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Effect of carbaryl on biochemical contents in *Escherichia coli* and soil isolate *Pseudomonas aeruginosa*

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ABSTRACT

The wide use of carbamate pesticides in agriculture has persuaded the rapid evolution and dissemination of specific degradative pathways for the compound in soil bacteria. 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, thereby impairing the physiological and metabolic activities of the cell. 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 biochemical contents such as DNA, RNA, protein, glucose utilization, growth and was further compared to the soil isolate- *Pseudomonas aeruginosa* at a given periods of 24 -72 hrs respectively. The results indicated that carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the levels of biochemical contents and increase in the % inhibition in these parameters was observed with an increase in dose and durational exposure to carbaryl 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 biochemical contents and growth in a dose and dependent manner and alterations in the parameters of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to carbaryl are can be due to carbaryl intoxication.

Keywords: Carbaryl, Biochemical contents, *Escherichia coli* and *Pseudomonas aeruginosa*

INTRODUCTION

Each year, pesticides are manufactured and used in massive quantities, and toxicity remains a major environmental problem associated with pesticide usage. 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).

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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).
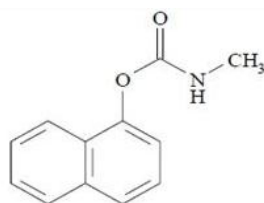

Fig.1: Chemical structure of carbaryl ($C_{12}H_{11}NO_2$, 1-naphthyl-*N*methylcarbamate), adapted from IPCS, Health and Safety Guide No. 78, WHO, 1992.

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. 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 biochemical contents such as DNA, RNA, protein, glucose utilization, growth 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.

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.

Isolation and Estimation of DNA and RNA

Perchloric acid (0.5 N, 4 ml) was added to the pellet of 10 ml culture and the mixture was allowed to stand in water bath at 70°C for 15 min with occasional shaking and centrifuged at 3,000 rpm for 15 min. The extraction was repeated twice with 0.5N perchloric acid (3 ml) each for 15 min, the extracts were combined and made up to 10 ml with 0.5N perchloric acid. From this extract, the estimation of DNA by diphenylamine method (Burton, 1956) and RNA by orcinol method (Brown, 1946) in respective samples and calculated by referring the standard graph as described.

Protein estimation

Protein estimation was done in respective samples by the method of Lowry et al. (1951). The amount of protein is calculated by referring the standard graph prepared by using Bouvine serum albumin (BSA).

Estimation of glucose utilization

The unutilized glucose in the medium was estimated by Anthrone method described by Hedge and Hofreiter (1962). The glucose content is calculated by referring the standard graph of glucose.

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).

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 $\pm$ SEM (n=10). $P \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

RESULTS

Dose dependent effect of carbaryl on DNA content
Escherichia coli

The level of DNA content 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 24-72 hrs and was compared to their corresponding controls. DNA content observed in the control group of free *Escherichia coli* cells was 103.1, 120.8 and 154.0 µg /ml respectively. However, graded concentrations from 10^{-8}-10^{-2} M

carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of DNA content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.2). With the increasing concentration of 10^{-8} M of carbaryl, the level of DNA content with inhibition (%) was [79.2(23.2%), 86.4 (28.5%) and 97.8 (36.5%) µg/ml]; 10^{-6} M [45.8(55.6%), 58.4(51.7%) and 65.6 (57.4%)µg/ml]; 10^{-4} M [24.4(76.3%), 28.4(76.5%) and 35.6 (76.9%) µg/ml]; and 10^{-2} M [14.8 (85.6%), 19.4(83.9%) and 24.00(84.4%)µg/ml] at a given periods of 24-72 hrs respectively.
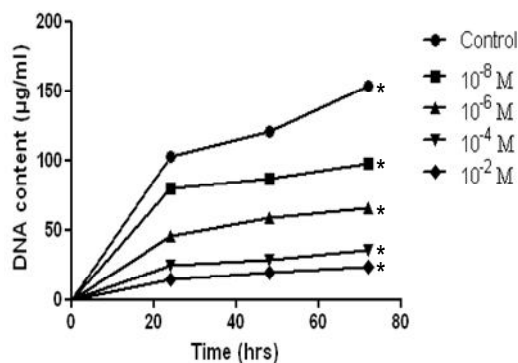

Fig. 2: Dose dependent effect of carbaryl on DNA content in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for DNA estimation and values are represented as DNA content (µg/ml). Values are mean $\pm$ SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

Immobilized Escherichia coli

The level of DNA content 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 24-72 hrs and was compared to their corresponding controls. DNA content observed in the control group of immobilized *Escherichia coli* cells was 129.4, 157.8 and 176.6 µg /ml respectively. However, graded concentrations from 10^{-8}- 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of DNA content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.3).
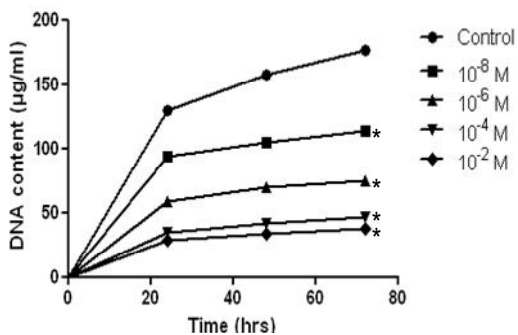

Fig. 3: Dose dependent effect of carbaryl on DNA content in immobilized *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for DNA estimation and values are represented as DNA content (µg/ml). Values are mean $\pm$ SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

With the increasing concentration of 10^{-8} M of carbaryl, the level of DNA content with inhibition was [93.8 (27.5%),105.0(33.5%) and 113.4(35.8%) µg/ml]; 10^{-6} M [58.2(55.0%), 69.2(56.1%) and 75.0(57.5%) µg/ml]; 10^{-4} M [35.0(72.9%), 42.0(73.4%) and 46.4(73.7%) µg/ml]; and 10^{-2} M [28.8(77.7%), 33.8(78.6%) and 38.0(78.5%) µg/ml] at a given periods of 24- 72 hrs respectively.

Pseudomonas aeruginosa

The level DNA content in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24-72 hrs and was compared to their corresponding controls. DNA content observed in the control group of *Pseudomonas aeruginosa* was 112.6, 134.8 and 165.0 µg /ml respectively. However, graded concentrations from 10^{-8}- 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of DNA content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.4). With the increasing concentration of 10^{-8} M of carbaryl, the level of DNA content with inhibition was [65.0(42.3%), 87.6 (35.0%) and 94.0(43.0%) µg/ml]; 10^{-6} M [38.0(66.3%), 42.8(68.3%) and 48.8(70.4%)µg/ml]; 10^{-4} M [23.0(79.6%), 26.8(80.1%) and 32.6(80.2%) µg/ml]; and 10^{-2} M [13.6(87.9%), 15.0(88.9%), and 19.0(88.5%)µg/ml] at a given periods of 24-72hrs respectively.
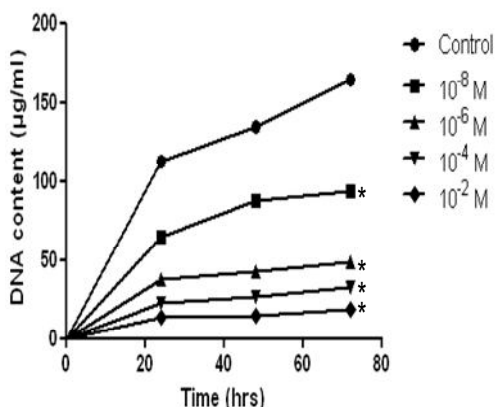

Fig. 4: Dose dependent effect of carbaryl on DNA content in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for DNA estimation and values are represented as DNA content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Dose dependent effect of carbaryl on RNA content
Escherichia coli

The level of RNA content 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 24-72 hrs and was compared to their corresponding controls. RNA content observed in the control group of free *Escherichia coli* cells was 17.0, 33.8 and 48.0 µg /ml respectively. However, graded concentrations from 10^{-8}- 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of RNA content when compared with that of the controls and increase in the % inhibition was observed with an

increase in dose and durational exposure to carbaryl (Fig.5). With the increasing concentration of 10^{-8} M of carbaryl, the level of RNA content with inhibition was [11.6(31.8%), 16.8(50.3%) and 20.8(56.7%) µg/ml]; 10^{-6} M [6.0(64.7%), 12.2(63.9%) and 16.0(66.7%)µg/ml]; 10^{-4} M [3.2(81.2%), 8.0(76.3%) and 12.2(74.6%) µg/ml]; and 10^{-2} M [2.8(83.5%),4.8(85.8%), and 8.4(82.5%) µg/ml] at a given periods of 24-72 hrs respectively.
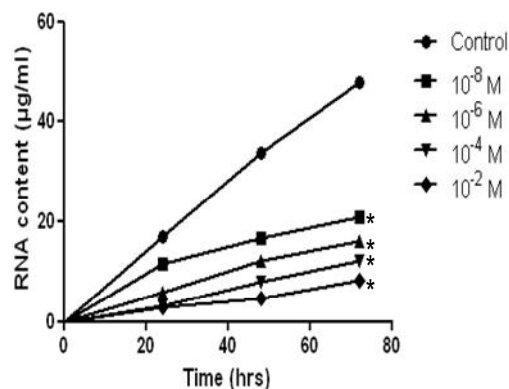

Fig. 5: Dose dependent effect of carbaryl on RNA Content in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for RNA estimation and values are represented as RNA content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized *Escherichia coli*

The level of RNA content 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 24-72 hrs and was compared to their corresponding controls. RNA content observed in the control group of immobilized *Escherichia coli* cells was 35.6, 43.6 and 61.0 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of RNA content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.6).
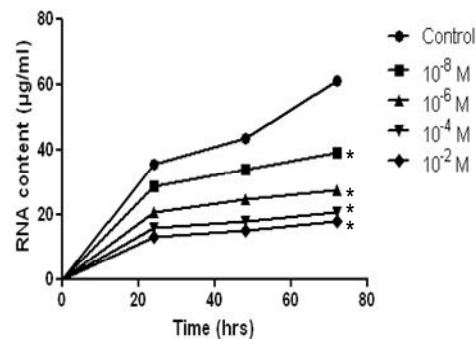

Fig. 6: Dose dependent effect of carbaryl on RNA Content in immobilized *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for RNA estimation and values are represented as RNA content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

With the increasing concentration of 10^{-8} M of carbaryl, the level of RNA content with inhibition was [28.8(19.1%), 33.8(22.5%) and 39.0(36.1%) µg/ml]; 10^{-6} M [20.6(42.1%), 24.6(43.6%) and 27.6(54.8%)µg/ml]; 10^{-4} M [16.0(55.1%),17.8(59.2%) and 20.8(65.9%) µg/ml];and 10^{-2} M [13.2(62.9%),15.0(65.6%), and 18.0(70.5%)µg/ml] at a given periods of 24-72 hrs respectively.

Pseudomonas aeruginosa

The level RNA content in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24-72 hrs and was compared to their corresponding controls. RNA content observed in the control group of *Pseudomonas aeruginosa* was 38.0, 55.8 and 68.0 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of RNA content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.7). With the increasing concentration of 10^{-8} M of carbaryl, the level of RNA content with inhibition was [24.2(36.3%), 28.8(48.4%) and 31.8(53.2%) µg/ml]; 10^{-6} M [16.8(44.2%), 21.6(61.3%) and 24.6(63.8%)µg/ml]; 10^{-4} M [11.6(55.8%), 15.6(72.0%) and 17.8(73.8%) µg/ml]; and 10^{-2} M [9.8(74.2%), 10.0(82.1%), and 12.0(82.4%)µg/ml] at a given periods of 24-72 hrs respectively.
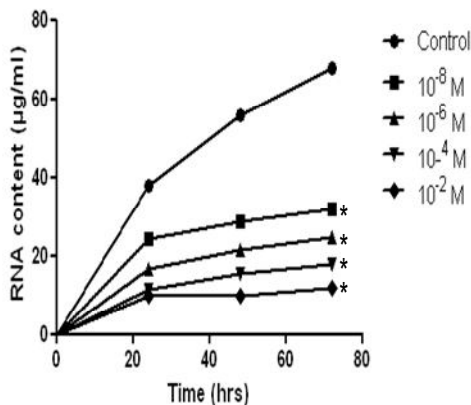

Fig. 7: Dose dependent effect of carbaryl on RNA Content in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for RNA estimation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

Dose dependent effect of carbaryl on protein content
Escherichia coli

The level of protein content 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 24-72 hrs and was compared to their corresponding controls. Protein content observed in the control group of free *Escherichia coli* cells was 65.0, 108.6 and 144.6 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of protein content when compared with that of the controls and increase in the % inhibition was observed

with an increase in dose and durational exposure to carbaryl (Fig.8). With the increasing concentration of 10^{-8} M of carbaryl, the level of protein content with inhibition was [43.4(33.2%), 74.6(31.3%) and 92.2(36.2%) µg/ml]; 10^{-6} M [35.2(45.8%), 42.2(61.1%) and 55.0(61.9%)µg/ml]; 10^{-4} M [21.4(32.9%), 35.4(67.4%) and 46.2(68.0%) µg/ml]; and 10^{-2} M [17.8(72.6%), 25.8(76.2%), and 34.2(76.3%) µg/ml] at a given periods of 24-72 hrs respectively.
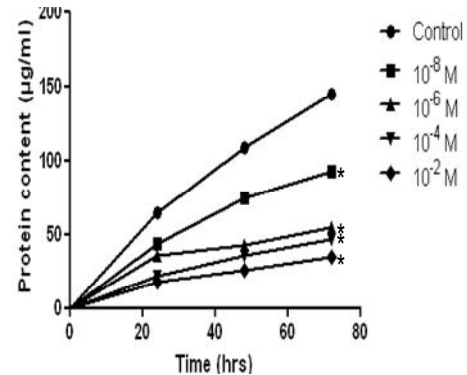

Fig. 8: Dose dependent effect of carbaryl on protein content in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for protein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

Immobilized Escherichia coli

The level of protein content 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 24-72 hrs and was compared to their corresponding controls. Protein content observed in the control group of immobilized *Escherichia coli* cells was 55.6, 85.2 and 125.4 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of protein content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.9).
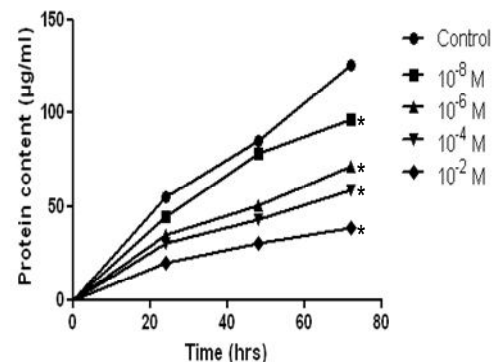

Fig. 9: Dose dependent effect of carbaryl on protein content in immobilized *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for protein estimation and values are represented as protein content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

With the increasing concentration of 10^{-8} M of carbaryl, the level of protein content with inhibition was [44.4(20.1%),78.4(7.9%) and 96.6(22,9%) µg/ml]; 10^{-6} M [35.0(37.1%), 51.0(40.1%) and 71.4(43.1%) µg/ml]; 10^{-4} M [30.6(44.9%),43.0(49.5%) and 59.00(52.9%) µg/ml]; and 10^{-2} M [19.8(64.4%), 30.6(64.1%), and 39.0(68.9%) µg/ml] at a given periods of 24-72 hrs respectively.

Pseudomonas aeruginosa

The level protein content in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24-72 hrs and was compared to their corresponding controls. Protein content observed in the control group of *Pseudomonas aeruginosa* was 35.8, 54.6 and 66.0 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of protein content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.10). With the increasing concentration of 10^{-8} M of carbaryl, the level of protein content with inhibition was [25.4(29.1%), 30.8(43.6%) and 37.2(43.6%) µg/ml]; 10^{-6} M [18.0(49.7%), 23.8(56.4%) and 28.0(57.6%)µg/ml]; 10^{-4} M [11.0(68.3%), 14.6(73.3%) and 16.8(74.5%) µg/ml]; and 10^{-2} M [6.6(81.6%), 7.0(87.2%), and 8.0(87.9%)µg/ml] at a given periods of 24-72 hrs respectively.
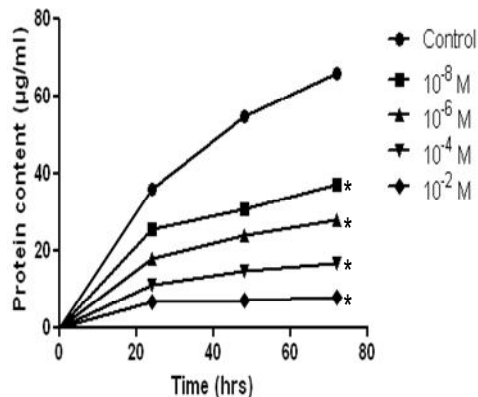

Fig. 10: Dose dependent effect of carbaryl on protein content in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for protein estimation and values are represented as protein content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Dose dependent effect of carbaryl on glucose content
Escherichia coli

The level of glucose content 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 24-72 hrs and was compared to their corresponding controls. Glucose content observed in the control group of free *Escherichia coli* cells was 32.4, 43.0 and 56.4 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of glucose content when compared with that

of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.11). With the increasing concentration of 10^{-8} M of carbaryl, the level of glucose content with inhibition was [27.4(15.4%), 30.2(29.8%) and 35.4(37.2%) µg/ml]; 10^{-6} M [17.6(45.7%), 22.2(48.4%) and 29.0(48.6%)µg/ml]; 10^{-4} M [12.8(60.5%), 14.1(67.2%) and 17.6(68.8%) µg/ml]; and 10^{-2} M [9.6(70.4%), 10.8(74.9%), and 12.4(78.0%)µg/ml] at a given periods of 24-72 hrs respectively.
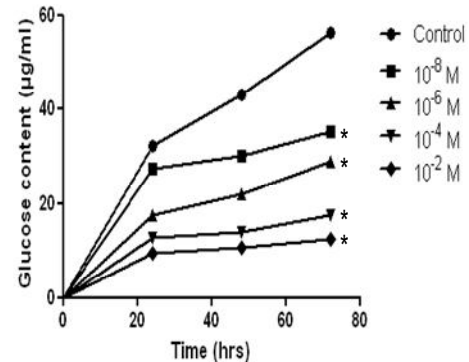

Fig. 11: Dose dependent effect of carbaryl on glucose content in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for glucose estimation and values are represented as glucose content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized *Escherichia coli*

The level of glucose content 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 24-72 hrs and was compared to their corresponding controls. Glucose content observed in the control group of immobilized *Escherichia coli* cells was 28.4, 38.0 and 46.6 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of glucose content when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.12).

Fig. 12: Dose dependent effect of carbaryl on glucose content 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, samples were assessed for glucose estimation and values are represented as glucose content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

With the increasing concentration of 10^{-8} M of carbaryl, the level of glucose content with inhibition was [24.8(12.7%),28.4(25.3%) and 34.0(27.0%) µg/ml]; 10^{-6} M [17.6(38.0%),20.4(46.3%) and 26.2(43.8%)µg/ml]; 10^{-4} M [13.6(52.1%), 16.2(57.4%) and 20.0(57.1%) µg/ml]; and 10^{-2} M [7.6(73.2%), 9.2(75.8%), and 11.8 (74.7%)µg/ml] at a given periods of 24-72 hrs respectively.

Pseudomonas aeruginosa

The level glucose content in *Pseudomonas aeruginosa* bacteria that were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24-72 hrs and was compared to their corresponding controls. Glucose content observed in the control group of *Pseudomonas aeruginosa* was 23.6, 33.0 and 40.0 µg /ml respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of glucose content when compared with that of the controls and increase in the % inhibition was observed with an in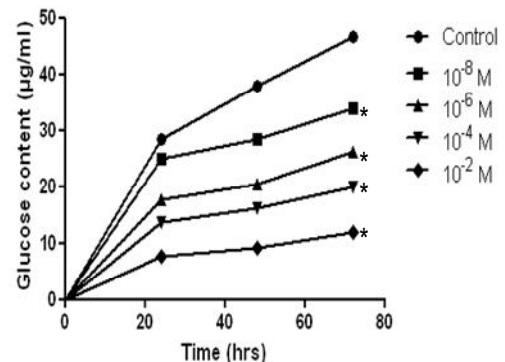crease in dose and durational exposure to carbaryl (Fig.13). With the increasing concentration of 10^{-8} M of carbaryl, the level of glucose content with inhibition was [16.0(32.2%),20.6(37.6%) and 24.0(40.0%) µg/ml]; 10^{-6} M [11.0(53.4%), 14.0(57.6%) and 18.0(55.0%)µg/ml]; 10^{-4} M [9.6(59.3%), 12.1(63.3%) and 14.0(65.0%) µg/ml]; and 10^{-2} M [8.4(64.4%), 9.8(70.3%), and 11.0(72.5%)µg/ml] at a given periods of 24-72 hrs respectively.
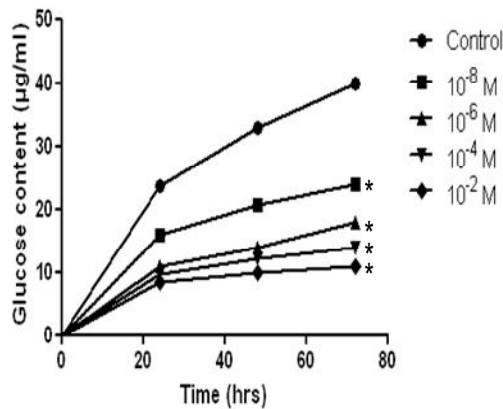

Fig. 13: Dose dependent effect of carbaryl on glucose content in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for glucose estimation and values are represented as glucose content (µg/ml). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Dose dependent effect of carbaryl on growth
Escherichia coli

The level of optical density 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 24-72 hrs and was compared to their corresponding controls. Optical density observed in the control group of free *Escherichia coli* cells was 0.233, 0.320 and 0.369 respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of optical density when compared with that of the controls and increase in the % inhibition was observed with an

increase in dose and durational exposure to carbaryl (Fig.14). With the increasing concentration of 10^{-8} M of carbaryl, the level of optical density with inhibition was [0.175(24.9%),0.231(27.8%) and 0.262 (28.9%)]; 10^{-6} M [0.129 (44.6%),0.165 (48.4%) and 0.202 (45.3%)]; 10^{-4} M [0.109 (53.2%),0.125 (60.9%) and 0.141 (61.8%)]; and 10^{-2} M [0.079 (66.1%),0.104 (67.5%), and 0.115 (68.8%)] at a given periods of 24-72 hrs respectively.
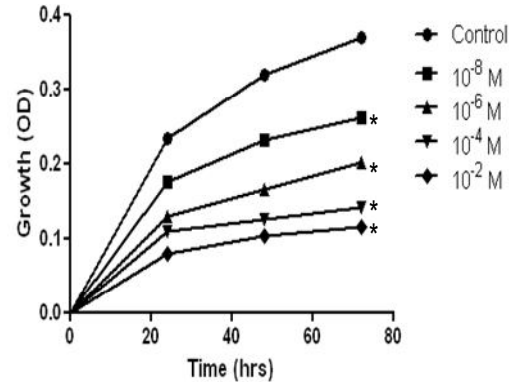

Fig. 14: Dose dependent effect of carbaryl on the growth level in free *Escherichia coli*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2}M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for growth concentration and values are represented as optical density (OD). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

Immobilized Escherichia coli

The level of optical density 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 24-72 hrs and was compared to their corresponding controls. Optical density observed in the control group of immobilized *Escherichia coli* cells was 0.264, 0.333 and 0.430 respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant (P ≤ 0.05) decrease in the level of optical density when compared with that of the controls and increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl (Fig.15).

Fig. 15: Dose dependent effect of carbaryl on the growth level 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, samples were assessed for growth concentration and values are represented as optical density (OD). Values are mean ± SEM (n = 10) and * indicates significant (P ≤ 0.05) compared to control.

With the increasing concentration of 10^{-8} M of carbaryl, the level of optical density with inhibition was [0.182 (31.1%),0.245 (26.4%) and 0.301 (30.0%)]; 10^{-6} M [0.145 (45.1%),0.190 (42.9%) and 0.251 (41.6%)]; 10^{-4} M [0.124 (53.0%),0.156 (53.2%) and 0.210 (51.2%)]; and 10^{-2} M [0.105 (60.2%),0.134 (59.8%), and 0.168 (60.9%)] at a given periods of 24-72 hrs respectively.

Pseudomonas aeruginosa

The level of optical density in *Pseudomonas aeruginosa* bacteria were exposed to different concentrations of carbaryl ranging from 10^{-8} to 10^{-2} M for a period of 24-72 hrs and was compared to their corresponding controls. Optical density observed in the control group of *Pseudomonas aeruginosa* was 0.177, 0.241and 0.292 respectively. However, graded concentrations from 10^{-8} to 10^{-2} M carbaryl treated groups exhibited a significant ($P \leq 0.05$) decrease in the level of optical density when compared with that of the controls and increase in the % inhibition was observed with an increase 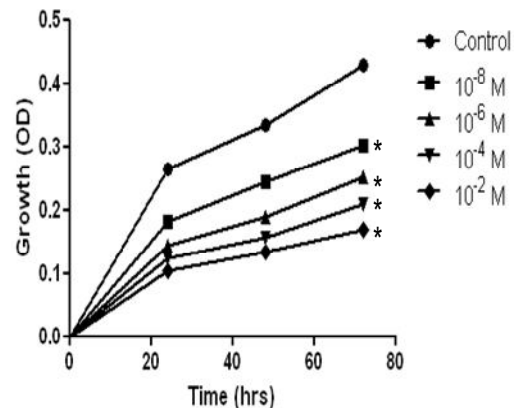in dose and durational exposure to carbaryl (Fig.16). With the increasing concentration of 10^{-8} M of carbaryl, the level of optical density with inhibition was [0.127 (28.2%),0.146 (39.4%) and 0.185 (36.6%)]; 10^{-6} M [0.081 (54.2%), 0.101 (58.1%) and 0.119 (59.2%)]; 10^{-4} M [0.069 (68.9%),0.089 (63.1%) and 0.101 (65.4%)]; and 10^{-2} M [0.055 (68.9%),0.068 (71.8%), and 0.079 (72.9%)] at a given periods of 24- hrs respectively.
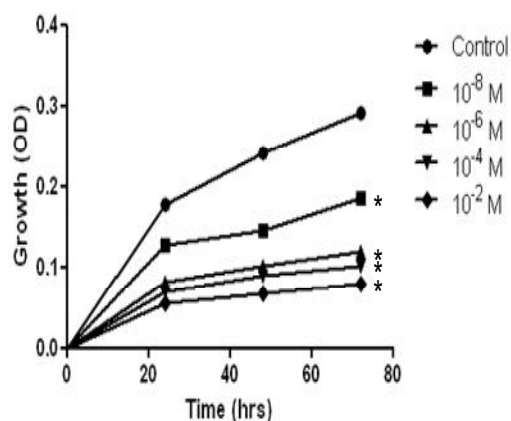

Fig. 16: Dose dependent effect of carbaryl on the growth level in *Pseudomonas aeruginosa*. Cells that were exposed to medium amended with increasing quantities of 10^{-8} to 10^{-2} M concentrations of carbaryl for a period of 24 to 72hrs respectively. At regular intervals, samples were assessed for growth concentration and values are represented as optical density (OD). Values are mean ± SEM (n = 10) and * indicates significant ($P \leq 0.05$) compared to control.

DISCUSSION

The present investigation was attempted to study the effect of carbaryl on biochemical parameters in *Escherichia coli* and *Pseudomonas aeruginosa* cells that were exposed to different concentrations ranging from 10^{-8}-10^{-2} M for a period of 24- 72 hrs. In this graded dose and durational exposure study, there was a significant decrease in the level of DNA content in all the treated groups in *Escherichia coli* and *Pseudomonas aeruginosa*. However, increase in the % inhibition was observed with an increase in dose and durational exposure to carbaryl. It has been

indicated that, the percent inhibition of all the parameters is more in free cells than in immobilized cells revealing that the immobilized system is less sensitive to the toxicant; it inhibited lesser stress and higher tolerance to carbaryl. It has been reported that carbamate pesticides cause various chromosomal aberrations such as chromosome breaks, dots, deletions and laggards and are able to decrease the RI (Replication Index) by preventing the replication of DNA (Saxena *et al.,* 1997**;** Rahman *et al.,* 2002). Nucleic acids are the most important macromolecules that perform all kinds of necessary biological information and involved in function which is essential in regulation of cell metabolism. It has been suggested that the biological targets for these pesticides highly reactive oxygen species are DNA, RNA, proteins and lipids. The activation of these responses greatly increases cellular resistance to oxidative agents (Cabiscol *et al.,* 2000). Reports shown that any xenobiotic, influence the micro-organisms by harmfully affecting their growth, morphology and biochemical activities, resulting in decreased biomass and diversity, and it has also been shown that both long term and short term stresses such as temperature, pH and chemical pollution often result in the altered metabolism, species diversity and plasmid incidence of the bacterial populations (Malik and Ahmed, 2002; Bahig *et al.,* 2008). Biochemical changes often ensure very early in the response to toxicants as a function of dose and duration of exposure to toxicants. From the reports on carbaryl is highly toxic, a pollutant of environmental concern because of its high solubility in water (120mg/L at 20° C) and has not shown any convincing evidence of genotoxic activity and no observed effect levels (NOELs) of 1000 and 1500 were demonstrated in the species for bladder, hepatic, thyroid and renal tumours (APVMA, 2007). According to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2001), the weight of evidence indicated that carbaryl is not an in vivo genotoxic agent. In mice and rats, carbaryl was found to be carcinogenic and likely to be carcinogenic to humans based on increased incidence of vascular tumours in mice. It has been reported that carbaryl known to cause sperm abnormalities, reduction in number of spermatononia, spermatozoa and degeneration of Leydig cells in mice (Shrivastava and Shrivastava, 1998). However, the extent of heritable genetic abnormalities associated with increased sperm anomalies remains unclear. It has also been shown that several pesticides cause reactive oxygen species and production of malondialdehyde which is one of the most important products of lipidperoxidation and interferes with protein biosynthesis by forming adduct with DNA, RNA and proteins (Doreswamy, 2004). Carbamate pesticides are known to cause chromosomal breaks by breaking phosphodiester backbone of DNA and induce decreased mitotic index (Barale *et al.,* 1993). It has been demonstrated that the stasis induced oxidation targets both DNA and protein and that some enzymes are specifically susceptible to oxidative attacks (Johnson *et al.,* 2001). Genetic responses to oxidative stress are known to occur in bacteria, yeast, mammalian cell line and in general in all aerobic organisms (Hidalgo and Demple, 1995). The decrease in DNA content and increase in the percent inhibition in DNA observed in the present

study may be due to possible genotoxic action of carbaryl or susceptibility to oxidative attacks or cleavage or breaking of phosphodiester bonds of DNA. RNA plays a vital role in cell metabolism by producing many proteins to catalyze the various biochemical reactions and various enzymes, which catalyzes various cellular reactions. Small chemical species can interact with proteins and other RNA's and even is the direct sensors of environmental inputs (Cases and De Lorenzo, 2005). RNA synthesis is known to be altered by chemical action and is one of the first metabolic alterations brought about that precedes the increase activities of many of the enzymes, RNA synthesis which would in turn influence the level of the protein synthesis. Thus the total RNA content of an organ is an index of functional status. It has been suggested that elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acid contents in response to pesticides with release of nucleases and proteases affecting RNA, DNA and protein metabolism (Awasthi *et al.,* 1984). The findings of the present study on dose and durational exposure of carbaryl to *Escherichia coli* and *Pseudomonas aeruginosa* revealed that, there was a significant decrease in the level of RNA content and increase in % inhibition may be due to the fact that the biological targets for the reactive oxygen species due to oxidative stress are RNA, DNA, proteins and lipids or inhibitory action of enzymes and induction of apoptosis which in turn cause damage to DNA and RNA.

Proteins function in different ways in the cell as enzymes, structural proteins and functional proteins. The major protein modification is observed due to stress and the loss of catalytic activity, amino acid modification, carbonyl group formation, increase in acidity, decrease in thermal stability, change in viscosity, fluorescence, fragmentation, formation of protein crosslink's, s-s bridges and increased susceptibility to proteolysis (Stadtman, 1992). It has been reported that in a wide range of bacteria, the activities of the protein family demonstrate an amazing flexibility through their ability to assimilate signals that indicate cellular metabolic activity in response to frequent biochemical changes in the environment (Baker *et al.,* 2006). Heavily oxidized proteins, extensively cross-linked and aggregated, are not only poor substrates for degradation but can also inhibit proteases to degrade other oxidized proteins (Stadtman 1992). The removal of damaged proteins is necessary to prevent their accumulation, which could compromise the correct metabolism of any cell exposed to oxidative stress. This phenomenon has been related with aging in higher organisms (Berlett and Stadtman 1997). The secretion of extra cellular proteins, including toxins and cellular effectors, is one of the key contributing factors in a bacterium's ability to thrive in diverse environments (Baker *et al.,* 2006). Sancho *et al.,* (1998) have suggested that the decline in protein level indicates the physiological adaptability to compensate for pesticide stress and to overcome the stress; they use more energy, which leads to stimulation of protein catabolism. The findings of the present study on dose and durational exposure of carbaryl to *Escherichia coli* and *Pseudomonas aeruginosa* revealed that there was a significant

decrease in the level of protein content in all the treated groups and increase in % inhibition may be due to increased susceptibility to proteolysis, oxidative stress, reactive oxygen species that interferes with protein biosynthesis by forming adduct with DNA, RNA and proteins.

Glucose is the main metabolic fuel and the most readily available source of energy. It is an essential substrate for the maintenance of cell integrity, for maximal oxygen uptake, energy production and protein synthesis. It has also been revealed that the most frequent combination of adverse factors, especially for aerobic or facultatively anaerobic microorganisms such as *Escherichia coli*, is the combination of glucose starvation and oxidative stress (Salakhetdinova *et al.,* 2000). At the cellular level, when proteins are exposed to reactive oxygen species, modifications of amino acid side chains occur and, consequently, the protein structure is altered. These modifications lead to functional changes that disturb cellular metabolism (Stadtman, 1992). Reactive oxygen species also attack DNA, producing chain breaks, modification of the carbohydrate parts and nitro bases, and this may lead to point mutation (Halliwell and Gutteridge, 1989). ROS are unstable free radical species in cells produced when oxidative stress occurs. These unstable free radical species can attack cellular components, inducing damage to lipids, proteins, DNA and carbohydrates (Mori *et al.,* 2007). The significant decrease in the in the glucose content and increase in percent inhibition observed in the present study may be due to glucose starvation and oxidative stress (Salakhetdinova *et al.,* 2000), disturbance in cellular metabolism (Stadtman, 1992), modification of carbohydrate (Halliwell and Gutteridge, 1989), unstable free radical species that can attack cellular components, inducing damage to lipids, proteins, DNA and carbohydrates (Mori *et al.,* 2007). Studies have been shown that methomyl carbamate exposure promotes the oxidation damage of liver cells by enhancing peroxidation of membrane lipids (Wafa *et al.,* 2011), which was evident by enhanced LDH activity in liver accompanied by decreased content of GSH as well as suppression of the activities of antioxidant enzymes (El-Khawaga, 2012).

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). 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 and in exponentially growing and starving *Escherichia coli* cultures, different systems are involved in cell defense against oxidative stress (Salakhetdinova, *et al.,* 2000). The increase in microbial population with time in most of the treatment options is an indication of utilization of the organic compounds in these options (Odokuma and Akubuenyi (2008). It has also been suggested that this decline in microbial counts must have been due

to the fact that microbial populations that were tolerant of treated pesticides were susceptible to the products of soil pesticide interactions, which could have possibly been bactericidal or fungicidal (Ayansina and Oso, 2006). Inhibition of DNA, RNA, proteins and growth was more in the free cells of *Bacillus* sp compared to immobilized cells (Gaddad *et al.,* 2005). Pesticides had shown that reduction in the total number of soil bacteria under laboratory and field conditions (Rangaswamy and Venkateshwarlu, 1992; Ahmed and Ahmad, 2006). In the present study, though in *Pseudomonas aeruginosa* growth was lesser when compared to *Escherichia coli,* the significant decrease and increase in % inhibition of growth of *Escherichia coli* and *Pseudomonas aeruginosa* may be due to interwoven relationships between organisms in different tropic levels, that will lead to many indirect effects or bacteria that display complex adaptive reactions in response to adverse environmental conditions in order to survive various combinations of stress factors, bactericidal or fungicidal action of the toxicant or as a consequence of secondary stress reaction of cells. The present study suggested that carbaryl is a toxicant affecting the synthesis of biochemical contents, growth and such parameters of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to carbaryl are dose and duration dependent. The alterations in the biochemical contents can be due to carbaryl intoxication. The decrease in the growth and biochemical parameters in the dose and duration of exposure of carbaryl was observed in both, *Escherichia coli* and *Pseudomonas* aeruginosa, however, in comparison with its free counterpart the activity was less in immobilized *Escherichia coli* cells revealing that immobilized system is less sensitive to the toxicant. 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. Therefore, further investigation is required to study the effect of carbaryl for its toxicity to *Escherichia coli* and soil isolate- *Pseudomonas aeruginosa* cells with emphasis on the enzymes, providing sensitive index to the changes due to pesticide toxicity, and such parameters are widely used for rapid detection and to predict early warming of pesticide toxicity.

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