3.1. INTRODUCTION

The major cause of environmental and agricultural soil pollution is usage of pesticides, these are most potential among all the pollutant for causing pollution among other toxicants (Hill, 1985). Although in India number of banned pesticides are being regularly used in large scale for the protection of standing crops. This conditions of utilization of pesticides will have adverse effect on the health and environment. To overcome the problems associated with the pesticides, many of the researchers from all over the world are working for finding the desire method to limit/stop the problems of pesticides, most of the researcher achieved through the biological weapons called microorganisms, microorganisms are adopted to the various toxic compounds for dissolution of toxic effects (Tien et al., 2013; Fang et al., 2014; Cai et al., 2015; Deng et al., 2015).

Pesticides pose either as a toxicant or appear as a somewhat broad range of activities against biological organisms (Rath and Misra, 1978) and physiological and biological harmfulness of pesticides on DNA, RNA and protein concentrations is well known (Dave et al., 1975). The reactive oxygen species aims the nucleic acids and proteins. During the nutritional stress and oxidative stress the DNA binding protein will produce and protect the DNA from physical oxidative distress and will allow to maintain the significant level of gene expression and if microorganisms survived against physiological and biological damaging effects they will gain the resistance over treated toxic compounds and also reported that the more damage will caused by the hydroxyl radicals produced from H$_2$O$_2$ through the Fenton reaction, which requires iron (or another divalent metal ion, such as copper) and a basis of decreasing equivalents (possibly NADH) to regenerate the metal (Cabisco et al., 2000). It is known that, organisms exposed to the pesticides will cause reproductive, carcinogenic, mutagenic
and oncogenic effects (Didla et al., 2011). Moreover, it was also shown that reactive oxygen species (ROS) will be produced excessively (Mathew et al., 1992; Bagchi et al., 1995).

From the above said reports it is clear that toxicants interact with different nucleic acids and enzymes of the targeted and non-targeted organisms, thus damaging the physiological and metabolic activities of the cell. Biochemical parameters are broadly applied for the detection and to forecast the pesticide toxicity. Therefore, propiconazole a triazole group of fungicide was evaluated for its toxicity on soil isolates *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains with emphasis on biochemical parameters such as DNA, RNA, protein, glucose and enzyme assays.
3.2. MATERIALS AND METHODS

3.2.1. Preparation of stock solution for propiconazole

The standard propiconazole (94%) was used for the experiment and it was obtained from the Nagarjuna Agrichem Co., Ltd. (Srikakullam, India). Stock solution of Propiconazole (1 mg/ml) was prepared by dissolving accurate amount in the methanol/acetonitrile and working standards were prepared by required amount by dissolving.

3.2.2. Maintenance of isolated pure cultures

The soil isolates *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 were maintained at 4°C on nutrient agar and were sub cultured very fortnight.

3.2.3. Medium used for the bacterial culture

The medium used for toxicity testing was an optimized medium dextrose - 0.65 g/l, Yeast extract - 1.05 g/l, KHPO₄ - 0.30 g/l, and NaCl - 0.25 g/l and pH (7.0 -7.2).

3.2.4. Preparation of inoculum

The inoculum was prepared by inoculating the loopful of fully grown bacteria from the 24 hrs incubated nutrient agar plate in a sterilized optimized dextrose medium and incubated for the 24 hrs at 35 °C in incubator shaker with 120 rpm.

3.2.5. Effect of propiconazole on biochemical content of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains

From the above mentioned medium five ml of the inoculum was aseptically inoculated to 250 ml Erlenmeyer’s flask containing 100 ml of sterilized optimized medium amended with different concentrations of propiconazole. Further, all the flasks were incubated at 35 °C for 72 hrs for *Pseudomonas aeruginosa* PS-4 and 96 hrs for
Burkholderia sp. BBK_9 strain under shaking conditions at 120 rpm on a rotary shaker. At regular intervals, fraction cultures were aseptically withdrawn from the each flasks for examination.

3.2.5.1. Nucleic acids Isolation and estimation

Perchloric acid of 0.5N strength was added to the bacterial pellet of 10 ml culture and preparation was allowed to incubate in the water bath at 70°C for 10 mins with constant shaking and further tubes were centrifuged at 3000 rpm for 20 min in 4 °C. The above mentioned extraction was repeated for two time with the 3ml of 0.5 N perchloric acid and from the obtained extracts both DNA was estimated by the diphenylamine method (DPA) (Burton, 1956). Conversely, RNA was estimated by the orcinol method (Brown, 1946).

3.2.5.1.1. DNA estimation by DPA method

DNA was estimated by the diphenylamine method (DPA) which was previously described by the Burton (1956).

Once DNA is treated with diphenyl amine with acid environments, a bluish green colored complex was formed which has an absorption at 595 nm and this reaction was forms of deoxypentose. In acidic solution deoxypentose was converted into a very sensitive β-hydroxylevulinaldehyde further which responds with diphenylamine forms bluish green colored complex.

4 ml of diphenylamine reagent was added to 2 ml of the bacterial extracts and mix thoroughly. Further, the mouth of the tubes were covered and incubated in boiling water bath for 10 mins and the tubes were cooled to room temperature and the optical density was taken at 595 nm against water blank and to find the concentration of DNA in bacterial extracts standard calibration graph was referred.
3.2.5.1.2. RNA estimation

RNA was estimated by the orcinol method which was previously described by the Brown (1946).

RNA was treated with orcinol reagent in the presence of ferric chloride and Hydrochloric acid (HCL) which can be estimated calorimetrically. This reaction of pentoses forms furfural rings when treated with concentrated HCL. Further, orcinol reacts with furfural in the presence of ferric chloride as a catalyst to form the green color which can be measured at 665 nm.

2 ml of cell filtrates of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* BBK_9 strains were supplemented with the 3 ml of orcinol reagent and heated on boiling water bath for 20 min. and optical density (OD) was determined at 665 nm against water blank. The amount of RNA was calculated by referring the standard graph.

3.2.5.2. Protein estimation

The protein was estimated by Lowry method which was previously described by the Lowry *et al.*, (1951).

3.2.5.2.1. Extraction of culture filtrates

10 ml of 24 hrs old cultures were treated with 2 ml of 0.5 N NaOH and incubated in a boiling water bath for 5 min and cooled to ambient temperature. Further, tubes were centrifuged at 10000 x g for 10 min (Eppendorf centrifuge) and obtained clear supernatant was for estimating the protein.

Proteins reacts with folinciocaltean reagent (FCR) to form blue colored complex and the color so formed was due to the reaction of alkaline copper with the peptide bonds in proteins. The reduction of phosphomolybdate and phosphotungstic of FCR by tryptophan and tyrosine residues present in the protein.
To 1 ml of the culture extracts of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* BBK_9 strains, 5 ml of alkaline copper reagent was added with thorough mixing and allowed it to stand at room temperature for 20 min. Then 0.5 ml of FCR was added, mixed well and incubate at room temperature for 30 min. Read the Optical density (OD) of blue colored solution at 660 nm against water blank. The amount of protein is calculated by referring the standard graph prepared by using Bovine serum albumin.

### 3.2.5.3. Estimation of glucose utilization

The utilization of glucose by the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* BBK_9 strains was estimated by Anthrone method which was previously described by the Hedge and Hofreiter, (1962).

Carbohydrates are first hydrolyzed into simple sugar using diluted HCL in hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural and this compound forms with anthrone a green colored product with absorption maximum at 630 nm.

24 hrs liquid culture medium of 0.1 ml was diluted up to 1 ml with distilled water and 4 ml of anthrone reagent was added, tube were mixed thoroughly and incubated over boiling water bath for 5 min and allow the tubes to cool at room temperature, a dark green colored product was formed and its optical density was recorded at 620 nm against water blank. The glucose content is calculated by referring the standard graph of glucose.

### 3.2.5.4. Extraction of enzymes

For the extraction of cell free filtrates the method described by Talwar *et al.*, (2013) was followed. *Pseudomonas aeruginosa* PS-4 and *Burkholderia* BBK_9 strains cells were washed and grown in Tris–HCl buffer pH 6.8 amended with 1 µg/l
propiconazole and sonicated (Sonics vibra cell) for 5 mins and centrifuged. The supernatant was used for the enzyme assay. The activity of amylase, protease, phosphatase, superoxide dismutase, catalase and peroxidase.

3.2.5.5. Amylase

Amylase activity was tested using the method described by Noelting and Bernfeld (1945). A tube containing 2 ml of the sample/ aliquot diluted with distilled water and further it was incubated with the addition of 2 ml of phosphate buffer pH 6.9 and 2 ml of 1% soluble starch at 37°C for 15 minutes. Further, 2 ml of DNS reagent was added and the tube was incubated over boiling water both for 10 min. The optical density (OD) was measured at 540 nm against a control. The quantity of reducing disaccharide released by the enzyme was calculated by using the standard graph of maltose. The unit of amylase activity is µmoles per ml per min of the sample.

3.2.5.6. Protease activity

Protease activity of Pseudomonas aeruginosa PS-4 and Burkholderia BBK_9 strains were estimated as per the method of Haddad et al., (1982).

2 ml of culture filtrates of Pseudomonas aeruginosa PS-4 and Burkholderia BBK_9 strains were incubated with 1% casein and phosphate buffer pH 7.6 for about 1.5 hrs at 37°C. To stop the enzymatic reaction 3 ml of 10% TCA solution was added. Further, the reaction mixture was filtered by using the Whattman filter paper and the obtained filtrates were examined for the protease activity by spectrophotometrically at 660 nm with addition of 0.5 ml of folin phenol reagent and 3 ml of 0.5 N. The unit of protease activity is calculated in micrograms of released tryptophan (per minute per ml of the sample).
3.2.5.7 Phosphatase activity

Alkaline phosphatase activity of *Pseudomonas aeruginosa* PS-4 and Burkholderia BBK_9 strains were was determined as per the method of Vastraete et al., (1976).

2 ml of culture filtrates were incubated with 2 ml of tris buffer pH 8.4 for 4 hrs. Further, 2 ml of the mixtures of reaction were transferred to the tubes containing 2 ml of 0.05 M EDTA and 2 ml of 0.5N NaOH. The activity of phosphatase was measured by spectrophotometrically at 420 nm against control. The quantity of released p-nitrophenol was estimated by using the standard graph. The unit of phosphatase activity is expressed as micro grams of p-nitrophenol released per minute per ml.

3.2.5.8. Superoxide dismutase (SOD)

SOD activity was estimated using the culture filtrates of *Pseudomonas aeruginosa* PS-4 and Burkholderia BBK_9 strains by following the method of Beauchamp and Fridovich, (1971).

0-150 µl of culture filtrates were added to tubes containing 50Mm phosphate buffer pH 7.8, 13 mM methionine, 75 mM nitrobluetetrazolium chloride monohydrate (NBT), 0.1 mM EDTA, 2µM riboflavin. Reaction mixtures were thoroughly mixed and tubes were incubated near light. Activity of SOD was measured at 560 nm. Based on the inhibition of NBT by volume of enzyme extracts, one unit of the enzyme was calculated and it was expressed as µmoles per ml per min.

3.2.5.9 Catalase (CAT)

Catalase activity of *Pseudomonas aeruginosa* PS-4 and Burkholderia BBK_9 strains was examined using the method employed by the Sadasivam and Manickam, (1996).
25 µl of the cell free culture extracts were added to the tubes containing 50Mm phosphate buffer pH 7.0 and 15mM hydrogen peroxide. The activity of catalase was measured at 240 nm against the control at regular interval of every 30 secs up to 3 min. The activity catalase was calculated using the following formula:

\[
\text{Activity} = \frac{\text{O.D}}{0.36 \times 1 \times \Delta t \times \text{vol. of reaction mixture}} \times 40 \text{ µmoles/ml/min}
\]

3.2.5.10. Peroxidase assay (POX)

The Peroxidase assay (POX) for *Pseudomonas aeruginosa* PS-4 and *Burkholderia BBK_9* strains were conducted as per the method of Sadashivam and Manickam (1996).

0.1 ml of cell free enzyme extract was transferred to the tubes containing 100Mm of potassium phosphate buffer pH 7.0 and 0.05 ml of 20 mM of guaiacol solution with the 0.03 ml of 12.3mM of hydrogen peroxide. The tubes were thoroughly shaken and in the meantime activity of POX was recorded at every 30 seconds up to 5 min against control at 436 nm. The activity of catalase was subsequently calculated using the below mentioned formula:

\[
\text{Activity} = \frac{\text{O.D}}{6.93 \times \Delta t \times \text{vol. of reaction mixture}} \times 10 \text{ µmoles/ml/min}
\]

3.2.5.11. Statistical analysis

All the experimental data were carried in three independent replicates and expressed as means ± standard errors. The statistical analyses of the data were performed using one way ANNOVA variance SPSS version 20.0 software with advanced models (SPSS Japan, Tokyo, Japan). Differences between means were located using Tukey’s test (\( P < 0.05 \)).
3.3. OBSERVATIONS

4.3.1. Effect of propiconazole on DNA content in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.1 and 3.2; Graph 3.1 and 3.2)

The impact of propiconazole on the DNA content in *pseudomonas aeruginosa* PS-4 strain, treated with the propiconazole concentration of 10, 20 and 30 µg/l and strain without treated with propiconazole was maintained as control (Table 3.1). The DNA content in the *pseudomonas aeruginosa* PS-4 strain in control was found to be 91.92, 138.43 and 172.46 µg/ml after 24, 48 and 72 hrs of incubation respectively. Similarly, the DNA content in PS-4 strain at 10 µg/l of propiconazole was found to be 77.16, 104 and 148.33 µg/ml after 24, 48 and 72 hrs of incubation respectively. In the meantime, bacterial cells of PS-4 strain treated with the 20 µg/l of propiconazole was showed the similar trend of DNA content and it was found to be 51.66, 87.77 and 98.45 µg/ml after 24, 48 and 72 hrs of incubation respectively. The DNA concentrations in the PS-4 strain treated with 30 µg/l was found to be 19.32, 43.42 and 47.34 µg/ml after 24, 48 and 72 hrs of incubation respectively (Graph 3.1).

The effect of propiconazole on the DNA concentration of *Burkholderia* sp. BBK_9 strain is given the table 3.2. The DNA content in the control (without exposure to propiconazole) was found to be 102.13, 147.53 and 169.08 µg/ml after the desired incubation period of 24, 48 and 72 hrs respectively. The DNA concentration of bacterial cells treated with 10 µg/l was found to be 85.73, 128.63 and 141.18 after the incubation period of 24, 48 and 72 hrs respectively. Similarly, the DNA concentration in the bacterial cells treated with 20 µg/l was 59.03, 91.68 and 88.63 µg/ml after 24, 48 and 72 hrs of given incubation respectively. The bacterial cells treated with 30 µg/ml exhibits the low amount of DNA content when above mentioned concentrations of propiconazole (10 and 20 µg/l) and the concentration of DNA was found to be 27.63, 64.12 and 57.96 µg/ml after 24, 48 and 72 hrs of incubation respectively (Graph 3.2).
The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on DNA content exhibits a reverse hierarchy (c to a) and denoted letter(s) were found significantly different and the DNA content was significantly decreased.

3.3.2. Effect of propiconazole on RNA content in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.3 and 3.4; Graph 3.3 and 3.4)

The effect of propiconazole on the RNA content in the *Pseudomonas aeruginosa* PS-4 was given the table 3.3. The bacterial cells (PS-4) which was not treated with the propiconazole (control) was found to be 29.49, 46.0 µg/ml and 71.96 at 24, 48 and 72 hrs respectively. The DNA concentration the cells treated with the 10 µg/l was found 23.7, 31.53 and 34.64 µg/ml after the incubation of 24, 48 and 72 hrs respectively. Conversely, bacterium treated with 20 µg/l of propiconazole showed the increasing trend in the RNA concentrations and it was found to be 17.24, 21.8 and 24.93 µg/ml in 24, 48 and 72 hrs respectively. In the other hand, cells treated with the 30 µg/l was found lethal and as a result the DNA was found to be 9.32, 11.75 and 11.0 µg/ml after 24, 48 and 72 hrs of incubation period respectively (Graph 3.3).

The effect of propiconazole on the RNA concentration of *Burkholderia* sp. BBK_9 strain is given the table 3.4. In the control (without treated with propiconazole) the RNA concentration of *Burkholderia* sp. BBK_9 strain was found 24.59, 53.50 and 76.64 µg/ml in desired incubation period of 24, 48 and 72 hrs respectively. Meanwhile, the cells BBK-9 strain treated with 10 µg/l of propiconazole was exhibits the increasing pattern of RNA contents with increases duration and was content of RNA was found to be 25.83, 36.42 and 41.74 µg/ml at 24, 48 and 72 hrs respectively. The bacterial strain
exposed to 20 µg/l of propiconazole showed different amount of RNA at different duration and the concentration of RNA was found to be 19.58, 27.72 and 32.91 µg/ml after the given incubation period. Propiconazole of 30 µg/l strength was showed toxic effect on the RNA content and it was found 11.2, 15.25 and 17.18 µg/ml after 24, 48 and 72 hrs respectively (Graph 3.4).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on RNA content exhibits a reverse hierarchy (c to a) and denoted letter(s) were found significantly different and the RNA content was significantly decreased.

3.3.3. Effect of propiconazole on protein content in Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains (Table 3.5 and 3.6; Graph 3.5)

The effect of propiconazole on protein content of Pseudomonas aeruginosa PS-4 strain is given in the table 3.5. In the controls the protein concentration was found to be 1.21, 1.25 and 1.12 mg/ml in the given incubation period of 24, 48 and 72 hrs respectively. On the exposure with the 10 µg/l of propiconazole, the protein concentration was 1.18, 1.13 and 1.04 mg/ml at the given incubation time of 24, 48 and 72 hrs respectively. Simultaneously, bacterial strain treated with 20 µg/l of propiconazole showed 1.16, 1.27 and 1.0 mg/ml after the 24, 48 and 72 hrs of incubation. On exposure with 30 µg/l of propiconazole to strain PS-4 exhibits 1.19, 1.07 and 0.89 0 mg/ml of protein concentration at 24, 48 and 72 hrs respectively (Graph 3.5).

The propiconazole effect on protein content of Burkholderia sp. strain BBK_9 is given in the table 3.6. In group of controls protein concentration was found to be
1.40, 1.54 and 1.27 mg/ml at desired incubation period. Conversely, the medium amended with 10 µg/l showed protein concentration 1.31, 1.26 and 1.13 mg/ml at 24, 48 and 72 hrs respectively. The strain treated with 20 µg/l of propiconazole showed protein concentration of 1.33, 1.24 and 1.39 in a 24, 48 and 72 hrs of incubation period. Likewise, 30 µg/l of propiconazole was also favors the isolate to express the protein concentration like 1.28, 1.18 and 1.10 at 24, 48 and 72 hrs respectively (Graph 3.5).

The results of the present study exposed that there was a significant decrease in the level of protein content in all the treated groups when compared with of corresponding parameters (controls).

3.3.4. Effect of propiconazole on utilization of glucose by *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.7 and 3.8; Graph 3.6 and 3.7)

The estimated utilized glucose concentration by the *Pseudomonas aeruginosa* PS-4 was given in the table 3.7. It was proved that the control groups which were not treated with propiconazole showed the good rate of glucose utilization than that of propiconazole treated groups. In control groups the utilization of glucose by *Pseudomonas aeruginosa* PS-4 strain enhances up to 32.84 to 50.88 µg/ml in all the given incubation period. Likewise, PS-4 strain treated with 10 µg/l of propiconazole was also enhances the glucose utilization like 29.60, 35.50 and 43.66 µg/ml in given incubation period of 24, 48 and 72 hrs respectively. Medium amended with 20 µg/l of propiconazole showed the similar trend of glucose utilize and exhibit the 21.46, 29.34 and 33.82 µg/ml of glucose. Similarly, strain (PS-4) exposed to 30 µg/l of propiconazole was exhibit glucose utilization 14.83, 15.76 and 17.89 µg/ml at 24, 48 and 72 hrs respectively (Graph 3.6).
The utilization of glucose by the *Burkholderia* sp. BBK_9 strain is given in the table 3.8. In the control (without treated with propiconazole) glucose utilization by *Burkholderia* sp. BBK_9 strain was found 35.65, 42.41 and 55.03 µg/ml in desired incubation period of 24, 48 and 72 hrs respectively. Meanwhile, the cells BBK-9 strain treated with 10 µg/l of propiconazole was exhibits the increasing pattern of Glucose utilization with increases duration and was content of glucose was found to be 29.98, 37.75 and 45.20 µg/ml at 24, 48 and 72 hrs respectively. The bacterial strain exposed to 20 µg/l of propiconazole showed different amount of glucose at different duration and the concentration of glucose was found to be 23.55, 30.41 and 35.94 µg/ml after the given incubation period. Propiconazole of 30 µg/l strength was showed little toxic effect on the glucose utilization and it was found 16.52, 21.76 and 25.59 µg/ml after 24, 48 and 72 hrs respectively (Graph 3.7).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on glucose content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the glucose content was significantly decreased.

### 3.3.5. Effect of propiconazole on amylase activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.9 and 3.10; Graph 3.8 and 3.9)

The activity of amylase in the *Pseudomonas aeruginosa* PS-4strain is given in the table 3.9. It was observed that the amylase activity was greatly influenced by addition of propiconazole to medium. In the group of controls the amylase activity was found to be 0.08, 0.11 and 0.12 U at interval of 24, 48 and 72 hrs respectively. On
exposure with 10 µg/l of propiconazole the activity of amylase was attained to be 0.12, 0.14 and 0.15 U at the given period of 24, 48 and 72 hrs respectively. Exposure with 20 µg/l of propiconazole exhibits an activity of 0.10, 0.14 and 0.14 U at the desired period of 24, 48 and 72 hrs respectively. On the exposure with 30 µg/l of propiconazole the activity of amylase was found to be 0.09, 0.10 and 0.11 U in 24, 48 and 72 hrs of incubation period respectively (Graph 3.8).

The activity of amylase in the *Burkholderia* sp. BBK_9 strain is given the table 3.10. In the control group amylase activity was found to be 0.10, 0.13 and 0.52 U at time period of 24, 48 and 72 hrs respectively. On the contact with 10 µg/l of propiconazole the activity of amylase was achieved to be 0.13, 0.15 and 0.18 U at the desired period of 24, 48 and 72 hrs respectively. On exposure with 20 µg/l of propiconazole showed an activity of 0.12, 0.14 and 0.16 U at the chosen period of 24, 48 and 72 hrs respectively. On the treatment with 30 µg/l of propiconazole the activity of amylase was found to be 0.10, 0.12 and 0.14 U in 24, 48 and 72 hrs of incubation period respectively (Graph 3.9).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on amylase content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the amylase content was significantly decreased.

### 3.3.6. Impact of propiconazole on protease activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.11 and 3.12; Graph 3.10 and 3.11)

The impact of propiconazole on the protease activity in the *Pseudomonas aeruginosa* PS-4 is given in the table 3.11. In the control groups the protease activity
was found to be 0.41, 0.62 and 0.63 U after the 24, 48 and 72 hrs respectively. *Pseudomonas aeruginosa* PS-4 strain treated with 10 µg/l of propiconazole was showed the activity of protease 0.62, 0.72 and 0.81 U at 24, 48 and 72 hrs of incubation period. On the treatment with 20 µg/l of propiconazole the protease activity was found to be 0.50, 0.62 and 0.69 U after 24, 48 and 72 hrs respectively. Conversely, with exposure to 30 µg/l of propiconazole the protease was 0.46, 0.59 and 0.67 U in the desired incubation period (Graph 3.10).

The influence of propiconazole on protease activity in the *Burkholderia* sp. BBK_9 strain is given in table 3.12. The protease activity of free cells of *Burkholderia* sp. BBK_9 strain in the control groups was 0.50, 0.61 and 0.73 U after 24, 48 and 72 hrs respectively. In the meantime 10 µg/l of propiconazole amended medium of *Burkholderia* sp. BBK_9 was showed the protease activity of 0.68, 0.77 and 86 U in the 24, 48 and 72 hrs respectively. On the exposure with 20 µg/l the activity of protease was found to be 0.54, 0.63 and 0.76 U after the 24, 48 and 72 hrs of incubation. On the treatment with 30 µg/l of propiconazole the protease activity was found to be 0.48, 0.62 and 0.78 U at the incubation period of the 24, 48 and 72 hrs respectively (Graph 3.11).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on protease content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the protease content was significantly decreased.

### 3.3.7. Influence of propiconazole on phosphatase activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.13 and 3.14; Graph 3.12 and 3.13)

Influence of propiconazole on phosphatase activity of *Pseudomonas aeruginosa* PS-4 strain is given in the table 3.13. Free cells of *Pseudomonas aeruginosa* PS-4 strain
which was not treated with propiconazole expressed the activity of phosphatase 0.34, 0.42 and 0.51 U at duration of 24, 48 and 72 hrs respectively. Conversely, in the propiconazole (10 µg/l) treated groups, the activity of phosphatase was found to be 0.43, 0.53 and 0.62 U after the desired incubation time. Meanwhile, in the medium amended with 20 µg/l of propiconazole the phosphatase activity was 0.53, 0.61 and 0.72 U at the duration of 24, 48 and 72 hrs respectively. The phosphatase activity was found to be 0.57, 0.66 and 0.74 in medium exposed to 30 µg/l of propiconazole after given incubation period (Graph 3.12).

Influence of propiconazole on phosphatase activity of *Burkholderia* sp. BBK_9 strain is given in the table 3.14. In group of controls the phosphatase activity was 0.32, 0.41 and 0.49 U at the period of 24, 48 and 72 hrs respectively. The category of *Burkholderia* sp. BBK_9 strain treated with 10 µg/l propiconazole, the phosphatase activity was found to be 0.40, 0.50 and 0.54 at given time period. Phosphatase activity was found to be 0.50, 0.59 U and 0.70 at 24, 48 and 72 hrs respectively in group of medium treated with 20 µg/l of propiconazole. Meanwhile, medium treated with 30 µg/l of propiconazole was showed activity of phosphatase 0.58, 0.68 and 0.76 U at the desired time of interval (Graph 3.13).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on phosphatase content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the phosphatase content was significantly increased.
3.3.8. Impact of propiconazole on Superoxide dismutase (SOD) activity of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains (Table 3.15 and 3.16; Graph 3.14 and 3.15)

The impact of propiconazole on SOD activity of Pseudomonas aeruginosa PS-4 strain is given in the table 3.15. In the sort of controls the SOD activity was 0.030, 0.038 and 0.046 U at the interval 24, 48 and 72 hrs respectively. The SOD activity was found to be 0.035, 0.041 and 0.048 at the optimum incubation period in cells of Pseudomonas aeruginosa PS-4 strain treated with 10 µg/l of propiconazole. In the groups of propiconazole (20 µg/l) exposed to the isolate, the activity of SOD was 0.056, 0.065 and 0.071 U at the regular time period. The SOD activity was 0.064, 0.073 and 0.080 U after the 24, 48 and 72 hrs incubation period in group of medium amended with 30 µg/l of propiconazole (Graph 3.14).

The activity of SOD in the Burkholderia sp. BBK_9 strain is given the table 3.16. In the control group SOD activity was found to be 0.022, 0.026 and 0.032 U at time period of 24, 48 and 72 hrs respectively. On the contact with 10 µg/l of propiconazole the activity of SOD was achieved to be 0.026, 0.032 and 0.038 U at the desired period of 24, 48 and 72 hrs respectively. On exposure with 20 µg/l of propiconazole showed an activity of 0.035, 0.042 and 0.045 U at the chosen period of 24, 48 and 72 hrs respectively. On the treatment with 30 µg/l of propiconazole the activity of SOD was found to be 0.044, 0.054 and 0.065 U in 24, 48 and 72 hrs of incubation period respectively (Graph 3.15).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on SOD content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the SOD content was significantly increased.
3.3.9. Impact of propiconazole on catalase (CAT) activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.17 and 3.18; Graph 3.16 and 3.17)

The impact of propiconazole on the CAT activity in the *Pseudomonas aeruginosa* PS-4 is given in the table 3.17. In the control groups the CAT activity was found to be 15.39, 17.53 and 19.42 U after the 24, 48 and 72 hrs respectively. *Pseudomonas aeruginosa* PS-4 strain treated with 10 µg/l of propiconazole was showed the activity of Cat 17.39, 20.50 and 23.91 U at 24, 48 and 72 hrs of incubation period. On the treatment with 20 µg/l of propiconazole the CAT activity was found to be 19.03, 22.17 and 26.09 U after 24, 48 and 72 hrs respectively. Conversely, with exposure to 30 µg/l of propiconazole the CAT was 22.15, 25.09 and 28.24 U in the desired incubation period (Graph 3.16).

The activity of CAT in the *Burkholderia* sp. BBK_9 strain is given the table 3.18. In the control group CAT activity was found to be 14.64, 16.21 and 18.45 U at time period of 24, 48 and 72 hrs respectively. On the contact with 10 µg/l of propiconazole the activity of CAT was achieved to be 16.34, 19.34 and 21.31 U at the desired period of 24, 48 and 72 hrs respectively. On exposure with 20 µg/l of propiconazole showed an activity of CAT 18.17, 21.36 and 24.25 U at the chosen period of 24, 48 and 72 hrs respectively. On the treatment with 30 µg/l of propiconazole the activity of CAT was found to be 20.23, 24.44 and 27.10 U in 24, 48 and 72 hrs of incubation period respectively (Graph 3.17).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on catalase content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the catalase content was significantly increased.
3.3.10. Influence of propiconazole on peroxidase activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains (Table 3.19 and 3.20; Graph 3.18 and 3.19)

The impact of propiconazole on peroxidase activity of *Pseudomonas aeruginosa* PS-4 is given in the table 3.19. In the sort of controls the peroxidase activity was 0.032, 0.040 and 0.049 U at the interval 24, 48 and 72 hrs respectively. The peroxidase activity was found to be 0.038, 0.047 and 0.055 at the optimum incubation period in cells of *Pseudomonas aeruginosa* PS-4 strain treated with 10 µg/l of propiconazole. In the groups of propiconazole (20 µg/l) exposed to the isolate, the activity of peroxidase was 0.053, 0.064 and 0.071 U at the regular time period. The peroxidase activity was 0.067, 0.073 and 0.085 U after the 24, 48 and 72 hrs incubation period in group of medium amended with 30 µg/l of propiconazole (Graph 3.18).

The activity of peroxidase in the *Burkholderia* sp. BBK_9 strain is given the table 3.20. In the control group peroxidase activity was found to be 0.028, 0.043and 0.053 U at time period of 24, 48 and 72 hrs respectively. On the contact with 10 µg/l of propiconazole the activity of peroxidase was achieved to be 0.038, 0.050 and 0.060 U at the desired period of 24, 48 and 72 hrs respectively. On exposure with 20 µg/l of propiconazole showed an activity of 0.054, 0.064 and 0.073 U at the chosen period of 24, 48 and 72 hrs respectively. On the treatment with 30 µg/l of propiconazole the activity of peroxidase was found to be 0.069, 0.073 and 0.085 U in 24, 48 and 72 hrs of incubation period respectively (Graph 3.19).

The present experimental results were employed for the analysis of statistical compatibility in all the treated and untreated groups. It was revealed that, the obtained values were denoted by different significant letter(s) (a-high, b-average and c-low) and the propiconazole effect on peroxidase content exhibits a reverse hierarchy of results (c to a) and denoted letter(s) were found significantly different and the catalase peroxidase was significantly increased.
3.4. DISCUSSION

In the era of modern agricultural system number of formers are dependent on the industrially manufactured pesticides (Imfeld and Vuilleumier, 2012). In addition, many of the pesticides are extensively applied to fields for the crop protection and better yielding and this condition of application of pesticides will have the chance of being stable in the agricultural soil. Thus, the condition of persistence of pesticides in soil will affect soil fertility, effect of non-target microorganisms and also will have capacity to alter the biochemical contents of the soil borne microorganisms.

3.4.1. Effect of propiconazole on the Nucleic acids of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains

It was observed that the content of DNA and RNA in the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains were affected by the exposure of propiconazole at 24, 48 and 72 hrs. It was also noticed that, DNA and RNA concentrations in the control groups were increased as the incubation time significantly increased and similar trend was also seen in the propiconazole treated groups. However, the higher concentration of propiconazole was done the damage on the production of DNA and RNA from *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9. In evidence to our experimental results Singh et al., (2015) have also reported the similar type of observation by saying the pesticides chlorpyrifos and cypermethrin have the toxic effect on the biochemical parameters of microorganisms. Similarly, pesticide butachlor will have capacity to reduce the population and biochemical parameters of *Azospirillum*. Contradictorily, pesticide carbofuran will have the ability to stimulate the growth and enhance the biochemical contents in *Azospirillum*. This type of mode of actions of pesticides on the microorganisms will witnessed for two kind of possibilities like some pesticide (beneficial) will have the ability to stimulate the growth and better
biochemical mechanisms in microorganisms and other pesticides (toxic) will affect/limit the growth and biochemical parameters in microbes (Lo, 2010), it is clear clue that our experimental results are falls under the category of toxic pesticides will have the ability to reduce the growth and biochemical parameters significantly. In addition, the Significativity of our results assures that the propiconazole will have the greater influence over the inhibition of DNA and RNA concentration in *Pseudomonas aeruginosa* PS4 and *Burkholderia* sp. BBK_9 strains

3.4.2. **Effect of propiconazole on protein content of the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains**

In the present study it was noted that the protein content in the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains was affected with the treatment of propiconazole at 24, 48 and 72 hrs. Similarly, protein content was decreased with exposure of toxic compounds to microbes (Gaddad et al., 2005). In addition, number of pesticides like captan, deltamethrin, isoproturon and pirimicarb have the effect on the bacterial activity and protein content (widcnfalk et al., 2004). It was also publicized that pesticide endosulfan triggered major decrease in the number of bacteria and protein activity (Nasim et al., 2005). Previously, Shetti and Kaliwal (2012) have reported that, *Brevundimonas* SP. MJ 15 strain treated with different concentrations of imidacloprid showed the toxic effect on the protein content and the significant decrease in the protein levels was observed. It was also observed that, in the control groups the protein content was found to be higher than the treated group and as duration of incubation period increases the protein content was also increased. Conversely, Kulkarni and Kaliwal (2010) have also reported that the different concentrations of methomyl exposed to the *Escherichia coli* and *Pseudomonas aeruginosa* also exhibits the similar trend of results. In the same way, in our study it
was observed that, the significant decrease in the protein contents of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains treated with propiconazole and in the control groups the level protein was found higher than the propiconazole treated groups. From the obtained results it was concluded that, the experimental data of the present study is reliable with previously published data and it was also noticed that pesticides will play a major role for reduction of bacterial protein.

**3.4.3. Effect of propiconazole on utilization of glucose by *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains**

Glucose is the energy substrate will act as carbon source for growth and biochemical mechanism in microorganism. From the results of the present study it was unveiled that, the glucose utilization by *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains was reduced with treatment of propiconazole at 24, 48 and 72 hrs. The decrease in the concentration of glucose in microorganisms with increased concentrations and time of contact with propiconazole may due to toxic impact of propiconazole on cells of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains. Similarly, Cabisco et al., (2000) have reported the same kind of observation and it was said that toxic compounds have the ability to disturb the regular mechanism of microorganisms and will cause the detriment in the content of glucose and biochemical parameters. Interestingly, relative oxygen species (ROS) will also damage the utilization of glucose and other biochemical parameters, resulting in the cell death when the level of ROS exceeds a microorganism detoxification (Kashmiri and Mankar, 2014).

The significant detriment in the glucose concentration in the present study may be due to glucose starvation and oxidative stress (Salakhetdinova, 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 proteins, DNA, carbohydrates and lipids (Mori et al., 2007). Soil isolates *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains were significantly reduced the content of glucose with dose and durational exposure to propiconazole. In addition, it was observed that the significativity of obtained results suggest that, the propiconazole have adverse impact over the production of glucose by the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.

### 3.4.4. Effect of propiconazole on amylase activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains

Amylase is the polysaccharide hydrolase and it will used in the simultaneous saccharification and starch fermentation (Blasheck, 1992; Karam and Nicell, 1997). The experimental data of the present study revealed that the activity of amylase in propiconazole exposed (10 and 20 µg/l) groups was increased when compare to the controls. However, *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains treated with the higher concentration of propiconazole (30 µg/l) was reduced the activity of amylase at 24, 48 and 72 hrs.

It was reported that endosulfan was found toxic to the *Bacillus subtilis* and inhibited the 50% of growth. Based on the amylase assay it was confirmed that in lower concentrations (endosulfan) the activity of amylase was slightly effected. In addition, higher concentrations inhibited the activity of amylase (Tolan, 2006). Moreover, earlier reports were found in agreement with our experimental data (Greve, 1972; Srivastava and Misra, 1982; Sangeeta et al., 1997). The failure in biochemical parameters level specifies the biological adaptableness to recompense for pesticide stress. To overcome the stress, they use energy, which leads to stimulation of protein, glycogen catabolism leading to increase in the amylase activity (Sancho et al., 1998). The significant increase
in the amylase activity in the propiconazole treated groups may enhance the response of microbes to stress environment. It was also shown in the present study that, the toxic effect of propiconazole have the tremendous impact over the activity of amylase in both the *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.

### 3.4.5. Effect of propiconazole on protease activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains

A protease (also called a peptidase or proteinase) is any enzyme that performs proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain. Proteases will contributing in the protein metabolism either by degradative or biosynthetic pathways release hormones and pharmacologically active peptides from precursor proteins (Hook *et al*., 2008). Results of present study revealed that the activity of protease in propiconazole treated category increased in lower concentrations 10 and 20 µg/l. However, in the 30 µg/l of propiconazole the protease activity was inhibited. It was also observed that, *Pseudomonas aeruginosa* express the protease activity slightly lower than the *Burkholderia* sp.

The extracellular hydrolytic enzyme proteases have relatively various possible practices in different areas such as food industry, feed additive, biomedical sciences, and chemical industries (Sánchez-Porro *et al*., 2003). Additionally, proteases are also play a vital role in biodegradation mechanism of pesticides and heavy metals (Karigar and Rao 2011). Moreover, culture filtrates of *E. coli* have been shown to degrade the damaged enzyme, but not the native protein, and several initial reports suggest that the *E. coli* protease that may be accountable for selective degradation of the altered proteins (Lee, 1987). The significant increase in the protease activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains free cells on dosage and time.
exposure of propiconazole detected in the present study may because of expression of intracellular proteins (Martin et al., 1998), hydrolysis of proteins that complete an essential part in the biodegradation (Martínez et al., 2012). In the treated groups, particularly in 30 µg/l of propiconazole concentration was found to be lethal for the significant detriment in the protease activity at the incubation period of 24, 48 and 72 hrs and in the other concentrations (10 and 20 µg/l) the activity was significantly increased at 24, 48 and 72 hrs. Thus, this state of propiconazole toxicity may reduce the possibility of microorganism adaptation to toxic environmental conditions.

3.4.6. Effect of propiconazole on phosphatase activity of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains

Phosphatase is well known for the removal of phosphate group from its substrates by the hydroxylation of phosphoric acid (Martin and Senior, 1980; Riley and Peters, 1981; Davies et al., 2012). It was clear that the degradation of propiconazole induced phosphatase production. For instance the activity of phosphatase in both Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains was found higher in propiconazole treated groups than the controls. It was also observed in the higher concentration (30 µg/l) that the phosphatase activity was slightly declined when compare to the lower concentrations of propiconazole (10 and 20 µg/l) at 24, 48 and 72 hrs.

Previously, Harishankar et al., (2002) shown that the role of phosphatase enzyme in the degradation process of chlorpyrifos (CP), it was addressed that degradation of CP was achieved from the induction of phosphatase (OPP) production in the supernatant of Lactobacillus fermentum and the CP was degraded up to 70% from the initial concentration. Additionally, the enzyme phosphatase, reported to be involved in biodegradation of organophosphorus compounds. Moreover, Monocrotophos (MCP)
was degraded by the induction of phosphatase (Bhadbhade et al., 2002). In support to our data, the enhanced degradation of five organophosphorus pesticides (OPPs) from the Lactobacillus brevis were achieved with production of phosphatase and in the pesticides treated groups the activity of phosphatase was found to higher than the controls (Zhang et al., 2014). While comparing our obtained results with the above mentioned reports, it give clear picture that our experimental results have similar match. The significant increase in the activity of phosphatase in propiconazole treated groups (10 and 20 µg/l) was observed. Hence, the activity of phosphatase will have impact over the degradation of propiconazole by Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains.

3.4.7. Effect of propiconazole on Superoxide dismutase (SOD) activity of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains

Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation of the superoxide radical into molecular oxygen (O₂) or hydrogen peroxide (H₂O₂). Results of present study revealed that the activity of SOD in treated category increased in lower concentrations 10 and 20 µg/l at 24, 48 and 72 hrs. However, in the 30 µg/l of propiconazole the SOD activity was slightly inhibited. It was also observed that, Pseudomonas aeruginosa express the SOD activity slightly lower than the Burkholderia sp. Moreover the up regulation of SOD is also common phenomena during the degradation of toxic chemicals (Su Seo et al., 2009).

Previously, researchers from china tested for reaction of SOD to the atrazine and the outcomes specifies that SOD was induced upon treatment with atrazine and the levels of SOD can varies in the selected bacteria (Zhang et al., 2012). Moreover, as can be seen in our results, the levels of SOD in both Pseudomonas aeruginosa and Burkholderia sp. slightly decreased. Conversely, oxidative stress response to the
number pesticides and pollutant have been widely studied (Hassett et al., 2000; Frederick et al., 2001). Importantly, Park et al., (2006) have carried out a research that affects the SOD concentration in the bacteria and it was suggested that the usage of higher dose will decrease the activity of SOD and other stress enzymes. In our study it was also shown that the higher concentration of propiconazole (30 µg/l) affected the concentration of SOD in the Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains. In lower concentration of propiconazole (10 and 20 µg/l), the activity of SOD was significantly increased with increased duration (24, 48 and 72 hrs). However, in the soil isolates treated with 30 µg/l of propiconazole showed the significantly lower activity of SOD. Thus, SOD activity was expressed significantly when it was treated with recommend doses.

3.4.8. Effect of propiconazole on catalase (CAT) activity of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). The experimental data of the present study revealed that the activity of CAT in propiconazole exposed groups was increased when compare to the controls at 24, 48 and 72 hrs. However, cells of Pseudomonas aeruginosa PS-4 and Burkholderia sp. BBK_9 strains treated with 30 µg/l of propiconazole was slightly reduced the activity of CAT.

To date, the oxidative stress replies to numerous contaminants have been broadly inspected in bacteria (Geckil et al., 2003). CAT treated with lower concentrations of atrazine seemed to be unresponsive. While, Gram-negative bacterium E. coli K12 exhibited higher sensitivity to atrazine stress. Consequently, Gram-negative bacterium E. coli K12 is fit organism for studies about the act of atrazine stress (Zhang...
et al., 2012). In contrast, in the lower concentration the activity of CAT was found promisingly good in our study and both of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains share usual mode of action while the utilization of propiconazole as source of carbon. Hence, we believe that both organisms used in the present study may have good impact over the stress responses. The CAT activity of *Burkholderia cepacia* is involved in the mechanisms of tolerance to the herbicides (Lu¨ et al.., 2004). In our study, it is possible that stimulation of CAT activity donates to the removal of ROS from the bacterial cell induced by propiconazole treatment. In the present study, the CAT activity was significantly enhanced in the treated groups of propiconazole (10, 20 and 30 µg/l) at 24, 48 and 72 hrs and in controls the activity was significantly lowered.

3.4.9. Effect of propiconazole on peroxidase activity of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains

Peroxidases are a large family of enzymes that characteristically catalyze a response of the form: ROOR' + electron donor (2 e−) + 2H+ → ROH + R'O'H. For numerous of these enzymes the optimum substrate is hydrogen peroxide, but others are further active with organic hydroperoxides such as lipid peroxides. Results of present study revealed that the activity of peroxidase in treated category increased in lower concentrations 10 and 20 µg/l at 24, 48 and 72 hrs. However, in the 30 µg/l of propiconazole the peroxidase activity was slightly inhibited. It was also observed that, *Pseudomonas aeruginosa* express the peroxidase activity slightly lower than the *Burkholderia* sp.

*Comamonas terrigena* Gram negative bacterium used for the degradation of phenolic compounds and this selected strain show it potentiality in the degradation process by expressing the functioning enzyme peroxidase (Zamock´y et al., 2001).
However, it has been shown that bacterial catalase-peroxidases show reversible association to dimers or even trimers of dimers (Zámocký 1999). It is known that peroxidases stated by the majority of aerobic microorganisms are of potential prominence in technology (Klibanov 1980; Klibanov 1981). Importantly, many authors reported that peroxidase is extremely reactive with toxic compounds and up regulation of peroxidase with toxic chemicals is common mechanism (Job 1976; Dunford 1986). In the same way, activity of peroxidase was significantly increased in the propiconazole treated groups (10, 20 and 30 µg/l) at 24, 48 and 72 hrs. Moreover, in the control groups the peroxidase activity was significantly lower and it also observed that, in the stress conditions *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains are being adopted themselves for the dissolution of propiconazole.
3.5. SUMMARY AND CONCLUSION

The enlarged usage of chemical pesticides in the field agricultural for the protection of standing plants from pests, causing the contamination of soil with toxic chemicals. When pesticides are sprayed, the possibility exists that these pesticides may exert certain effects on non-target organisms, including soil microorganisms. The microbial biomass plays an important role in the soil ecosystems where they achieve a critical role in nutrient cycling and decomposition. Pesticides might affect the microbial population by regulatory the survival and reproduction of individual species.

- The present study is aimed to interpret the graded dose and durational exposure of propiconazole to *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.

- Exposure with dose and duration of propiconazole triggered substantial decrease in the level of DNA, RNA, proteins and glucose content in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains when related with their respective controls. However,

- Treatment with higher dose (30 µg/l) of propiconazole affected no significant increase in the amylase activity. However, increased activity of amylase was observed in the lower concentrations (10 and 20 µg/l) of propiconazole in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.

- Protease activity exposed with higher content of propiconazole (30 µg/l) of propiconazole triggered less amount of protease activity. Although, increased activity of protease was observed in the lower dose (10 and 20 µg/l) of propiconazole in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.
• Exposure with dose and duration of propiconazole affected a significant increase in the phosphatase activity in all the groups of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains when matched with their respective controls.

• Super oxide dismutase and catalase activity in the treated groups improved significantly in lower doses (10 and 20 µg/l) and there was slight decrease was observed in the higher dose (30 µg/l) of propiconazole exposed to *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.

• Exposure with dose and duration of propiconazole affected a significant increase in the peroxidase activity in all the groups of *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains when compared with their respective controls.

Therefore, the results of present investigation suggests that the propiconazole will affect the regular mechanism of biochemical parameters in *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains. It was also noted that the toxic effect of propiconazole affect the enzyme activities. Thus it is concluded propiconazole induced toxicity and stress in bacterial soil isolates *Pseudomonas aeruginosa* PS-4 and *Burkholderia* sp. BBK_9 strains.