBT) assay of Beauchamp and Fridovich (1971). Using the supernatant which was centrifuged (15000 rpm) for 12 min at 4^0 C homogenate in 50mM sodium phosphate buffer of (pH 7.8), then 3ml of the reaction mixture containing 50Mm phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitroblue tetrazolium chloride monohydrate (NBT), 0.1 mM EDTA, 2µM riboflavin and 0-150µl of the enzyme extract was added. The tubes were shaken and placed 30 cm below the light source. Reaction was stopped by switching off the light and covering the tubes with black paper. The reaction was carried out for 15 minutes and the absorbance was read at 560 nm. One unit of the enzyme is calculated as the volume of enzyme extract which could inhibit the NBT reduction by 50% and expressed in µmoles per ml per min.

Catalase activity

Catalase activity was determined according to the method of Sadasivam and Manickam (1996). 3 ml reaction mixture was prepared by adding 50mM phosphate buffer pH 7.0. 25 µl of the enzyme extract and 15mM hydrogen peroxide and the absorbance was recorded at 240 nm wavelength against a blank without hydrogen peroxide for every 30 sec up to 3 min. One unit of catalase activity was defined as 1 µmol of H_2O_2 consumed per min.

Peroxidase activity

The enzyme activity was estimated using the method of Sadasivam and Manickam (1996). The 3 ml reaction mixture in the cuvette consists of 10 mM potassium phosphate buffer (pH 7.0), 0.05 ml of 20 mM guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml of 12.3 mM H_2O_2. The reaction mixture was mixed well and ongoing reaction was recorded at every 30 sec at 436 nm up to 3 min against the blank without enzyme. One unit of the enzyme activity was expressed as µmol/ml/min.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) using the Graph Pad Prism software method, followed by Dunnet test by comparing all treated groups against controls. Values represented are mean ± SEM (n=10). P ≤ 0.05 is considered to indicate a significant difference between experimental and controls.

RESULTS

Dose dependent effect of carbaryl on amylase activity

Escherichia coli

The amylase enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of amylase observed in the control group of free *Escherichia coli* cells was 0.071, 0.096 and 0.112µmol/ml/min respectively. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) in amylase activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Escherichia coli* cells when compared to controls (Fig.2). With the increasing concentration of 10^{-8} M carbaryl, the amylase activity was (0.096, 0.115 and 0.127); 10^{-6} M (0.127, 0.136 and 0.149); 10^{-4} M (0.134, 0.158 and 0.164); and 10^{-2} M (0.148, 0.159 and 0.169) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

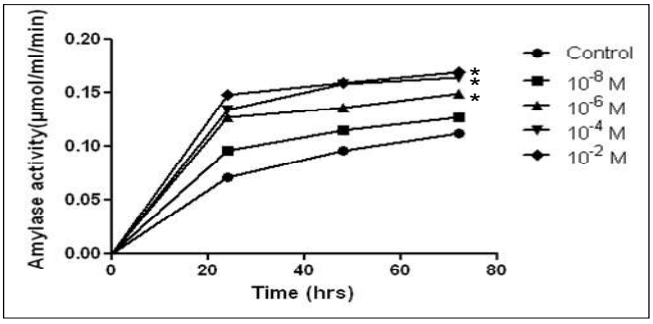

Fig. 2. Dose dependent effect of carbaryl on amylase activity in free Escherichia coli. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for amylase activity estimation and values are represented as unit of amylase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

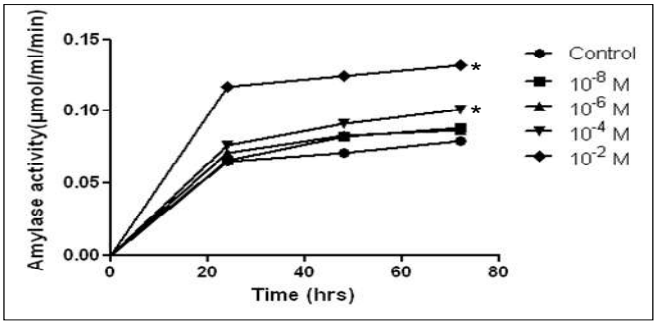

Fig. 3. Dose dependent effect of carbaryl on amylase activity in immobilized Escherichia coli. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for amylase activity estimation and values are represented as unit of amylase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized *Escherichia coli*

The amylase enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was

compared to their corresponding controls. The activity of amylase observed in the control group of immobilized *Escherichia coli* cells was 0.065, 0.071 and 0.079µmol/ml/min respectively. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in amylase activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in immobilized *Escherichia coli* cells when compared to controls (Fig.3). With the increasing concentration of 10^{-8} M of carbaryl, the amylase activity was (0.066, 0.082 and 0.088); 10^{-6} M (0.071, 0.083 and 0.087); 10^{-4} M (0.076, 0.091 and 0.101); and 10^{-2} M (0.117, 0.124 and 0.132) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

Pseudomonas aeruginosa

The amylase enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of amylase observed in the control group of *Pseudomonas aeruginosa* was 0.081, 0.112 and 0.157µmol/ml/min respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) increase in the amylase activity when compared to controls (Fig.4). With the increasing concentration of 10^{-8} M of carbaryl, the amylase activity was (0.107, 0.133 and 0.185); 10^{-6} M (0.126, 0.162 and 0.194); 10^{-4} M (0.138, 0.175 and 0.216); and 10^{-2} M (0.163, 0.192 and 0.234) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

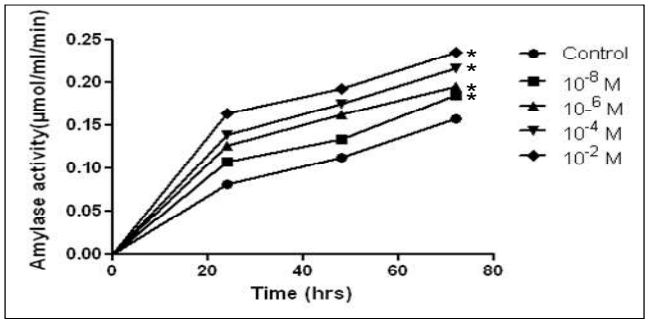

Fig. 4. Dose dependent effect of carbaryl on amylase activity in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for amylase activity estimation and values are represented as unit of amylase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Dose dependent effect of carbaryl on protease activity

Escherichia coli

The protease enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of protease observed in the control group of free *Escherichia coli* cells was 0.380, 0.438 and 0.528 µg tryptophan released/ml/min respectively. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in protease activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Escherichia coli* cells when compared to controls (Fig.5). With the increasing concentration of 10^{-8} M of carbaryl, the protease activity was (0.464, 0.558 and 0.658); 10^{-6} M (0.508, 0.658 and 0.860); 10^{-4} M (0.564, 0.762 and 0.958); and 10^{-2} M (0.648, 0.860 and 0.160) µg tryptophan released/ml/min respectively, at a given periods of 24 to 72 hrs.

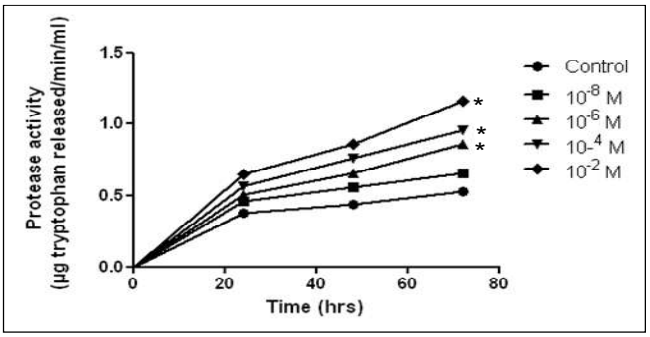

Fig. 5. Dose dependent effect of carbaryl on protease activity in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for protease activity estimation and values are represented as unit of protease activity is µg of tryptophan released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Immobilized *Escherichia coli*

The protease enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of protease observed in the control group of immobilized *Escherichia coli* cells was 0.256, 0.322 and 0.372 µg tryptophan released/ml/min respectively. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in protease activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in immobilized *Escherichia coli* cells when compared to controls (Fig.6). With the increasing concentration of 10^{-8} M of carbaryl, the protease activity was (0.334, 0.384 and 0.418); 10^{-6} M (0.374, 0.410 and 0.448); 10^{-4} M (0.432, 0.462 and 0.556); and 10^{-2} M (0.492, 0.532 and 0.610) µg tryptophan released/ml/min respectively, at a given periods of 24 to 72 hrs.

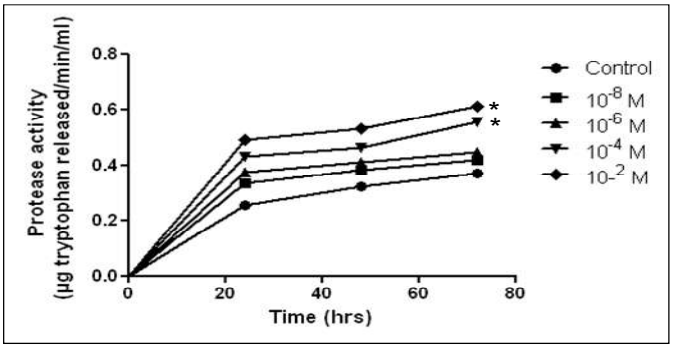

Fig. 6. Dose dependent effect of carbaryl on protease activity in immobilized *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for protease activity estimation and values are represented as unit of protease activity is µg of tryptophan released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Pseudomonas aeruginosa

The protease enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of protease observed in the control group of *Pseudomonas*

aeruginosa was 0.364, 0.404 and 0.460 µg tryptophan released/ml/min respectively. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in protease activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Pseudomonas aeruginosa* when compared to controls (Fig.7). With the increasing concentration of 10^{-8} M of carbaryl, the protease activity was (0.426, 0.464 and 0.498); 10^{-6} M (0.484, 0.526 and 0.594); 10^{-4} M (0.536, 0.594 and 0.640); and 10^{-2} M (0.608, 0.688 and 0.730) µg tryptophan released/ml/min respectively, at a given periods of 24 to 72 hrs.

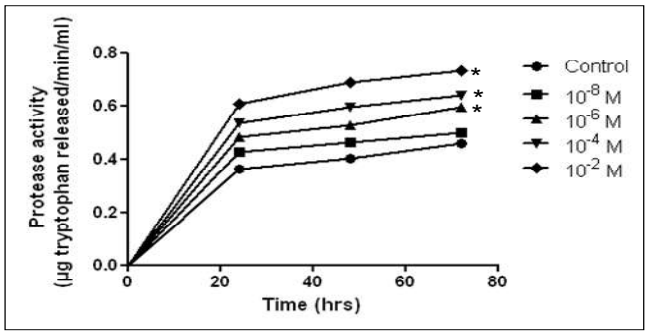

Fig. 7. Dose dependent effect of carbaryl on protease activity in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for protease activity estimation and values are represented as unit of protease activity is µg of tryptophan released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Dose dependent effect of carbaryl on phosphatase activity

Escherichia coli

The phosphatase enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of phosphatase observed in the control group of free *Escherichia coli* cells was 0.432, 0.506 and 0.542 µg p-nitrophenol released/min/ml respectively. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in phosphatase activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in *Escherichia coli* cells when compared to controls (Fig.8). With the increasing concentration of 10^{-8} M of carbaryl, the phosphatase activity was (0.500, 0.564 and 0.606); 10^{-6} M (0.534, 0.642 and 0.664); 10^{-4} M (0.562, 0.720 and 0.762); and 10^{-2} M (0.602, 0.782 and 0.828) µg p-nitrophenol released/min/ml respectively, at a given periods of 24 to 72 hrs.

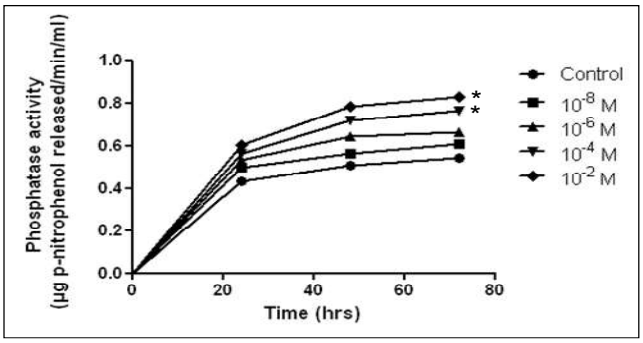

Fig. 8. Dose dependent effect of carbaryl on alkaline phosphatase activity in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for protease activity estimation and values are represented as unit of phosphatase activity is µg of p-nitrophenol released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Immobilized *Escherichia coli*

The phosphatase enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of phosphatase observed in the control group of immobilized *Escherichia coli* cells was 0.084, 0.106 and 0.154 µg p-nitrophenol released/min/ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) increase in the phosphatase activity when compared to controls (Fig.9). With the increasing concentration of 10^{-8} M of carbaryl, the phosphatase activity was (0.196, 0.254 and 0.322); 10^{-6} M (0.236, 0.296 and 0.356); 10^{-4} M (0.266, 0.360 and 0.392); and 10^{-2} M (0.294, 0.386 and 0.416) µg p-nitrophenol released/min/ml respectively, at a given periods of 24 to 72 hrs.

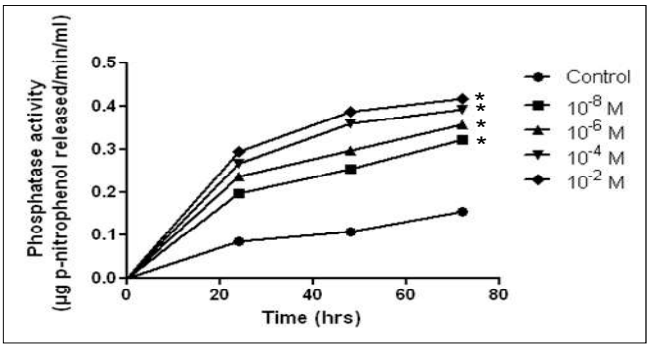

Fig. 9. Dose dependent effect of carbaryl on alkaline phosphatase activity in immobilized *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for phosphatase activity estimation and values are represented as unit of phosphatase activity is µg of p-nitrophenol released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

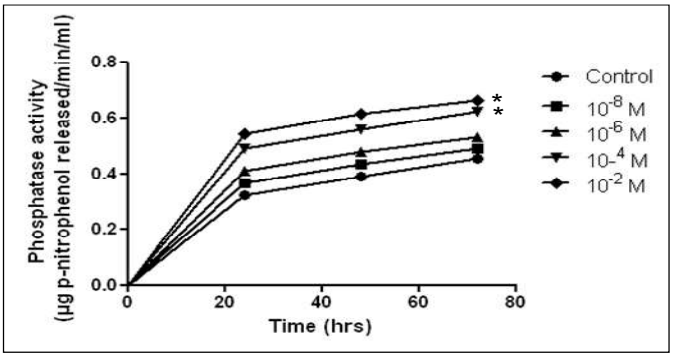

Fig. 10. Dose dependent effect of carbaryl on alkaline phosphatase activity in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for phosphatase activity estimation and values are represented as unit of phosphatase activity is µg of p-nitrophenol released per min per ml of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Pseudomonas aeruginosa

The phosphatase enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of phosphatase observed in the control group of *Pseudomonas aeruginosa* was 0.321, 0.389 and 0.453 µg p-nitrophenol released/ min/ml respectively. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq$

0.05) in phosphatase activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in *Pseudomonas aeruginosa* when compared to controls (Fig.10). With the increasing concentration of 10^{-8} M of carbaryl, the phosphatase activity was (0.365, 0.433 and 0.491); 10^{-6} M (0.411, 0.479 and 0.529); 10^{-4} M (0.491, 0.557 and 0.621); and 10^{-2} M (0.541, 0.613 and 0.661) µg p-nitrophenol released/min/ml respectively, at a given periods of 24 to 72 hrs.

Dose dependent effect of carbaryl on super oxide dismutase (SOD) activity

Escherichia coli

The SOD enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of SOD observed in the control group of free *Escherichia coli* cells was 0.020, 0.023 and 0.026µmol/ml/min respectively. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in SOD activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in *Escherichia coli* cells when compared to controls (Fig.11). With the increasing concentration of 10^{-8} M of carbaryl, the SOD activity was (0.022, 0.025 and 0.027) ; 10^{-6} M (0.027, 0.029 and 0.031); 10^{-4} M (0.029, 0.033 and 0.035); and 10^{-2} M (0.031, 0.034 and 0.038)µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

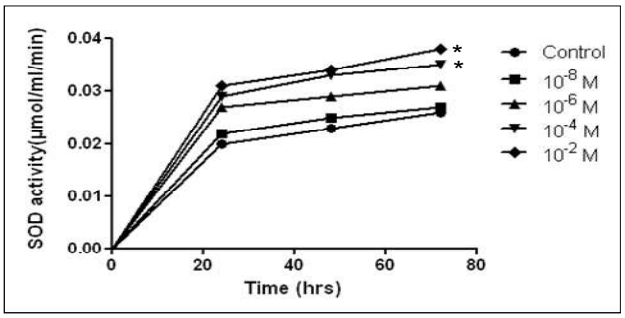

Fig. 11. Dose dependent effect of carbaryl on superoxide dismutase (SOD) activity in free *Escherichia coli.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for SOD activity estimation and values are represented as unit of the SOD activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Immobilized *Escherichia coli*

The SOD enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of SOD observed in the control group of immobilized *Escherichia coli* cells was 0.017, 0.020 and 0.022µmol/ml/min. However, higher doses of 10^{-4} and 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in SOD activity, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M carbaryl in immobilized *Escherichia coli* cells when compared to controls (Fig.12). With the increasing concentration of 10^{-8} M of carbaryl, the SOD activity was (0.020, 0.021 and 0.023); 10^{-6} M (0.024, 0.025 and 0.027); 10^{-4} M (0.027, 0.028 and 0.029); and 10^{-2} M (0.029, 0.031 and 0.033) µmol/ml/min respectively, at a given periods of 24 to72 hrs.

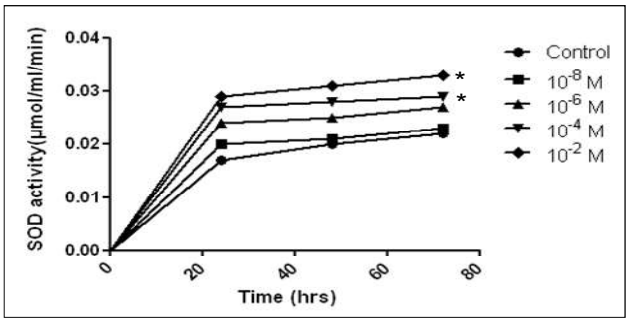

Fig. 12. Dose dependent effect of carbaryl on superoxide dismutase (SOD) activity in immobilized *Escherichia coli.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for SOD activity estimation and values are represented as unit of the SOD activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Pseudomonas aeruginosa

The SOD enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of SOD observed in the control group of *Pseudomonas aeruginosa* was 0.014, 0.016 and 0.018µmol/ml/min respectively. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in SOD activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Pseudomonas aeruginosa* when compared to controls (Fig.13). With the increasing concentration of 10^{-8} M of carbaryl, the SOD activity was (0.016, 0.019 and 0.020); 10^{-6} M (0.019, 0.023 and 0.026); 10^{-4} M (0.022, 0.026 and 0.028); and 10^{-2} M (0.027, 0.031 and 0.035) µmol/ml/min respectively, at a given periods of 24 to72 hrs.

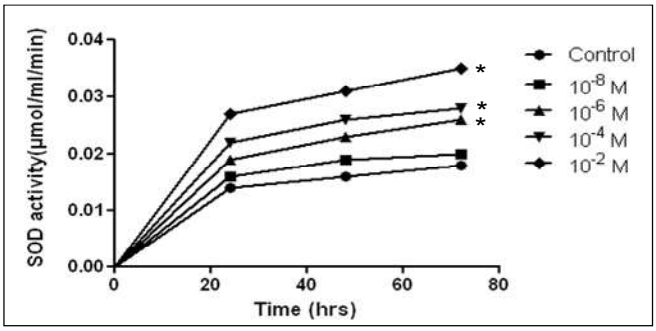

Fig. 13. Dose dependent effect of carbaryl on superoxide dismutase (SOD) activity in *Pseudomonas aeruginosa.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for SOD activity estimation and values are represented as unit of the SOD activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Dose dependent effect of carbaryl on catalase activity

Escherichia coli

The catalase enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of catalase observed in the control group of free *Escherichia coli* cells was 13.14, 14.04 and 15.24µmol/ml/min. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) increase in the catalase activity when compared to controls (Fig.14). With the increasing concentration of 10^{-8} M of carbaryl, the catalase

activity was (17.28, 18.66 and 20.54); 10^{-6} M (20.67, 21.56 and 22.28); 10^{-4} M (25.06, 27.12 and 28.12); and 10^{-2} M (29.44, 31.66 and 32.12) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

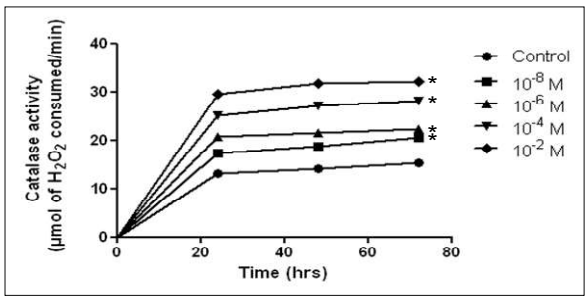

Fig. 14. Dose dependent effect of carbaryl on catalase activity in free *Escherichia coli.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for catalase activity estimation and values are represented as unit of catalase activity is µmol of H_2O_2 consumed per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized *Escherichia coli*

The catalase enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of catalase observed in the control group of immobilized *Escherichia coli* cells was 11.04, 12.04 and 13.34µmol/ml/min. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) in catalase activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in immobilized *Escherichia coli* cells when compared to controls (Fig.15). With the increasing concentration of 10^{-8} M of carbaryl, the catalase activity was (13.20, 14.02 and 15.76); 10^{-6} M (18.06, 19.06 and 19.90); 10^{-4} M (23.26, 24.06 and 24.98); and 10^{-2} M (27.56, 28.78 and 30.06) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

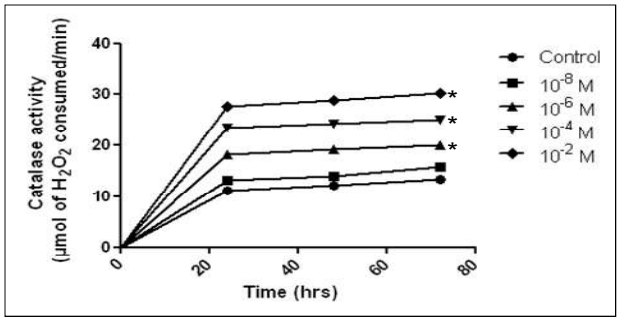

Fig. 15. Dose dependent effect of carbaryl on catalase activity in immobilized *Escherichia coli.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for catalase activity estimation and values are represented as unit of catalase activity is µmol of H_2O_2 consumed per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Pseudomonas aeruginosa

The catalase enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of catalase observed in the control group of *Pseudomonas aeruginosa* was 15.04, 16.22 and 18.22µmol/ml/min. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) increase in the catalase activity when compared to controls (Fig.16). With the increasing concentration of 10^{-8} M of carbaryl, the catalase activity was (18.54, 21.04 and 23.96); 10^{-6} M (22.04, 23.44 and 25.76); 10^{-4} M (24.40, 27.06 and 31.06); and 10^{-2} M (29.12, 32.52 and 34.02) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

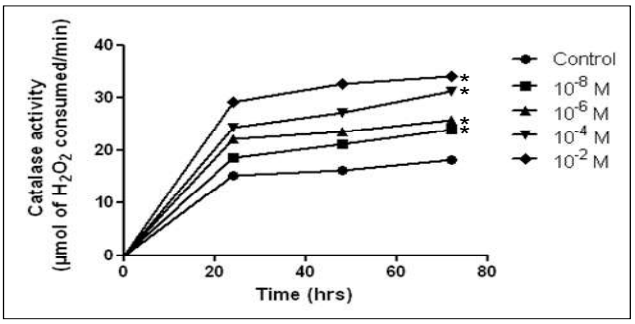

Fig. 16. Dose dependent effect of carbaryl on catalase activity in *Pseudomonas aeruginosa.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for catalase activity estimation and values are represented as unit of catalase activity is µmol of H_2O_2 consumed per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Dose dependent effect of carbaryl on peroxidase activity

Escherichia coli

The peroxidase enzyme activity in free *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of peroxidase observed in the control group of free *Escherichia coli* cells was 0.035, 0.038 and 0.040µmol/ml/min. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) in peroxidase activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Escherichia coli* cells when compared to controls (Fig.17). With the increasing concentration of 10^{-8} M of carbaryl, the peroxidase activity was (0.044, 0.047 and 0.051); 10^{-6} M (0.048, 0.050 and 0.057); 10^{-4} M (0.056, 0.059 and 0.063); and 10^{-2} M (0.061, 0.064 and 0.067) µmol/ml/min respectively, at a given periods of 24 to72 hrs.

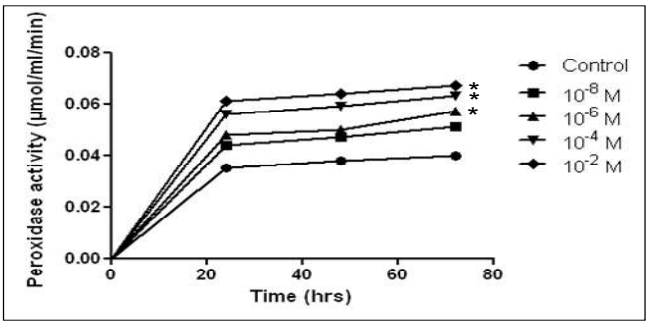

Fig. 17. Dose dependent effect of carbaryl on peroxidase activity in *Pseudomonas aeruginosa.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for peroxidase activity estimation and values are represented as unit of the peroxidase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized *Escherichia coli*

The peroxidase enzyme activity in immobilized *Escherichia coli cells* that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs

and was compared to their corresponding controls. The activity of peroxidase observed in the control group of immobilized *Escherichia coli* cells was 0.032, 0.035 and 0.036µmol/ml/min. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in peroxidase activity, whereas, no significant increase was observed in the lower dose of 10^{-8} carbaryl in immobilized *Escherichia coli* cells when compared to controls (Fig.18). With the increasing concentration of 10^{-8} M of carbaryl, the peroxidase activity was (0.040, 0.042 and 0.044); 10^{-6} M (0.044, 0.047 and 0.049); 10^{-4} M (0.051, 0.053 and 0.055); and 10^{-2} M (0.055, 0.057 and 0.059) µmol/ml/min respectively, at a given periods of 24 to 72 hrs.

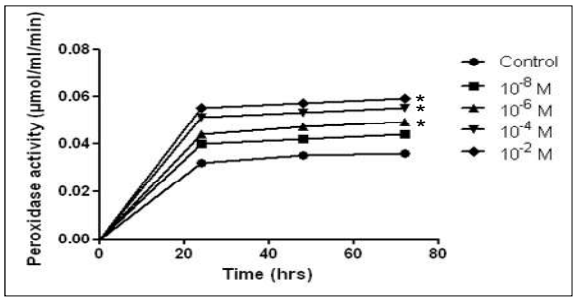

Fig. 18. Dose dependent effect of carbaryl on peroxidase activity in *Pseudomonas aeruginosa.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for peroxidase activity estimation and values are represented as unit of the peroxidase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Pseudomonas aeruginosa

The peroxidase enzyme activity in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24 to72 hrs and was compared to their corresponding controls. The activity of peroxidase observed in the control group of *Pseudomonas aeruginosa* was 0.035, 0.038 and 0.040µmol/ml/min. However, graded doses of 10^{-6} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) in peroxidase activity, whereas, no significant increase was observed in the lower dose of 10^{-8} M carbaryl in *Pseudomonas aeruginosa* when compared to controls (Fig.19). With the increasing concentration of 10^{-8} M of carbaryl, the peroxidase activity was (0.040, 0.044 and 0.047); 10^{-6} M (0.044, 0.049 and 0.052); 10^{-4} M (0.052, 0.056 and 0.060); and 10^{-2} M (0.059, 0.061 and 0.065) µmol/ml/min respectively, at a given periods of 24 to72 hrs.

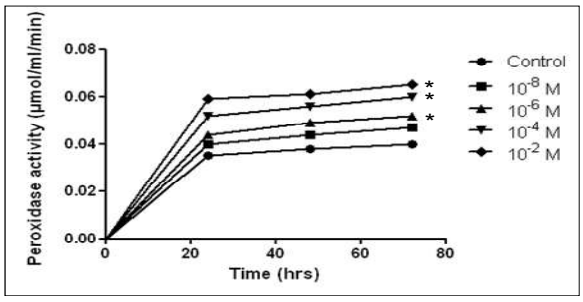

Fig. 19. Dose dependent effect of carbaryl on peroxidase activity in *Pseudomonas aeruginosa.* Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, supernatant of the respective samples were assessed for peroxidase activity estimation and values are represented as unit of the peroxidase activity is µmoles per ml per min of the sample. Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

DISCUSSION

The present study revealed that there was dose and duration dependent increased in the enzyme activities of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to carbaryl. The response of exposure to carbaryl in both free and immobilized cells with its free counterpart the activity was less in immobilized *Escherichia coli* cells revealing that immobilized system is less sensitive to the carbaryl. Exogenous sources like, environmental agents, pharmaceuticals and industrial chemicals exert their toxic effects by generating ROS (Klaunig and Kamendulis, 2004). The main role of amylase is the starch metabolism in the extracellular medium and microorganisms depend on amylases for survival (Jones *et al*., 1999). Some cells show increased catabolism to meet the energy demand under stress induced by the pesticides (Ivanova, 1982). Our previous data has been shown that the decline in protein and glucose levels in *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to carbaryl indicates the physiological adaptability to compensate for pesticide stress (communicated data).

To overcome the stress, they use more energy, which leads to stimulation of protein, glycogen catabolism and hence increase in the amylase activity (Sancho *et al*., 1998). Oxidative stress results when the rate of production of ROS exceeds the capacity of the cell for disposal (Sies, 1991). Under physiological conditions, ROS are continuously generated in cells from metabolic processes such as respiration and fatty acid biosynthesis in aerobic organisms or environmental agents (Georgiou, 2002). It has been reported a significant increases in the specific enzyme activities of extracellular α amylase and protease and consequent increases in total enzyme concentration with increase in the levels of intracellular ROS in *Bacillus subtilis* cultures were a result of adaptive responses induced under oxidative stress (Mishraa *et al.,* 2005). In present study, the increase in the amylase activity observed treated groups may be due to the toxic effect of the carbaryl by generating ROS or an adaptive response induced under oxidative stress. The protease activity observed in the treated groups was increased significantly in the higher doses with duration of exposure to carbaryl in *Escherichia coli* and *Pseudomonas aeruginosa*.

The proteases are a widespread group of enzymes that catalyze the hydrolysis of different proteins and perform a pivotal role in the degradation and turnover of intracellular proteins (Chapman *et al*., 1997). It has been shown that the increase in the protease activity due to the expression of intracellular proteins, which require cell lysis for purification which will result in exposure to proteases (Martin *et al.,* 1998). Protease is essential for *Escherichia coli* to survive at elevated temperatures and the protein selective proteolytic degradation appears to be rather significant in homeostasis maintaining and metabolism regulation in the cell (Strauch and Beckwith, 1988). Therefore, the significant decrease in the protease activity of *Escherichia coli* and *Pseudomonas aeruginosa* cells on dose and durational exposure of carbaryl observed in the present study may be due to hydrolysis of different proteins that perform a pivotal role in the degradation and turnover of intracellular proteins. The phosphatase activity was increased significantly in higher dose of 10^{-4} and 10^{-2} M exposure to carbaryl treated groups of free *Escherichia coli* and *Pseudomonas aeruginosa bacteria,* while in immobilized *Escherichia coli* there was a significant increase in the activity

in all treated groups with dose and duration. Phosphatase being nonspecific phosphomonoesterase, widely found in various organisms, indicating their important role in metabolism of different phosphorus containing organic compounds (Bjorkman and Karl, 1994). Report has been shown that metal-stress conditions enhanced phosphatase production by *Aspergillus niger* and suggested that the overproduction of phosphatase and enhanced metal uptake can be postulated as detoxification mechanisms in this fungus. (Tsekovaa, *et al*., 2002). In contrast, Periasamy and Raman (1995) have reported that the increase of copper concentration decreased the levels of the acid phosphatase activity, as well as the culture growth. One hypothesis widely reported at bacteria suggests overproduction of phosphatases as detoxification mechanisms, with precipitation of metals away from sensitive cellular sites (Montgomery *et al*., 1995).

The elevated level of phosphatase was observed on exposure to carbamates which may be indicative of an adaptive rise in enzyme activity in response to persistent stress (Murphy and Porter, 1966). Hence, the significant increase in phosphatase activity observed in the present study may be due to its synthesis as an adaptive mechanism to stress or a positive correlation that exists between phosphate solubilizing capacity and phosphatase enzyme activity. SOD activity in the treated groups increased significantly in higher doses of 10^{-4} and 10^{-2} M in both free and immobilized *Escherichia coli* on carbaryl exposure, whereas, no significant increase was observed in the lower doses of 10^{-8} and 10^{-6} M. However, this activity increased significantly with dose and duration dependent on exposure to carbaryl in *Pseudomonas aeruginosa*. SOD is one of the antioxidant known in nature and changes in the activity of this enzyme also used as indirect measures of oxidative stress (Scott and Easton, 1997) and increased in SOD activity might be in response to increased oxidative stress or might be due to compensatory response to oxidative stress induced by this xenobiotic (Maunteanu *et al*., 2005). Various pesticide chemicals may induce oxidative stress leading to generation of free radicals and alternation in antioxidants or oxygen free radical scavenging enzyme system (Banerjee *et al*., 1999; Lu, 2004).

Studies demonstrates that *Escherichia coli* possess antioxidant enzymes which are induced in response to oxidative stress (Smith *et al*., 2003) and the genetic responses to superoxide stress in *Pseudomonas putida* differ noticeably from those seen in *Escherichia coli* and *Salmonella* (Park *et al.*, 2006). The significant increase in the SOD activity observed in *Escherichia coli* and *Pseudomonas aeruginosa* in the present study may be due to the oxidative stress or specific enzymes in response to oxidative stress induced by the carbaryl. Catalase activity was significantly increased in all the groups with increase in the dose and durational exposure of carbaryl to *Escherichia coli* and *Pseudomonas aeruginosa*. Catalase is the one of among efficient antioxidants, known to act on toxic compounds by per oxidative reactions (Kirkman and Geatani, 1984). Antioxidant enzymes participate a major role in the cellular defense mechanism, they are susceptible to inactivation by ROS. The oxidative processes result in the loss of key antioxidant enzymes which may exacerbate oxidative stress-mediated cytotoxicity (Tabatabaie and Floyd, 1994). Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. In *Escherichia coli* as in almost all other aerobic cells, the first line of defense against oxygen-derived free radicals includes superoxide dismutases and catalases. The increase in 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 (Cabiscol *et al*., 2000). Acetamiprid oxidative stress on *Escherichia coli*, *Pseudomonas* sp and *Bacillus subtilis* elevated SOD and catalase activities to antagonize oxidative stress (Yao *et al*., 2006). The significant increase in the catalase activity observed in the present study in 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.

Peroxidase activity in the treated groups increased significantly in higher doses of 10^{-6} to10^{-2} M exposure to carbaryl in both *Escherichia coli* and *Pseudomonas aeruginosa* on carbaryl exposure, whereas, no significant increase was observed in the lower dose of 10^{-8} M. Peroxidase is widely distributed and they perform essential roles in metabolism (Spatafora *et al*., 2002). 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. The most frequent combination of adverse factors, especially for aerobic or facultatively anaerobic microorganisms such as *Escherichia coli*, is the combination of starvation and oxidative stress (Salakhetdinova *et al*., 2000). Antioxidants, such as catalases, peroxidases and SOD form the first line of defense against ROS and a gradual increase of these enzymes production in aging cultures because of radical scavenging enzymes activity in cells in response to oxidative stress (Yun and Lee, 2000).

These antioxidants have been reported to play protective roles in *Escherichia coli* (Kim *et al*., 1996), in *Pseudomonas* sp. (Hassett *et al*., 1996) and in *Bacteroides* sp. (Rocha *et al*., 1996) and also these enzymes are known to act as radical scavengers in response to the oxidative stress in *Escherichia coli, Pseudomonas putida, Streptomyces coelicolor* and *Arcobacter nitrofigilis* (Zaid *et al*., 2003). Hence, in this study, the significant increase in peroxidase activity in treated groups may be due to regulated adaptive responses of microorganisms to oxidative stress or participation of these enzymes in the protection against ROS. The present study suggested that carbaryl is a toxicant affecting the synthesis of enzyme activities of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to carbaryl are dose and duration dependent. Synthesis of these enzymes occurs as an adaptive mechanism to chemical stress or participation of these enzymes in the protection against ROS and thereby overcome stress or as a compensatory response to oxidative stress induced by this carbaryl. It is very much clear that carbaryl may interact with different components and enzymes of the target and non-target organisms, thereby impairing the physiological and metabolic activities of the cell. Although, adaptive responses of microorganisms to oxidative stress have been extensively studied, nevertheless the oxidative stress response of common soil microorganisms has been poorly known for the *Pseudomonas* species (Park *et al* 2006) and the increase in peroxidase activity in *Pseudomonas aeruginosa* bacteria 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 (Cabiscol *et al*., 2000). Therefore, further investigation is required to study the effect of carbaryl on *Escherichia coli* and soil isolate-*Pseudomonas aeruginosa* cells with emphasis on the molecular mechanisms like, proteomic profiling of *Escherichia coli* and *Pseudomonas aeruginosa* and underlying responses of cells against carbaryl in a dose and duration manner.

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