Chapter 4: Results

Effect of pesticides:

The extent of toxicity of various concentrations of two common pesticides used in rice fields (herbicide-2,4-D ethyl ester and fungicide-pencycuron) has been studied on three cyanobacterial species i.e. *Anabaena fertilissima*, *Aulosira fertilissima* and *Westiellopsis prolifica*. These pesticides differently affected the morphological characters, heterocyst frequency, photosynthetic pigments, total carbohydrates, total amino acids, total proteins and total phenols, nitrate reductase, glutamine synthetase, succinate dehydrogenase, genomic DNA and 16S rDNA in test algae. The detailed observations are as under:

4.1 2,4-D ethyl ester:

4.1.1.1 Morphological characters:

(A) *Anabaena fertilissima*:

The culture treated with 15 ppm was not observed to be affected, while 30 ppm treatment of 2,4-D ethyl ester resulted into slight reduction of cell size after 4-days of exposure. Further, the highest concentration i.e. 60 ppm drastically altered the cell size and color when matched with untreated control sample. After 16-days, 15 ppm and 30 ppm treatments modified the culture color and size, in addition 60 ppm treatments also transformed and bleached the cell color from green to yellow. Disintegration from long trichomes to smaller ones along with some isolated cells, change in size (constriction) and irregular arrangement of vegetative cells in the filaments was observed too (Fig. 4.1).

The heterocyst frequency was recorded after 4-days and 16-days (till the experiment was terminated). Reduction in heterocyst frequency was encountered to be concentration-dependent and followed the course of growth. It was observed that under the influence of 2,4-D ethyl ester the heterocyst frequency declined gradually with increasing concentration and the maximum retardation of heterocyst formation was resulted at highest concentration i.e. 60 ppm by 50% after 4-days and by 53% after 16-days of treatment (Table 4.1).
(B) *Aulosira fertilissima*:

Initially after 4-days, the untreated culture and the cultures incubated with 20 ppm and 40 ppm concentrations of 2,4-D ethyl ester were alike with respect to cell size, cell shape and color but in 80 ppm treated culture immediate decrease in the pigmentation of the cells was manifested. The culture exposed to all the three concentrations for 16-days demonstrated the toxic effect of 2,4-D ethyl ester on cyanobacterial cells as the cells were observed to be shriveled and decolorized that might have been caused because of degradation of chlorophyll (Fig. 4.2).

When compared with control the percentage heterocyst frequency followed the similar trend as was observed in case of *Anabaena fertilissima*. In this case also the heterocyst frequency was observed after 4-days and 16-days, and the reduction was found to be concentration-dependent which followed the course of experiment. However, the highest values of retardation achieved were 38% after 4-days and 41% after 16-days at 80 ppm dose of 2,4-D ethyl ester (Table 4.1).

(C) *Westiellopsis prolifica*:

After 4-days of exposure to 30 ppm, 60 ppm and 120 ppm dose of 2,4-D ethyl ester, the test organism remained unaffected when compared with control with reference to the studied morphological features. Even at the end of the experiment i.e. 16-days, the 30 ppm and 60 ppm treated culture was almost unchanged with slight decolorization, however 120 ppm treated cultures showed intense change in color with degeneration of compartmentalization of trichome which is a normal characteristic of the filamentous cyanobacteria. Also, the existing barrel-shaped cell shape was found to be distorted with shrinkage in size after the treatment with 120 ppm of 2,4-D ethyl ester for 16-days (Fig. 4.3).

A gradual decline in the heterocyst frequency with respect to increasing concentration of 2,4-D ethyl ester and duration of exposure was observed. The 2,4-D ethyl ester treatment in *Westiellopsis prolifica*, at all the estimated concentrations, recorded highest heterocyst frequency under stress and less diminution in heterocyst formation when compared with other two species since maximum reduction in heterocyst frequency was at 120 ppm, by 32% after 4-days and 38% after 16-days (Table 4.1).
Table 4.1 Effect of different selected concentrations of 2,4-D ethyl ester on heterocyst frequency of three selected species of cyanobacteria.

<table>
<thead>
<tr>
<th>Cyanobacteria</th>
<th>Concentration (ppm)</th>
<th>Heterocyst frequency (%)</th>
<th>4-days</th>
<th>16-days</th>
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<tr>
<td></td>
<td>Control</td>
<td></td>
<td>4</td>
<td>9.5</td>
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<tr>
<td><strong>Anabaena fertilissima</strong></td>
<td>15</td>
<td>2.9 (28)</td>
<td>6.8 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.5 (38)</td>
<td>5.6 (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.0 (50)</td>
<td>4.5 (53)</td>
<td></td>
</tr>
<tr>
<td><strong>Aulosira fertilissima</strong></td>
<td>Control</td>
<td>3.2</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.7 (16)</td>
<td>5.0 (20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.4 (25)</td>
<td>4.5 (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>2.0 (38)</td>
<td>3.7 (41)</td>
<td></td>
</tr>
<tr>
<td><strong>Westiellopsis prolifica</strong></td>
<td>Control</td>
<td>3.4</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.0 (12)</td>
<td>4.6 (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.8 (18)</td>
<td>4.0 (27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2.3 (32)</td>
<td>3.4 (38)</td>
<td></td>
</tr>
</tbody>
</table>

Data in parenthesis denotes percentage inhibition.
Figure 4.1 Photomicrograph (100 X) of *Anabaena fertilissima* exposed to 15ppm, 30ppm and 60ppm of 2,4-D ethyl ester for 4-days (A) and 16-days (B) with reference to control.

An investigation of differential effects of 2,4-D and Penycuron on three species of Cyanobacteria in-vitro — *A Biochemical and Molecular approach*
Figure 4.2 Photomicrograph (100 X) of *Aulosira fertilissima* exposed to 20ppm, 40ppm and 80ppm of 2,4-D ethyl ester for 4-days (A) and 16-days (B) with reference to control.
Figure 4.3 Photomicrograph (100 X) of *Westiellopsis prolifica* exposed to 30ppm, 60ppm and 120ppm of 2,4-D ethyl ester for 4-days (A) and 16-days (B) with reference to control.
4.1.1.2 Chlorphyll a:

(A) *Anabaena fertilissima*:

The chlorophyll a content in untreated samples ranged from 0.21 µg/20ml to 0.34 µg/20ml. There was a linear fall in chlorophyll a at 15 ppm (13%), 30 ppm (26%) and 60 ppm (36%) dose after 4-days of incubation which gradually diminished after 8-days by 25% at 15 ppm, 32% at 30 ppm and 78% at 60 ppm treatment. After 12-days the retardation of chlorophyll a by 27% at 15 ppm, 45% at 30 ppm and 83% at 60 ppm treatment was observed when compared with control. The maximum fall in chlorophyll a content was recorded at 60 ppm concentration (86%) followed by 30 ppm (50%) and 15 ppm (33%) dose after 16-days. Thus 2,4-D ethyl ester had a significant (P<0.05, ANOVA) effect on chlorophyll a content at all the tested intervals and concentrations (Appendix DP1 A; Fig. 4.4 (a)).

(B) *Aulosira fertilissima*:

In *Aulosira fertilissima*, chlorophyll a content (0.21 µg/20ml to 0.15 µg/20ml) at the end of 4-days was less affected (P>0.05, ANOVA) by 10% at 20 ppm, 21% at 40 ppm and 30% at 80 ppm concentration of 2,4-D ethyl ester, however after 4-days the results had gained the significant level of suppression (Appendix DP1 B). Similar drop off in dose dependent manner was also followed after 8-days from 0.25 µg/20ml to 0.20 µg/20ml (20%), 0.18 µg/20ml (28%) and 0.11 µg/20ml (56%) and from 0.35 µg/20ml to 0.26 µg/20ml (26%), 0.23 µg/20ml (43%) and 0.09 µg/20ml (74%) after 12-days at 20 ppm, 40 ppm and 80 ppm treatment, respectively. At the end of 16th day, the chlorophyll a content ranged from 0.39 µg/20ml to 0.09 µg/20ml with retardation of 33% at 20 ppm, 51% at 40 ppm and 77% at 80 ppm treatment (Fig. 4.4 (b)).

(C) *Westiellopsis prolifica*:

An increase of chlorophyll a content from 0.31 µg/20ml to 0.41 µg/20ml was achieved in control with the progress in time. However all the three treatments showed gradual but significant fall (P<0.05, ANOVA-Appendix DP1 C) in chlorophyll a after 4-days by 10% at 30 ppm, 19% at 60 ppm and 26% at 120 ppm treatment, while at 8th day by 14% at 30 ppm, 23% at 60 ppm and 43% at 120 ppm treatment, whereas after 12-days by 21% at 30 ppm, 37% at 60 ppm and 47% at 120 ppm dose and 16-days by 27% at 30 ppm, 51% at 60 ppm and 54% at 120 ppm dose(Fig. 4.4 (c)).

An investigation of differential effects of 2,4-D and Pendecuron on three species of Cyanobacteria in-vitro –
A Biochemical and Molecular approach
4.1.1.3 Total Carotenoids:

(A) *Anabaena fertilissima*:
The carotenoid content ranged from 2.8 μg/20ml to 3.6 μg/20ml and extent of toxicity increased significantly with increasing time and concentration of 2,4-D ethyl ester (Appendix DP₂ A). The carotenoid content was initially suppressed (P>0.05, ANOVA) by 7%, 21% and 32% after 4-days while after 8-day treatment it was recorded by 18%, 43% and 64% at 15 ppm, 30 ppm and 60 ppm concentration of 2,4-D ethyl ester, respectively. The decline in the carotenoid content with respect to increase in exposure periods was more prominent, since the increase in reduction level was observed after 12-days (23% at 15 ppm, 60% at 30 ppm and 70% at 60 ppm) and 16-days (30% at 15 ppm, 69% at 30 ppm and 80% at 60 ppm) when compared with initial incubation period (Fig. 4.4 (a)).

(B) *Aulosira fertilissima*:
The carotenoid content was observed to decrease in concentration and time dependent manner, since after 4-days the results were insignificant (P>0.05, ANOVA) which gradually reached to highly significant level with increase in time (Appendix DP₂ B). The increase in carotenoid content of untreated culture ranged from 3.6 μg/20ml to 4.7 μg/20ml, whereas, in treated culture it reduced from 3.5 μg/20ml to 1.3 μg/20ml. After 16-days the highest concentration treatment (80 ppm) showed 72% reduction which was uppermost when compared with 4-days (22%), 8-days (36%) and 12-days (64%) results (Fig. 4.4 (b)).

(C) *Westiellopsis prolifica*:
The toxic effect of 2,4-D ethyl ester on carotenoids was comparatively less by only 5% at 30 and 60 ppm and by 15% at 120 ppm after 4-days of exposure (P>0.05, ANOVA), whereas about 9%, 27% and 35% after 8-days and 12%, 32%, and 39% of significant inhibition (P<0.05, ANOVA) after 12-days was observed at 30 ppm, 60 ppm, and 120 ppm dose respectively (AppendixDP₂ C; Fig. 4.4 (c)). However, the highest concentration i.e. 120 ppm after 16-days of incubation was found to be more deleterious as the carotenoid content was reduced from 7.6 μg/20ml to 2.7 μg/20ml (64%) than other two concentrations (14% at 30 ppm and 58% at 60 ppm).
Figure 4.4 Effect of different concentrations of 2,4-D ethyl ester on chlorophyll $a$ and carotenoids content of cyanobacteria.
4.1.1.4 Phycocyanin:

(A) *Anabaena fertilissima:*

The raise in phycocyanin content of control from 4-days to 16-days ranged from 0.026 µg/20ml to 0.038 µg/20ml. After 4-days, reduction in phycocyanin content by 30%, 42% and 53% was noticed following supplementation of 15 ppm, 30 ppm and 60 ppm of 2,4-D ethyl ester. Even 8 (41% at 15 ppm, 54% at 30 ppm and 68% at 60 ppm) and 12 (63% at 15 ppm, 66% at 30 ppm and 79% at 60 ppm) days of 2,4-D ethyl ester incubation showed deleterious effect, which was increased with the progress in time (after 16-days) by 83% at 15 ppm, 88% at 30 ppm and 93% at 60 ppm dose. Nevertheless, phycocyanin of *Anabaena fertilissima* revealed a positive relation with 2,4-D ethyl ester treated cultures, being significantly (P<0.05, ANOVA) depressed at all concentrations in a time-dose response manner (Appendix DP3 A; Fig. 4.5 (a)).

(B) *Aulosira fertilissima:*

As compared with the phycocyanin content in control which ranged from 0.024 µg/20ml to 0.039 µg/20ml, the lower concentration and LC$_{50}$ concentration followed similar trend of inhibition after 4-days (21% at 20 ppm and 22% at 40 ppm) and 8-days (28% at 20 ppm and 28% at 40 ppm). However, the results indicated insignificant level of reduction in phycocyanin content (P>0.05, ANOVA) after 4-days which reached at significant level with increase in time (Appendix DP3 B; Fig. 4.5 (b)). The highest retardation of phycocyanin content was noticed at the end of the experiment (16-days) after supplementation of 20 ppm (41%), 40 ppm (54%) and 80 ppm (64%) of 2,4-D ethyl ester treatments.

(C) *Westiellopsis prolifica:*

The phycocyanin content of untreated cultures ranged from 0.025 µg/20ml to 0.038 µg/20ml. The higher concentrations of 2,4-D ethyl ester were significantly inhibitory for phycocyanin content after 16-days (24% at 30 ppm, 50% at 60 ppm and 60% at 120 ppm) and 12-days (21% at 30 ppm, 28% at 60 ppm and 40% at 120 ppm) of treatment, as compared with 4-days (9% at 30 ppm, 17% at 60 ppm and 21% at 120 ppm) and 8-days (14% at 30 ppm, 23% at 60 ppm and 27% at 120 ppm) as the gradual reduction (P>0.05, ANOVA) in phycocyanin

An investigation of differential effects of 2,4-D and Penicycuron on three species of Cyanobacteria in-vitro – A Biochemical and Molecular approach
content was registered (Appendix DP3 C). Effect of different concentrations of test pesticide on phycocyanin is presented in Fig. 4.5 (c).

### 4.1.1.5 Phycoerythrin:

**A** - *Anabaena fertilissima:*

Depletion in phycoerythrin content ranged from 0.024 μg/20ml to 0.001 μg/20ml with the progress in time. After 4-days, the phycoerythrin content was reduced by 30% at 15 ppm, 45% at 30 ppm and 60% at 60 ppm dose which were continued further in the experiment after 8-days by 38% at 15 ppm, 62% at 30 ppm and 72% at 60 ppm treatment and 12-days by 71% at 15 ppm, 79% at 30 ppm and 84% at 60 ppm treatment. When compared with other incubation periods, 16-days cultures were more significantly (P<0.001, ANOVA) affected at 15 ppm (89%), 30 ppm (91%) and 60 ppm dose (95%) of 2,4-D ethyl ester treatments (Appendix DP4 A; Fig. 4.5 (a)).

**B** - *Aulosira fertilissima:*

Fig. 4.5 (b) showed the results of 2,4-D ethyl ester on phycoerythrin at different time duration and concentrations when compared with untreated culture which ranged from 0.020 μg/20ml to 0.029 μg/20ml. In all the times of exposure i.e. 4, 8, 12 and 16 days the hierarchy of toxicity (80>40>20) with a significant inhibition (P<0.05, ANOVA) remained the same (Appendix DP4 B). Depletion of 26% of phycoerythrin was noticed on day 4 in 80 ppm treated cultures. The cultures on day 16, however, encountered greatest fall in phycoerythrin content by 45% at 20 ppm, 66% at 40 ppm and 79% at 80 ppm.

**C** - *Westiellopsis pralifica:*

After 4-days of exposure to herbicide levels, phycoerythrin contents demonstrated minor (P>0.05, ANOVA) fall of 12% at 30 ppm, 18% at 60 ppm and 23% at 120 ppm treatment in relative to control (0.023 μg/20ml). The maximum reduction of phycoerythrin content was achieved by the end of 16th day by 22% at 30 ppm, 48% at 60 ppm and 67% at 120 ppm treatment (Appendix DP4 C; Fig. 4.5 (c)).
4.1.1.6 Allophycocyanin:

(A) *Anabaena fertilissima*:

Cultures incubated with 2,4-D ethyl ester depicted persistent inhibition from 0.022 μg/20ml to 0.003 μg/20ml. A significant reduction in allophycocyanin content by 16%, 24% and 35% after 4-days, 28%, 38% and 57% after 8-days and 57%, 67% and 80% after 12-days was registered at 15 ppm, 30 ppm and 60 ppm treatment, respectively (P<0.05, ANOVA). Maximum loss of allophycocyanin concentration by 81% at 15 ppm, 88% at 30 ppm and 94% at 60 ppm treatment at the end of the experiment (16th day) further confirms the greater toxic potential of 2,4-D ethyl ester on *Anabaena fertilissima* (Appendix DP5 A; Fig. 4.5 (a)).

(B) *Aulosira fertilissima*:

The allophycocyanin content in untreated culture ranged from 0.029 μg/20ml to 0.042 μg/20ml. The presence of 2,4-D ethyl ester in the growth medium resulted into decrease (P>0.05, ANOVA) in allophycocyanin content by 14% at 20 ppm, 21% at 40 ppm and 28% at 80 ppm treatment after 4-days, of which the latter concentration was more significantly (P<0.001, ANOVA) affected after 16-days by 42% at 20 ppm, 59% at 40 ppm and 76% at 80 ppm of incubation (Appendix DP5 B; Fig. 4.5 (b)).

(C) *Westiellopsis prolifica*:

The allophycocyanin content in control ranged from 0.031 μg/20ml to 0.040 μg/20ml however, in treated cultures it was from 0.030 μg/20ml to 0.015 μg/20ml. At lower concentration (30 ppm) the percentage inhibition was less as compared with higher concentrations after 4-days (4%), 8-days (10%), 12-days (17%) and 16-days (21%). As the time progressed from 4 to 16-days there was gradual fall, but significant decrease (P<0.05) at higher concentration (120 ppm) by 26% after 4-days, 31% after 8-days, 39% after 12-days and 63% after 16-days (Appendix DP5 C; Fig. 4.5 (c)).
**Results**

<table>
<thead>
<tr>
<th>Phycocyanin</th>
<th>Phycoerythrin</th>
<th>Allophycocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Anabaenafisulissima" /></td>
<td><img src="image" alt="Anabaenafisulissima" /></td>
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</tr>
</tbody>
</table>

Figure 4.5 Effect of different concentrations of 2,4-D ethyl ester on phycocyanin, phycoerythrin and allophycocyanin content of cyanobacteria.
4.1.2.1 Total Carbohydrates:

(A) *Anabaena fertilissima:*

The carbohydrate content was found to be higher in untreated cells in all the tested periodic intervals and ranged from 0.222 mg/20ml to 0.347 mg/20ml. The toxicity of 2,4-D ethyl ester on total carbohydrate content was lower at 15 ppm concentration (32%), than at 30 ppm (42%) and 60 ppm treatment (48%). As the concentration and time increased, the toxicity also raised significantly (P<0.05, ANOVA) and proportionately from 36% (15 ppm) to 63% (60 ppm) after 8-days, 41% (15 ppm) to 74% (60 ppm) after 12-days and 57% (15 ppm) to 81% (60 ppm) after 16-days (Appendix DM; A; Fig. 4.6 (a)).

(B) *Aulosira fertilissima:*

The carbohydrate content was repressed in cells grown in BG-11 medium containing 2,4-D ethyl ester and was highest (0.289 mg/20ml) in the control. Of the different concentrations of 2,4-D ethyl ester tested, 80 ppm dose showed maximum (34%) antagonistic effect on the production of carbohydrates by cyanobacterium after 4-days (P<0.05, ANOVA) and this was followed by 60%, 65% and 70% at the end of 8 (P<0.01, ANOVA), 12 (P<0.001, ANOVA) and 16-days (P<0.001, ANOVA) of experiment, respectively (Appendix DM; B; Fig. 4.6 (b)).

(C) *Westiellopsis prolifica:*

Maximum carbohydrate content increase, from 0.254 mg/20ml to 0.351 mg/20ml, was reflected in untreated cells. As compared to other two tested organisms, where higher inhibition of carbohydrate concentrations was noticed, the *Westiellopsis prolifica* showed the reduction by 41% at 30 ppm, 53% at 60 ppm and 65% at 120 ppm treatment after 16-days. Furthermore, the initial period of 2,4-D ethyl ester exposure i.e. 4-days (17% at 30 ppm, 22% at 60 ppm and 32% at 120 ppm), 8-days (28% at 30 ppm, 35% at 60 ppm and 45% at 120 ppm) and 12-days (33% at 30 ppm, 44% at 60 ppm and 57% at 120 ppm) were found less toxic when compared with 16-day treatment. However, the results were highly significant (P<0.001, ANOVA) at all the four different intervals (Appendix DM; C; Fig. 4.6 (c)).
4.1.2.2 Total Amino acids:

(A) *Anabaena fertilissima*:

A significant variation in total amino acid content was noticed from 4-days (P<0.05, ANOVA) to 16-days (P<0.001, ANOVA) of the experiment. The percentage loss in total amino acid content was by 20%, 31%, 40% after 4-days, 33%, 42%, 53% after 8-days, 45%, 46%, 60% after 12-days and 51%, 57%, 75% after 16-days at 15 ppm, 30 ppm and 60 ppm treated culture respectively (Appendix DM2 A; Fig. 4.6 (a)). At the end of 16-days treatment, the untreated culture showed higher amino acid content (0.030 mg/20ml) than that of the treated cultures.

(B) *Aulosira fertilissima*:

The doses of 20 ppm, 40 ppm and 80 ppm of 2,4-D ethyl ester supplementation in growth medium rendered concentration dependent inhibitory pattern of interaction from 0.017 mg/20ml to 0.012 mg/20ml after 4-days and from 0.027 mg/20ml to 0.009 mg/20ml after 16-days of exposure. Thus showing decline by 18%, 23%, 27% after 4-days ((P>0.05, ANOVA) and 44%, 51%, 66% after 16-days (P<0.001, ANOVA) respectively, over the control. Almost similar pattern of significant interaction (P<0.001, ANOVA) was observed when the culture was incubated for 8-days (31% at 20 ppm, 40% at 40 ppm and 49% at 80 ppm) and 12-days (41% at 20 ppm, 45% at 40 ppm and 50% at 80 ppm) (Appendix DM2 B; Fig.4.6 (b)).

(C) *Westiellopsis prolifica*:

The amino acid content in untreated cultures was determined in the range of 0.017 mg/20ml -0.029 mg/20ml. Though a similar trend of suppression prevailed for the 16-days treatment cultures, the reduction values under 30 ppm, 60 ppm and 120 ppm were 44%, 47%, 57%, respectively and higher than 4-days (8% at 30 ppm,17% at 60 ppm and 17% at 120 ppm), 8-days (29% at 30 ppm, 36% at 60 ppm and 43% at 120 ppm) and 12-days (36% at 30 ppm, 40% at 60 ppm and 42% at 120 ppm). This was further confirmed statistically as insignificant result after 4-days (P>0.05, ANOVA) was followed by a significant level after 8-days (P<0.01, ANOVA) and finally by highly significant level (P<0.001, ANOVA) after 12 and 16-days (Appendix DM2 C; Fig.4.6 (c)).
Results

**Carbohydrates**

**Amino acids**

![Graphs showing effect of different concentrations of 2,4-D ethyl ester on total carbohydrates and total amino acid content of cyanobacteria.](image)

**Figure 4.6** Effect of different concentrations of 2,4-D ethyl ester on total carbohydrates and total amino acid content of cyanobacteria.
4.1.2.3 Total Proteins:

(A) *Anabaena fertilissima*:

No change in total proteins was observed at 15 ppm while slight induction in protein content from 0.0585 mg/20ml to 0.0601 mg/20ml was recorded at 30 ppm (2%) and 60 ppm (2%) treatment after 4-days of inoculation, which was succeeded by a period of slow depression (P>0.05, ANOVA) after 8-days by 17% at 15 ppm, 21% at 30 ppm and 29% at 60 ppm and by 26% at 15 ppm, 31% at 30 ppm and 48% at 60 ppm after 12-days. Further, the reduction in protein content by 30% at 15 ppm, 43% at 30 ppm and 63% at 60 ppm was recorded after 16-days of incubation (Appendix DM3 A; Fig.4.7 (a)).

(B) *Aulosira fertilissima*:

The protein content increased from 0.088 mg/20ml to 0.0945 mg/20ml after initial incubation of 4-days (2% at 20 ppm, 8% at 40 ppm and 8% at 80 ppm) but as the incubation time increased i.e. after 8-days (5% at 20 ppm, 8% at 40 ppm and 10% at 80 ppm) and 12-days (10% at 20 ppm, 14% at 40 ppm and 15% at 80 ppm) the values were lower (P>0.05, ANOVA) when compared with control. In the same pattern, the reduction was observed in protein content after 16-days treatment by 26%, 37% and 56% in 15 ppm, 30 ppm and 60 ppm concentration, respectively (Appendix DM3 B; Fig.4.7 (b)).

(C) *Westiellopsis prolifica*:

A gradual increase in protein content was noticed and total increase ranged from 0.098 mg/20ml to 0.103 mg/20ml. The pattern of stimulation after 4-days of treatment followed the same course of induction by 6% at 30 ppm, 7% at 60 ppm and 11% at 120 ppm treatment when compared with other two species but the percentage incremental values were dissimilar. The protein content was reduced (P>0.05, ANOVA) by 5%, 7%, 10% after 8-days and by 11%, 13%, 17% after 12-days at 30 ppm, 60 ppm and 120 ppm treatment respectively. While maximum repression was observed after 16-days (P<0.001, ANOVA) at 120 ppm by 50% followed by 60 ppm by 34% and 30 ppm by 22% (Appendix DM3 C; Fig.4.7 (c)).
4.1.2.4 Total Phenols:

(A) *Anabaena fertilissima:*

A more pronounced effect of 2,4-D ethyl ester was observed on phenol content of the cyanobacterium since the phenol content was higher in treated samples when compared with control and values ranged from 0.024 mg/20ml to 0.035 mg/20ml. The release of phenol content was slightly stimulated (P>0.05, ANOVA) after 4-days of exposure and was higher in all treatments (2% at 15 ppm, 7% at 30 ppm and 10% at 60 ppm) as compared to untreated cultures. Maximum and significant upsurge in phenols was recorded after 16-days (P<0.05) by 10% at 15 ppm, 31% at 30 ppm and 39% at 60 ppm treatment, when compared with control (Appendix DM4 A; Fig. 4.7 (a)).

(B) *Aulosira fertilissima:*

The levels of increment of phenol content after 4 (P>0.05, ANOVA), 8 (P>0.05, ANOVA), 12 (P<0.01, ANOVA) and 16-days (P<0.05, ANOVA) were from 0.014 mg/20ml (1%) to 0.017 mg/20ml (13%), from 0.019 mg/20ml (3%) to 0.021 mg/20ml (11%), from 0.026 mg/20ml (7%) to 0.030 mg/20ml (24%) and from 0.032 mg/20ml (10%) to 0.037 mg/20ml (27%) respectively (Appendix DM4 B; Fig.4.7 (b)).

(C) *Westiellopsis prolific a:*

An increase in the phenol content of *Westiellopsis prolific a* was noticed in treatments which ranged from 0.016 mg/20ml to 0.026 mg /20ml when compared to control (Fig. 4.7 (c)). However, slight difference in stimulation pattern was recorded as the phenol content remained stable with no change at 30 ppm treatment until 12-days, when matched to other two organisms, at various concentration and time exposure levels i.e. on 4th day (P>0.05, ANOVA) by 0%, 4% and 10%, on 8th day (P>0.05, ANOVA) by 0%, 6% and 11%, on 12th day (P<0.05, ANOVA) by 0%, 10% and 16% and on 16th day (P>0.05, ANOVA) by 9%, 19% and 24% at 30 ppm, 60 ppm and 120 ppm treatment, respectively (Appendix DM4 C).
Figure 4.7 Effect of different concentrations of 2,4-D ethyl ester on total protein and total phenol content of cyanobacteria.
4.1.3.1. Nitrate reductase:

(A) Anabaena fertilissima:
The NR activity was affected by the different concentrations of 2,4-D ethyl ester and was highest in cells grown in medium without 2,4-D ethyl ester i.e. control (7.56 μg of NO\(^2^-\)/20ml/min). The cultures spiked with 15 ppm, 30 ppm and 60 ppm dose for 4-days gave 19%, 38% and 42% reduction in NR activity, correspondingly. However, increase in exposure time, such as 8-days and 12-days, depicted increase in % reduction by 29% at 15 ppm, 41% at 30 ppm, 59% at 60 ppm and 38% at 15 ppm, 64% at 30 ppm, 73% at 60 ppm treatment respectively. Interaction of test alga with 2,4-D ethyl ester till 16\(^{th}\) day showed almost similar pattern within concentrations but the inhibition was more pronounced (43% at 15 ppm, 69% at 30 ppm and 78% at 60 ppm) and highly significant (P<0.001, ANOVA) when compared with other experimental periods (Appendix DE\(_1\) A; Fig. 4.8 (a)).

(B) Aulosira fertilissima:
The NR activity in cultures treated with 2,4-D ethyl ester was substantially inhibited and the degree of inhibition increased from 3.54 μg of NO\(^2^-\)/20ml/min to 2.2 μg of NO\(^2^-\)/20ml/min with increase in concentration of 2,4-D ethyl ester and time. All the concentrations of 2,4-D ethyl ester significantly inhibited (P<0.001, ANOVA) NR activity. The initial (after 4-days) rate of inhibition was by 18% at 20 ppm, 32% at 40 ppm and 38% at 80 ppm concentration which gradually increased by 37% at 20 ppm, 59% at 40 ppm and 73% at 80 ppm treatment at the end of the experiment (Appendix DE\(_2\) B; Fig. 4.8 (b)).

(C) Westiellopsis prolifica:
Experiments on NR activity revealed that different concentrations of 2,4-D inhibited the synthesis of NR from 4.8 μg of NO\(^2^-\)/20ml/min to 3.2 μg of NO\(^2^-\)/20ml/min. Of various concentrations tested, 120 ppm of 2,4-D ethyl ester was found to be a comparatively potent inhibitor (P<0.001, ANOVA) of NR activity, since the values recorded were 32% after 4-days, 41% after 8-days, 53% after 12-days and 62% after 16-days (Appendix DE\(_3\) C; Fig. 4.8 (c)).
4.1.3.2 Glutamine Synthetase (GS):

(A) *Anabaena fertilissima*:

The GS activity was repressed in cells grown in medium containing 2,4-D ethyl ester (from 3.9 to 0.2 μg of γ-glutamyl hydroxamate/20ml/min) and was highest (6 μg of γ-glutamyl hydroxamate/20ml/min) in the control. 2,4-D ethyl ester at 15 ppm level caused significant decrease in GS activity by 23%, 29%, 46% and 50%, while at LC₅₀ concentration i.e. 30 ppm, the reduction was recorded by 41%, 49%, 65% and 92% after 4, 8, 12, and 16 days (P<0.05, ANOVA) respectively. It was further, shown that the culture was adversely affected at 60 ppm dose by 51% after 4, 58% after 8, 65% after 12 and 97% after 16-days of investigation period (Appendix DE₂ A; Fig. 4.8 (a)).

(B) *Aulosira fertilissima*:

The maximum GS activity (5.6 μg of γ-glutamyl hydroxamate/20ml/min) was exhibited by the untreated cells whereas the cells grown with 2,4-D ethyl ester represented decrease in GS activity from 3.7 to 1.3 μg of γ-glutamyl hydroxamate/20ml/min. Effects of initial application of 2,4-D ethyl ester on GS activity of *Aulosira fertilissima* cultures demonstrated less but highly significant levels (P<0.001, ANOVA) of inhibition (21% at 20 ppm, 31% at 40 ppm and 43% at 80 ppm). The GS inhibition levels were relatively slow and steady after 8-days (31% at 20 ppm, 44% at 40 ppm and 57% at 80 ppm) and 12-days (35% at 20 ppm, 46% at 40 ppm and 67% at 80 ppm). However, after 16-days GS activity reached a maximum fall by 37%, 58% and 76% upon treatments of 20 ppm, 40 ppm and 80 ppm, respectively (Appendix DE₂ B; Fig. 4.8 (b)).

(C) *Westiellopsis prolifica*:

GS activity was stimulated from 5.4 to 6 μg of γ-glutamyl hydroxamate/20ml/min in cells grown in BG-11 medium; on the other hand, the enzyme activity was inhibited from 4.4 to 2 μg of γ-glutamyl hydroxamate/20ml/min in culture grown in the presence of 2,4-D ethyl ester. The exposure concentration i.e. 30 ppm of 2,4-D ethyl ester showed less toxicity towards the test organism where the in vitro GS activity was reduced to 20% after 4-days, 24% after 8-days, 26% after 12-days, and 27% after 16 days, however, at 120 ppm dose, the GS activity was significantly suppressed (P<0.001, ANOVA) by 40% after 4-days, 43% after 8-days, 57% after 12-days and 69% after 16-days (Appendix DE₂ C; Fig. 4.8 (c)).
4.1.3.3 Succinate Dehydrogenase (SDH):

(A) *Anabaena fertilissima:*

SDH activity decreased linearly from 0.133 to 0.022 µg of TTC reduced/20ml/min at highly significant levels (P<0.001, ANOVA) as the concentration and time of exposure increased. The SDH activity rates of treated alga with different concentrations of 2,4-D ethyl ester i.e. 15 ppm, 30 ppm and 60 ppm concentration represented inhibition by 25%, 50%, 62% after 4-days, 30%, 54%, 69% after 8-days and 44%, 59%, 82% after 12-days. After 16-days of growth, SDH activity was declined by 73%, 73%, 91% at 15 ppm, 30 ppm and 60 ppm concentration respectively, in comparison with the control (Appendix DE3 A; Fig.4.8 (a)).

(B) *Aulosira fertilissima:*

The levels of SDH activity in untreated and treated cells ranged from 0.222 to 0.044 µg of TTC reduced/20ml/min. After 4-days, the SDH activity of the cyanobacterium treated with three concentrations of 2,4-D ethyl ester (10% at 20 ppm, 30% at 40 ppm and 50% at 80 ppm) was significantly (P<0.001, ANOVA) dissimilar when compared with that of the control, while after 16-days, it apparently (P<0.001, ANOVA) decreased by 61% at 20 ppm, 77% at 40 ppm and 85% at 80 ppm treatment (Appendix DE3 B; Fig. 4.8 (b)).

(C) *Westiellopsis prolifica:*

SDH activity was affected by the different concentrations of 2,4-D ethyl ester and was lowest in the cells grown in 120 ppm of 2,4-D ethyl ester for 16-days where minimum value recorded was 0.089 µg of TTC reduced/20ml/min when compared with control (0.333 µg of TTC reduced/20ml/min). Consequently, 120 ppm dose of 2,4-D ethyl ester exhibited a severe dwindle by 40%, 47%, 51% and 73% in comparison with the controls during the 4-day, 8-day, 12-day and 16-day (P<0.001, ANOVA) respectively of experimental period (Appendix DE3 C; Fig. 4.8 (c))
Results

Nitrate reductase  Glutamine synthetase  Succinate dehydrogenase

Figure 4.8  Effect of different concentrations of 2,4-D ethyl ester on nitrate reductase, glutamine synthetase and succinate dehydrogenase activities of cyanobacteria.
4.1.4.1: Analysis of functional groups using Fourier Transform Infrared Spectroscopy (FTIR):

(A) *Anabaena fertilissima:*

FTIR spectrum of the 2,4-D ethyl ester adsorbed biomass of *Anabaena fertilissima* showed many changes of band over the herbicide-free biomass. Although different treatments of 2,4-D ethyl ester after 4-days reflected a consistent absence of 1703 cm\(^{-1}\) which attributed to C=O of \(\alpha, \beta\) unsaturated aldehydes, ketones and 1321 cm\(^{-1}\) to C-N stretch of aromatic amines, and newer peaks of 3511 cm\(^{-1}\) indicating O-H of phenols and alcohols were detected in all the treatments (Fig. 4.9). After 16-days of incubation, the absorption peak of 1348 cm\(^{-1}\) was recorded in all the three concentrations and this was due to presence of N-O symmetric stretch (nitro compounds) (Fig. 4.10). However, the peaks of 869 cm\(^{-1}\) and 1599 cm\(^{-1}\) frequency representing C-H of aromatics were recorded at the highest concentration (60 ppm) after 4-days and 16-days, respectively.

(B) *Aulosira fertilissima:*

An induction of new functional groups such as nitro compounds (N-O symmetric stretch at 1347 cm\(^{-1}\)), \(1^\circ, 2^\circ\) amines (N-H wag at 869 cm\(^{-1}\)), aromatic amines (C-N stretch at 1285 cm\(^{-1}\)) and aromatics (C-C stretch (in-ring) at 1425 cm\(^{-1}\)) were observed solely at the highest concentration (80 ppm) of 2,4-D ethyl ester treated *Aulosira fertilissima* after 4-days of exposure (Fig. 4.11). Whereas, after 16-days two groups, \(1^\circ\) amines (N-H bend at 1630 cm\(^{-1}\)) and nitro compounds (N-O symmetric stretch at 1347 cm\(^{-1}\)) were registered at 40 ppm and 80 ppm treatment of 2,4-D ethyl ester (Fig. 4.12).

(C) *Westiellopsis prolifica:*

As compared to the control, the initial spectra obtained after all the treatments of 2,4-D ethyl ester on *Westiellopsis prolifica* biomass resulted in the occurrence of two common functional groups, aromatic amines (C-N stretch) with a wavelength of 1288 cm\(^{-1}\) and aliphatic amines (C-N stretch) with a wavelength range of 1237-1247 cm\(^{-1}\). While in addition to above mentioned groups two more groups i.e. nitro compounds (N-O symmetric stretch) with a wavelength range of 1479-1339 cm\(^{-1}\) and aromatics (C-H “oop”) with a wavelength of 867 cm\(^{-1}\) were developed at 120 ppm treatment (Fig. 4.13). On the other hand, after 16-days one general functional group of aromatic amines (C-N stretch) with a wavelength of 1288 cm\(^{-1}\) was formed in 30 ppm and 120 ppm concentration of 2,4-D ethyl ester (Fig. 4.14).
Figure 4.9 FTIR chromatogram of 2,4-D ethyl ester treated *Anabaena fertilissima* after 4-days of incubation.
Figure 4.10  FTIR chromatogram of 2,4-D ethyl ester treated *Anabaena fertilissima* after 16-days of incubation.
Figure 4.11 FTIR chromatogram of 2,4-D ethyl ester treated *Aulosira fertilissima* after 4-days of incubation.
Figure 4.12 FTIR chromatogram of 2,4-D ethyl ester treated *Aulosira fertilissima* after 16-days of incubation.
Figure 4.13  FTIR chromatogram of 2,4-D ethyl ester treated *Westiellopsis prolifica* after 4-days of incubation.

An investigation of differential effects of 2,4-D and Penconuron on three species of Cyanobacteria in-vitro –  
A Biochemical and Molecular approach
Figure 4.14 FTIR chromatogram of 2,4-D ethyl ester treated *Westiellopsis prolifica* after 16-days of incubation.
4.1.4.2: Biotransformation studies using Gas Chromatography–Mass Spectrometry (GC-MS):

(A) *Anabaena fertilissima*:

After 4-days of 2,4-D ethyl ester exposure *Anabaena fertilissima* produced 2,4-D butyl ester at highest concentration (60 ppm) while the 15 ppm and 30 ppm treatments did not induced any new biotransformation and even the mother compound i.e 2,4-D ethyl ester was not observed. However GC-MS chromatogram of 30 ppm and 60 ppm 2,4-D ethyl ester treatment proved the formation of isobutyric acid allyl ester and 3-bromobutyric acid respectively after 16-days of incubation. After 16-days also it was observed that 15 ppm treatment of 2,4-D ethyl ester did not yielded any degradation compound of 2,4-D ethyl ester (Fig. 4.15).

(B) *Aulosira fertilissima*:

As a result of biotransformation earned out by *Aulosira fertilissima* a new compound – hydroxyurea was generated in response to 80 ppm dose of 2,4-D ethyl ester after 4-days of exposure whereas other two lower concentrations did not produced any biotransformation product. Moreover, trifluoroacetic acid, 2-methyl propyl ester and acetic acid 2-propenyl ester were important biotransformation products which were persistently recorded after 16-days in all the three chosen tested treatments (20 ppm, 40 ppm and 80 ppm) when compared with 100 ppm 2,4-D ethyl ester standard (Fig. 4.16).

(C) *Westiellopsis prolifica*:

An induction of new products such as 2,4-D methyl ester and acetic acid (2,3-dichlorophenoxy) were observed at the highest concentration (120 ppm) of 2,4-D ethyl ester treated *Westiellopsis prolifica* after 4-days and 16-days of exposure. No new by-product was identified with 15 ppm and 30 ppm treatment of 2,4-D ethyl ester and also it did not illustrated the presence of 2,4-D ethyl ester (Fig. 4.17).
Figure 4.15 GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated *Anabaena fertilissima*
Figure 4.16  GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated *Aulosira fertilissima*.
Figure 4.17 GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated Westiellopsis prolifica.
4.1.4.3 Protein profiling using SDS-PAGE:

Intracellular proteins of different cyanobacterial cells were extracted after concentrating the cells by centrifugation, lysing the cells using sample buffer and were resolved by SDS-PAGE. Equal amounts of protein from the control and various treatment samples were loaded onto SDS-PAGE gel and significant changes were recorded after 4 and 16-days, showing variations in the protein profile of untreated and pesticide treated cyanobacteria.

(A) *Anabaena fertilissima*:

Synthesis of several proteins declined with increasing duration of exposure to the 2,4-D ethyl ester. At the same time, synthesis of new proteins was induced. A total of 9 bands of 74 kDa, 64 kDa, 59 kDa, 50 kDa, 47 kDa, 28 kDa, 24 kDa, 23 kDa and 21 kDa were observed in control after 4 and 16-days. *Anabaena fertilissima* treated with 15 ppm of 2,4-D ethyl ester had lead to eradication of four protein bands (74 kDa, 24 kDa, 23 kDa and 21 kDa) whereas other protein bands (64 kDa, 59 kDa, 50 kDa, 47 kDa and 28 kDa) were identical to that of the untreated cultures after 4-days and 16-days of exposure. In contrast, after 4-days of incubation 30 ppm and 60 ppm 2,4-D ethyl ester treated cultures showed a new band of around 46 kDa but protein bands of 74 kDa, 50 kDa, 28 kDa, 24 kDa, 23 kDa and 21 kDa disappeared (Fig.4.18 (a) & (b)). However, loss of all the protein bands was registered in the culture treated with 30 ppm and 60 ppm of 2,4-D ethyl ester after 16-days of incubation.

(B) *Aulosira fertilissima*:

The protein profiles of untreated cells of *Aulosira fertilissima* resulted in 7 standard protein bands of 65 kDa, 60 kDa, 55 kDa, 48 kDa, 29 kDa, 18 kDa and 16 kDa after 4 and 16-days of incubation. While exposure to 2,4-D ethyl ester for 4-days resulted in low expression of protein bands of molecular weight of 18 kDa and 16 kDa at 20 ppm and 80 ppm treatment. Further at 40 ppm dose there was diminution in the intensity of two protein bands of 65 kDa and 60 kDa, whereas at 80 ppm dose these bands were dense as compare to control and other treatments after 4-days of incubation. After 16-days of exposure to 20 ppm dose 65 kDa, 60 kDa and 55 kDa proteins were not detected, while at 40 ppm dose weak bands of the same kDa were recovered. However, the intensity of 48 kDa protein sharply decreased at 80 ppm, while 65 kDa...
and 60 kDa proteins were less affected than 29 kDa, 18 kDa and 16 kDa which were completely eliminated after 16-days of incubation (Fig.4.18 (c) & (d)).

(C) *Westiellopsis prolifica*:

The different concentrations of 2,4-D ethyl ester seemed to have less effect on the protein synthesis (62 kDa, 59 kDa, 30.4 kDa and 14 kDa) in *Westiellopsis prolifica* after 4-days of incubation. At the end of 16-days three protein bands of 22 kDa, 16 kDa and 14 kDa were observed at 60 ppm and 120 ppm dose of 2,4-D ethyl ester and the rest of the bands of 62 kDa, 59 kDa and 30.4 kDa which otherwise appeared in the untreated control and 30 ppm were completely disappeared (Fig.4.18 (e) & (f)).
Figure 4.18  Effects of 2,4-D ethyl ester on polypeptide pattern of total proteins. Lanes C, 1, 2 and 3 represent proteins extracted from control, 15 ppm, 30 ppm and 60 ppm treated Anabaena fertilissima (a & b); control, 20 ppm, 40 ppm and 80 ppm treated Aulosira fertilissima (c & d); control, 30 ppm, 60 ppm and 120 ppm treated Westiellopsis prolifica (e & f), respectively.
4.1.4.4 DNA Profiling using RAPD:

A total of 4 primers (OPAH-02, OPB-09, OPG-04 and OPA-08) of arbitrary nucleotide sequence were initially used to amplify DNA segments from the genomic DNA and to generate RAPD patterns for three different cyanobacterial cultures. The decisive factor for choosing these primers was the generally accepted bias towards oligonucleotides of high G+C content. All the 4 primers were tried and 3 primers were found to produce informative and reproducible genetic markers for different cyanobacterial cultures. Hence, RAPD was carried out employing three primers i.e. OPAH-02 for *Anabaena fertilissima*, OPG-04 for *Aulosira fertilissima* and OPB-09 for *Westiellopsis prolifica*.

(A) *Anabaena fertilissima*:

The primer OPAH-02 produced total 5 amplified DNA fragments of size ranging from 1500 bp to 200 bp. All the three concentrations of 2,4-D ethyl ester resulted in rigorous inhibition of template activity of DNA after 4-days incubation that might have caused because of changes at various places in genomic DNA. It is evident from the electrophoretic pattern of DNA fragments of RAPD that at the end of 16-days, DNA of untreated cultures of *Anabaena fertilissima* showed five distinct bands of 1500 bp, 1300 bp, 700 bp, 500 bp and 200 bp, whereas DNA from cultures exposed to 2,4-D ethyl ester exhibited loss of all the fragments even at the lowest concentration (Fig. 4.19 (a) & (b)).

(B) *Aulosira fertilissima*:

*Aulosira fertilissima* treated with different concentrations of 2,4-D ethyl ester exhibited a time and concentration dependent inhibition in the polymorphic DNA pattern of the organism after 4 and 16-days. Although, untreated control cultures of *Aulosira fertilissima* produced bands of 2142 bp, 1795 bp, 1500 bp, 1160 bp and 900 bp. The number of bands generated by untreated culture were similar to both the lower concentrations (20 ppm and 40 ppm) of 2,4-D ethyl ester while at 80 ppm dose of 2,4-D ethyl ester, bands of 2142 bp, 1795 bp and 1160 bp were lost but one new DNA fragment of 600 bp was induced after 4-days incubation. However, after 16-days of exposure of 2,4-D ethyl ester there was almost complete disappearance of all bands at 40 ppm and 80 ppm treatment, whereas 20 ppm treated culture was analogous to control (Fig. 4.19 (c) & (d)).
(C) *Westiellopsis prolifica:*

A total of fourteen different DNA bands were observed in all the lanes, ranging from 10,000 bp to 600 bp after 4-days and sixteen DNA fragments ranging from 1700 bp to 400 bp after 16-days were encountered for the primer OPB-09. After 4-days, 30 ppm treatment in *Westiellopsis prolifica* showed banding pattern which was generally close to the untreated cultures while in addition to bands present in control some new bands of 2500 bp, 1400 bp, 1300 bp, 950 bp, 900 bp, 600 bp and 500 bp were also observed for 60 and 120 ppm 2,4-D ethyl ester treatments. However after 16-days of incubation one band of 1500 bp was found absent at 60 ppm and recovered at 120 ppm treatment, at the same time three bands of 900 bp, 700 bp, 500 bp and 400 bp were absent when compared with control (Fig. 4.19 (e) & (f)).
Figure 4.19 RAPD profile of 2,4-D ethyl ester treated cyanobacterial cultures. Lane 1, 2, 3 and 4 represent control, 15 ppm, 30 ppm and 60 ppm treated *Anabaena fertilissima* (a & b); control, 20 ppm, 40 ppm and 80 ppm treated *Aulosira fertilissima* (c & d); control, 30 ppm, 60 ppm and 120 ppm treated *Westiellopsis prolifica* (e & f), respectively. Lane M: Standard molecular weight DNA markers.
4.1.4.5 Sequencing of 16S rDNA Gene:

Cyanobacteria were grown in the presence of pesticides for 16-days and the effect on 16S rDNA sequence was analyzed by DNA sequencing (DNA sequencing services; Chromous Biotech Ltd., India) of the PCR amplified amplicons, amplified using primers specific for 16S rDNA. The results of sequencing appeared as different peaks, for different nucleotides, in the form of a chromatogram. The DNA sequences obtained from pesticide treated and control cyanobacteria were aligned using BLAST. A local alignment without gaps consists simply of a pair of equal length segments but presence of large regions of mismatch having no homology can be because of strand breakage and incorrect arrangement of nucleotides in the sequence. The gaps in the two sequences results from mismatching of purines and pyrimidines (replacement of purines instead of pyrimidines and vice-versa, purine-purine interactions or pyrimidine-pyrimidine interactions), formation of thymine dimers, or mismatch of bases, due to errors in DNA replication in which the wrong DNA base is incorporated in a newly forming DNA strand or a DNA base is skipped over or mistakenly inserted. Damage to DNA can also be caused by oxidation of bases and generation of DNA strand interruptions from reactive oxygen species, alkylation (methylolation) of nucleotide bases, hydrolysis (deamination, depurination, and depyrimidination) of the bases or crosslinking of DNA because of some mutagens.

(A) *Anabaena fertilissima*:

Significant changes were observed in the 16S rDNA sequence of *Anabaena fertilissima* after 16-days treatment with 2,4-D ethyl ester at a concentration of 60 ppm treatment. The impact of 2,4-D ethyl ester was to such an extent that there was no homology in the region of 39 basepairs (i.e. nucleotide 203 to 242) of control when aligned with sequence of pesticide treated culture. Also in the remaining sequence, several mismatches and gaps were observed. There was observed 94% identity in the nucleotides 1-203 while only 83% sequence similarity was recorded from nucleotide 242-1159 of control 16S rDNA sequence with gaps at 60 places (Fig. 4.20). Gaps refer to places where sequences preceding and succeeding the gap are exactly similar suggesting that a nucleotide is missed during the DNA replication process. The mismatch refers to the change in the nucleotide with another nucleotide (purine with purine, pyrimidine with pyrimidine, purine with pyrimidine or pyrimidine with purine). The most common mismatch in the 16S rDNA sequence of *Anabaena fertilissima* resulted because of purine-pyrimidine substitutions in the two sequences (63 places) followed by pyrimidine-pyrimidine mismatches (at 25 places) and purine-purine mismatches (at 24 places).
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**Figure 4.20** DNA sequence alignment of 16S rDNA sequence of control and 2,4-D ethyl ester (60ppm; 16-days) treated *Anabaena fertilissima* using BLAST.

(B) *Aulosira fertilissima*:

Substantial differences were observed in the 16S rDNA sequence in case of *Aulosira fertilissima* cultures in presence of 2,4-D ethyl ester at a concentration of 80 ppm after 16-days when compared to its untreated control. Identities of 83% were observed suggesting a 17% difference in the sequence after treatment and along-with this, 5% gaps were also present (Fig. 4.21). The main deviation was because of purine-pyrimidine counterparts (43 places), pyrimidine-pyrimidine mismatches (19 places) and purine-purine mismatches (16 places).
Results

<table>
<thead>
<tr>
<th>Score = 547 bits (296), Expect = 5e-160</th>
<th>Identities = 532/642 (83%), Gaps = 32/642 (5%)</th>
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Figure 4.21 DNA sequence alignment of 16S rDNA sequence of control and 2,4-D ethyl ester (80ppm; 16-days) treated *Aulosira fertilissima* using BLAST.

An investigation of differential effects of 2,4-D and Picloram on three species of Cyanobacteria in vitro – A Biochemical and Molecular approach.
(C) *Westiellopsis prolifica*:

Even at the end of experiment (16-days), very minor dissimilarities were observed in the 16S rDNA sequence of *Westiellopsis prolifica* cultures when treated with 2,4-D ethyl ester at a concentration of 120 ppm, as compared to that observed in other two selected organisms, with respect to its untreated control. There were 1133 similarities out of total 1151 basepairs i.e. 98% identities and 2 gaps between the sequences of control and pesticide treated cultures (Fig.4.22). In *Westiellopsis prolifica* there were 14 purine-pyrimidine mismatches while only one purine-purine (position 273) and one pyrimidine-pyrimidine mismatch was observed (position 888). The gaps in the sequence were observed at positions 517 (where a cytosine was incorporated) and 892 (where a thymine was incorporated).
Results

Figure 4.22 DNA sequence alignment of 16S rDNA sequence of control and 2,4 D ethyl ester (120ppm; 16-days) treated Westiellopsis prolifica using BLAST.
4.2 Pencycuron:

4.2.1.1 Morphological characters:

(A) *Anabaena fertilissima:*

Morphological characters such as filamentous structure, cell shape, pigmentation of the cells, and size of the cells remained unaffected throughout the experimental period (4-days to 16-days) at 15 ppm and 30 ppm treatments of pencycuron to the cyanobacterial culture. However, 60 ppm treated culture initially (4-days) resulted into considerable bleaching of color and increase in size of the cells which intensified after 16-days and the cells appeared more plump while no change was observed with reference to degeneration of filamentous structure and shape of the cells (Fig. 4.23).

The heterocyst frequency was found to be affected slightly after the treatment with pencycuron. The percentage inhibition of heterocysts was recorded to be 15% at 15 ppm, 20% at 30 ppm and 33% at 60 ppm dose of pencycuron after 4-days of incubation whereas after 16-days the values for reduction in heterocyst frequency were 16% at 15 ppm, 24% at 30 ppm and 35% at 60 ppm (Table 4.2).

(B) *Aulosira fertilissima:*

During the exposure period of 4-days the culture treated with 15 ppm, 30 ppm and 60 ppm of pencycuron was similar to the corresponding controls in terms of cell size, shape, filament structure and pigmentation. On the other hand the culture treated with 30 ppm and 60 ppm was rigorously affected after 16-days of observation. At both the concentrations, prompt degradation of dominating green pigment was observed as the counter-stain (safranin) was taken up efficiently by the cells. Also, increase in the size of the cell compartments and change in cell shape (from elongated barrel-shaped to spherical) was registered which verified the interference of pencycuron doses with the morphology of *Aulosira fertilissima* (Fig. 4.24).

The number of heterocysts decreased was concentration dependent by 25% and 30% of the reduction was recorded at highest concentration (60 ppm) after 4-days and 16-days (Table 4.2). Whereas heterocyst frequency progressively declined by 6% to 10% at 15 ppm dose and by 16% and 20% at 30 ppm dose of pencycuron.
(C) *Westiellopsis prolifica:*

Tolerant behavior of *Westiellopsis prolifica* was explained morphologically since the culture exposed to 50 ppm, 100 ppm and 200 ppm dose was not significantly affected after initial treatments (4-days) of pencycuron. However, with increase in exposure period (at the end of 16-days), concentration of 50 ppm pencycuron resulted in marked changes such as decolorization of green colored cells and disintegration of long filaments to smaller fragments. Further, the effect of pencycuron was exemplified with increase in concentration (100 ppm). Similar observations with higher intensities of the impact, associated to 200 ppm of pencycuron treatment, were recorded after 16-days (Fig. 4.25).

Heterocyst frequency of *Westiellopsis prolifica* was not affected by 50 ppm of pencycuron; however a minor decrease in heterocyst production by 9% was generated after 16-days. Raising pencycuron concentrations with increasing incubation periods explained the progressive dose and time dependent decrease in the heterocyst frequency since maximum retardation percentage in heterocyst formation i.e. by 24 % was recorded at 200 ppm dose after 16-days of exposure (Table 4.2).

<table>
<thead>
<tr>
<th>Cyanobacteria</th>
<th>Concentration (ppm)</th>
<th>Heterocyst frequency (%)</th>
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</thead>
<tbody>
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<td></td>
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<td>4-days</td>
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<tr>
<td>Control</td>
<td>4</td>
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</tr>
<tr>
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<td></td>
<td>3.4 (15)</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>3.2 (20)</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>2.7 (33)</td>
</tr>
<tr>
<td><strong>Aulosira fertilissima</strong></td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>3.0 (6)</td>
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<tr>
<td>30</td>
<td></td>
<td>2.7 (16)</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>2.4 (25)</td>
</tr>
<tr>
<td><strong>Westiellopsis prolifica</strong></td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>3.4 (0)</td>
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<td>100</td>
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<td>3.2 (6)</td>
</tr>
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<td>200</td>
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</table>

Data in parenthesis denotes percentage inhibition.

An investigation of differential effects of 2,4-D and Pencycuron on three species of Cyanobacteria in-vitro — A Biochemical and Molecular approach
Figure 4.23 Photomicrograph (100 X) of Anabaena fertilissima exposed to 15ppm, 30ppm and 60ppm of pencycuron for 4-days (A) and 16-days (B) with reference to control.
Figure 4.24 Photomicrograph (100 X) of *Aulosira fertilissima* exposed to 15ppm, 30ppm and 60ppm of pencycuron for 4-days (A) and 16-days (B) with reference to control.
Figure 4.25 Photomicrograph (100 X) of Westiellopsis prolifica exposed to 50ppm, 100ppm and 200ppm of pencycuron for 4-days (A) and 16-days (B) with reference to control.
4.2.1.2 Chlorphyll a:

(A) *Anabaena fertilissima*:

The effect of pencycuron was pronounced, because all the three concentrations of pencycuron markedly depressed the chlorophyll a content of the cyanobacterium from 0.20 µg/20ml to 0.10 µg/20ml in a time-dose dependent manner. Initially after 4-days of experiment, minor reduction (5% at 15 ppm, 7% at 30 ppm and 15% at 60 ppm) was observed (P>0.05, ANOVA) which was followed by highly significant reductions (P<0.001, ANOVA) after 8-days (19% at 15 ppm, 31% at 30 ppm and 54% at 60 ppm) and 12-days (21% at 15 ppm, 43% at 30 ppm and 64% at 60 ppm). Finally at the end of the experiment i.e. after 16-days, 15 ppm, 30 ppm and 60 ppm treatment resulted into reduction of 41%, 50% and 70% respectively (Appendix PP1 A; Fig. 4.26 (a)).

(B) *Aulosira fertilissima*:

Exposure of *Aulosira fertilissima* cells to different concentrations of pencycuron showed that the chlorophyll a inhibition was concentration dependent and ranged between 0.23 µg/20ml to 0.14 µg/20ml. The decline in chlorophyll a with respect to increasing exposure periods was more prominent and highly significant (P<0.001, ANOVA) at higher concentrations of pencycuron as compared with the lower doses. The percentage reduction at highest concentration (60 ppm) was 12% after 4-days, 52% after 8-days, 61% after 12-days and 64% after 16-days (Appendix PP1 B; Fig. 4.26 (b)).

(C) *Westiellopsis prolifica*:

The chlorophyll a values in pencycuron treated cultures tended to drop down with time, reaching 0.17 µg/20ml below control (0.40µg /20ml). Maximum rate of retardation, as reflected by quantification of chlorophyll a content, was observed in cells subjected to pencycuron exposure for 16-days (P<0.001, ANOVA) by 25% at 50 ppm, 50% at 100 ppm and 57% at 200 ppm treatment (Appendix PP1 C; Fig. 4.26 (c)).
4.2.1.3 Total Carotenoids:

(A) *Anabaena fertilissima*:
The level of total carotenoids decreased from 2.6 µg/20ml to 1.3 µg/20ml in all pencycuron treated cultures in a time-dose response manner. The lowest concentration (15 ppm) of fungicide reduced carotenoid contents by 7%, 14%, 13%, and 19%, after 4, 8, 12 and 16-days of incubation respectively. It is evident from the results that the declining trend in the carotenoid content continued with the rising concentration of fungicide as 60 ppm of pencycuron treatment demonstrated sharp decrease in the carotenoid content i.e. by 28%, 31%, 43% and 65% after 4, 8, 12 and 16-days respectively (Fig. 4.26 (a)). However, the results were highly significant (P<0.001, ANOVA) after 16-days of exposure period (Appendix PP2 A).

(B) *Aulosira fertilissima*:
Reduction in carotenoid content from 3.5 µg/20ml to 2.1 µg/20ml matched with an increase in the exposure period. The cells grown with 60 ppm of pencycuron showed the highest reduction by 50% in carotenoid content after 16-days of exposure, whereas the lowest reduction was observed initially (after 4-days) by 5% 15 ppm, 13% at 30 ppm and 22% at 60 ppm treatment (Fig. 4.26 (b)). Nevertheless, the statistical analysis verified the insignificant effect (P>0.05, ANOVA) after 4 and 8-days which was followed by significant effect of pencycuron on reduction in carotenoid content (P<0.05, ANOVA) after 12 and 16-days (Appendix PP2 B).

(C) *Westiellopsis prolifica*:
The values of carotenoid content of untreated and pencycuron treated *Westiellopsis prolifica* varied from 7.7 µg/20ml to 4.1 µg/20ml after 16-days of incubation. Of the three concentrations used in the study, 200 ppm dose had the most deleterious effect (10% reduction after 4-days, 25% reduction after 8-days, 29% reduction after 12-days and 48% reduction after 16-days) on the culture while 50 ppm dose had the least effect (5% reduction after 4-days, 5% reduction after 8-days, 6% reduction after 12-days and 6% reduction after 16-days) (Appendix PP2 C; Fig.4.26 (c)).
Figure 4.26  Effect of different concentrations of pencycuron on chlorophyll $a$ and carotenoids content of cyanobacteria.
4.2.1.4 Phycocyanin:

(A) *Anabaena fertilissima*:

Significant reduction in phycocyanin content from 0.03 µg/20ml to 0.009 µg/20ml was recorded at the highest dosage i.e. 60 ppm by the end of 16-days in *Anabaena fertilissima*. At the end of 4-days incubation, the reduction in phycocyanin content was somewhat sluggish (P>0.05, ANOVA; 26% at 15 ppm, 33% at 30 ppm and 44% at 60 ppm) than that in 8-days (P>0.05; 27% at 15 ppm, 36% at 30 ppm and 49% at 60 ppm) followed by 12-days (P<0.01, ANOVA; 35% at 15 ppm, 44% at 30 ppm and 66% at 60 ppm), but after 16-days the diminution was much higher (P<0.01, ANOVA; 40% at 15 ppm, 47% at 30 ppm and 70% at 60 ppm) than other exposure periods (Appendix PP3 A; Fig.4.27 (a)).

(B) *Aulosira fertilissima*:

In contrast to the control cultures where phycocyanin content ranged from 0.027 µg/20ml to 0.034 µg/20ml, pencycuron-supplemented culture of *Aulosira fertilissima* failed to maintain phycocyanin content (P<0.05, ANOVA). The inhibitory effect of pencycuron was more pronounced and accelerated with increase in time and concentration as 32%, 44% and 65% of reduction was recorded at 15 ppm, 30 ppm and 60 ppm treatments respectively after 16-days of incubation (Appendix PP3 B; Fig.4.27 (b)).

(C) *Westiellopsis prolifica*:

Phycocyanin content surpassed from 0.027 µg/20ml to 0.039 µg/20ml in untreated cells after 4-days till the end of the experiment. The addition of pencycuron to the culture caused an initial suppression in phycocyanin content (P>0.05, ANOVA) by 15% at 50 ppm, 15% at 100 ppm and 26% at 200 ppm treatment which was increased (P<0.01, ANOVA) to 20% at 50 ppm, 33% at 100 ppm and 49% at 200 ppm dose at the end of the experiment (Appendix PP3 C; Fig.4.27 (C)).

4.2.1.5 Phycoerythrin:

(A) *Anabaena fertilissima*:

Phycoerythrin content of cells exposed to pencycuron was reduced significantly from 0.020 µg/20ml to 0.004 µg/20ml. The inhibition of phycoerythrin production after 4-days of treatment with 15 ppm concentration was low (17%), whereas in 30 and 60 ppm treatments the...
phycoerythrin content was inhibited by 25% and 46%, respectively (P<0.01, ANOVA). Pencycuron at 60 ppm dose gave an inhibition of 50% after 8-days and 61% after 12-days of treatment, but later the reduction increased to 86% after 16-days and became highest (P<0.001, ANOVA) among all the tested concentrations and exposure times (Appendix PP4 A; Fig.4.27 (a)).

(B) *Aulosira fertilissima:*

In all treatments, phycoerythrin content was always less (0.019 to 0.010 µg/20ml) than that in the corresponding controls (0.022 to 0.030 µg/20ml). In cultures treated with 15 ppm (14%), 30 ppm (23%) and 60 ppm treatment (36%) the rate of inhibition was slow until 4-days of treatment (P<0.05, ANOVA) which increased with exposure period. At the end of the experiment, the cultures treated with 15 ppm, 30 ppm and 60 ppm concentration showed highest inhibition (P<0.001, ANOVA) by 27%, 53% and 67% respectively when compared with other incubation periods (Appendix PP4B; Fig.4.27 (b)).

(C) *Westiellopsis prolifica:*

The cultures exposed to different pencycuron concentrations showed different patterns of phycoerythrin content from control as the values were changed from 0.022 µg/20ml to 0.018 µg/20ml. The ANOVA test demonstrated the significant results after 4 (P<0.01) and 8-days (P<0.001) of pencycuron treatment. However, pencycuron at 200 ppm concentration showed significant inhibition which was around 29% after 4-days, 30% after 8-days, 33% after 12-days and 40% after 16-days of treatments, respectively (Appendix PP4C; Fig.4.27 (c)).

4.2.1.6 Allophycocyanin:

(A) *Anabaena fertilissima:*

It was noticed that the toxic effect on allophycocyanin content was amplified in the presence of pencycuron since the values were reduced from 0.024 µg/20ml to 0.010 µg/20ml. Under pencycuron exposure the pigment content was reduced (P<0.05, ANOVA) by 11%, 17%, 21% and 27% at 15 ppm treatment; 22%, 27%, 42% and 57% at 30 ppm treatment; as well as by 33%, 37%, 42% and 75% at 60 ppm treatment after 4, 8, 12 and 16-days, respectively, in comparison with the controls (Appendix PP5 A; Fig.4.27 (a)).
(B) *Aulosira fertilissima*:

Allophycocyanin content was significantly increased from 0.028 µg/20ml to 0.041 µg/20ml in the control. On the other hand, the effects of the three concentrations of pencycuron appeared to differ in a time-dependent manner, with slight inhibition (P>0.05, ANOVA) during 4-days (7% at 15 ppm, 18% at 30 ppm and 28% at 60 ppm) followed by enlarged inhibition (P<0.05, ANOVA) during 8th day (14% at 15 ppm, 28% at 30 ppm and 32% at 60 ppm) and 12th day (19% at 15 ppm, 32% at 30 ppm and 49% at 60 ppm) of test period and finally followed by severe inhibition (P<0.001, ANOVA) at the end of 16-days of treatment (22% at 15 ppm, 34% at 30 ppm and 56% at 60 ppm) (Appendix PP5B; Fig.4.27 (b)).

(C) *Westiellopsis prolifica*:

The insignificant results of effects of pencycuron (P>0.05, ANOVA) were obtained after 4-days of incubation which gradually transformed to highly significant inhibition at the end of the experiment (P<0.001, ANOVA). In absence of pencycuron, allophycocyanin content was stimulated from 0.033 µg/20ml to 0.041 µg/20ml. The toxicity of pencycuron on allophycocyanin content was lowest at 15 ppm (17% reduction after 16-days) among the three concentrations tested even at the end of the experiment, whereas the maximum toxicity i.e. 41% reduction was recorded after 60 ppm treatment (Appendix PP5C; Fig.4.27 (c)).
Figure 4.27 Effect of different concentrations of pencycuron on phycocyanin, phycoerythrin and allophycocyanin content of cyanobacteria.
4.2.2.1 Total Carbohydrates:

(A) *Anabaena fertilissima*:

The inhibition in total carbohydrate content ranged from 0.19 mg/20ml to 0.13 mg/20ml. The recorded carbohydrates inhibitory levels were relatively low at 15 ppm dose (21%) and reached a maximum of 42% upon treatment by 60 ppm dose after 4-days (P>0.05, ANOVA). The results revealed that carbohydrate formation was slowly hindered by pencycuron treatment for observable effects (P<0.05, ANOVA) on *Anabaena fertilissima* at exposure period of 8-days (25%, 36% and 50%), 12-days (33%, 47% and 57%) and 16-days (41%, 56% and 62%) at 15 ppm, 30 ppm and 60 ppm treatment, respectively (Appendix PM1 A; Fig.4.28 (a)).

(B) *Aulosira fertilissima*:

All the three selected concentrations, after 4 and 8-days, were found to be less affected as compared to that after 12 and 16-days cultures, however, the total carbohydrate content was reduced from 0.19 mg/20ml to 0.12 mg/20ml by the end of the experiment. On day 4\(^\text{th}\) of incubation, release of carbohydrates in *Aulosira fertilissima* was suppressed (P<0.05, ANOVA) by 5% at 15 ppm, 15% at 30 ppm and 25% at 60 ppm followed by 17% at 15 ppm, 25% at 30 ppm and 33% at 60 ppm treatment after 8\(^\text{th}\) day of incubation (P<0.01). However, level of inhibition was accelerated with increase in incubation time by 26% at 15 ppm, 44% at 30 ppm and 48% at 60 ppm after 12-days (P<0.05, ANOVA) which was followed by 16-days (P<0.001, ANOVA) of exposure by 36% at 15 ppm, 50% at 30 ppm and 57% at 60 ppm treatment (Appendix PM1 B; Fig.4.28 (b)).

(C) *Westiellopsis prolifica*:

*Westiellopsis prolifica* also followed the same trend (as recorded for other two cyanobacterial species) of time–concentration dependent decrease in carbohydrate content from 0.21 mg/20ml to 0.18 mg/20ml after 4-days and from 0.29 mg/20ml to 0.20 mg/20ml after 16-days, whereas the highest percentage reduction was recorded at 200 ppm treatment by 22% after 4-days, 26% after 8-days, 37% after 12-days and 46% after 16-days, when compared with other two lower concentrations (Fig.4.28 (c)). After 4-days of exposure the data explained insignificant results (P>0.05, ANOVA) but with increase in exposure time the significant effect (P<0.01, ANOVA) was observed (Appendix PM1 C).
4.2.2.2 Total Amino acids:

(A) *Anabaena fertilissima*:

In *Anabaena fertilissima* synthesis of amino acids declined in response to the exposure period, since less significant effect was recorded at initial incubation (P>0.05, ANOVA) period and highly significant effect was observed at the end of the experiment (P<0.001, ANOVA). The amino acid content in *Anabaena fertilissima* ranged from 0.019 mg/20ml to 0.030 mg/20ml. At 15 ppm dose, the inhibition level of 10% after 4-days was enhanced to 15%, 21% and 23% after 8, 12 and 16-days, respectively. Similarly, at LC50 value i.e. 30 ppm, the inhibition was extended by 21%, 27%, 38% and 60% while at the highest concentration i.e. 60 ppm it was recorded to be 31%, 38%, 55% and 70% after 4-days, 8-days, 12-days and 16-days of incubation (Appendix PM2 A; Fig.4.28 (a)).

(B) *Aulosira fertilissima*:

Different concentrations of pencycuron significantly reduced total amino acids from 0.017 mg/20ml to 0.013 mg/20ml. A gradual reduction of 10%, 21% and 31% of the total amino acids content at the end of 4 days (P<0.05, ANOVA) was recorded when the cells were treated with 15 ppm, 30 ppm and 60 ppm of pencycuron, respectively. Nevertheless, this gradual reduction was replaced with drastic decrease (P<0.01, ANOVA) of 21%, 39% and 53% at the end of experiment i.e. 16th day (Appendix PM2 B; Fig.4.28 (b)).

(C) *Westiellopsis prolifica*:

The results showed less toxic effect (P>0.05, ANOVA) after 4-days (0.019 mg/20ml to 0.015 mg/20ml) and 8-days (0.025 mg/20ml to 0.018 mg/20ml) while after 12-days (P<0.01, ANOVA) and 16-days (P<0.05, ANOVA) significant reduction from 0.027 mg/20ml to 0.016 mg/20ml and 0.030 mg/20ml to 0.016 mg/20ml, respectively, were recorded (Appendix PM2 C). Increasing the concentration of pencycuron from 50 to 200 ppm on the 12th day of treatment, total amino acids declined by 18% at 50 ppm, 26% at 100 ppm, and 41% at 200 ppm, as compared with the control, depicting a concentration-dependent inhibition of amino acids. While after 16-days of treatment, 50 ppm, 100 ppm and 200 ppm concentrations of pencycuron reduced the amino acid content of cyanobacterium by 20%, 37% and 47%, respectively (Fig.4.28 (c)).
Results

Figure 4.28  Effect of different concentrations of pencycuron on total carbohydrates and total amino acid content of cyanobacteria
4.2.2.3 Total Proteins:

(A) *Anabaena fertilissima:*

The untreated cultures had higher protein content (0.113 mg/20ml) while treated cultures resulted into reduction from 0.113 mg/20ml to 0.060 mg/20ml. However, in all the three concentrations of pencycuron treatment, the reduction level (2% to 47% of reduction) was less than 50% from starting (P>0.05, ANOVA) till the end of the experiment (P<0.001, ANOVA). The maximum fall in protein content was recorded by 12% at 15 ppm, 35% at 30 ppm and 47% at 60 ppm treatment after 16-days of exposure (Appendix PM3 A; Fig.4.29 (a)).

(B) *Aulosira fertilissima:*

In resemblance to above results, *Aulosira fertilissima* also showed slow retardation from 0.084 mg/20ml to 0.081 mg/20ml in protein content after 4-days of exposure period. Nevertheless, at the end of the experiment (16-days) the protein reduction (P>0.05, ANOVA) was achieved by 12%, 21% and 32% at 15 ppm, 30 ppm and 60 ppm treatment, correspondingly (Appendix PM3 B; Fig.4.29 (b)).

(C) *Westiellopsis prolifica:*

A significant enhancement in total protein content from 0.092 mg/20ml to 0.246 mg/20ml had been shown in control. In all the three treatments, only 1% reduction (P>0.05, ANOVA) was observed at the end of 4th day, while the parallel experiment for 50 ppm, 100 ppm and 200 ppm dose after 8-days resulted into slight but negligible reductions (P>0.05, ANOVA) by 4%, 8% and 10% followed by 6%, 12% and 16% after 12-days (P>0.05, ANOVA). Highest reduction (P>0.05, ANOVA) among all the test conducted was recorded after 16-days by 9% at 50 ppm, 19% at 100 ppm and 23% at 200 ppm treatment (Appendix PM3 C; Fig.4.29 (c)).

4.2.2.4 Total Phenols:

(A) *Anabaena fertilissima:*

Pencycuron stress induced and stimulated the phenols in the *Anabaena fertilissima* and its content was raised from 0.018 mg/20ml to 0.039 mg/20ml (P>0.05, ANOVA) at all the four different levels of exposure periods when compared with the control (Appendix PM4 A). Remarkably the increase in phenol content was accelerated with the increase in time as it was 0% at 15 ppm dose, 5% at 30 ppm dose and 7% at 60 ppm dose after 4-days which was elevated up.
to 30% at 60 ppm dose after 8-days and 37% at 60 ppm dose after 12-days. However, a maximum upsurge was recorded after 16-days by 50% at 60 ppm dose (Fig.4.29 (a)).

(B) **Aulosira fertilissima**: The stimulation recorded from initial time (4-day) till the end of the experiment (16-day) was from 0.015 mg/20ml to 0.035 mg/20ml. It is clear from the results that among the three different treatments, 15 ppm and 30 ppm cultures were equally affected as the same rate of increment in phenol content was recorded after 8-days (9%), 12-days (11%) and 16-days (13%), whereas at the end of 4th day there was no change in 15 ppm treated cultures as compared with control. The maximum rate of enhancement in phenol content was registered at 60 ppm dose by 17% after 16-days (P>0.05, ANOVA) of exposure to pencycuron (Appendix PM4 B; Fig.4.29 (b)).

(C) **Westiellopsis prolifica**: No change in the phenol content was observed in cultures exposed to 50 ppm dose of pencycuron when compared with untreated culture after all the four selected experimental phases (P>0.05, ANOVA). The raise in phenol content of 100 ppm (5%) pencycuron treated cultures after 4- days was stable till the end of 16th day, however at 200 ppm dose a regular and gradual stimulation by 6% (0.017 to 0.018 mg /20ml) after 4-days, 10% (0.019 to 0.021 mg /20ml) after 8-days, 10% (0.020 mg /20ml to 0.022 mg /20ml) after 12-days and 14% (0.021 to 0.024 mg /20ml) after 16-days was achieved (Appendix PM4 C, Fig.4.29 (c)).
Results

Figure 4.29 Effect of different concentrations of pencycuron on total protein and total phenol content of cyanobacteria.
4.2.3.1 Nitrate reductase:

(A) *Anabaena fertilissima*:

The diminution in the NR activity ranged from 3.4 to 3.1 μg of NO\(_2\)/20ml/min. The initial reduction of 17% at 15 ppm treated culture was increased to 24% after 8-days, 28% after 12-days and 35% after 16-days, while at 30 ppm dose it was reduced by 20% on day 4, 28% on day 8, 46% on day 12 and 50% on day 16 when compared with untreated control cultures. Likewise 60 ppm treatment also showed the similar pattern of inhibition by 30%, 37%, 51% and 60% with respect to 4, 8, 12 and 16-days of incubation, but the level of inhibition was greater than other two tested concentrations of the pencycuron (Fig.4.30 (a)). However, decrease in NR activity was highly significant (P<0.001, ANOVA) at all the four exposure periods tested in the study (Appendix PE1 A).

(B) *Aulosira fertilissima*:

The highest values i.e. from 4.7 to 8 μg of NO\(_2\)/20ml/min for NR activity were dealt in case of untreated cultures. On day 16 of growth, NR activity was conspicuously (P<0.001, ANOVA) reduced by 26%, 47% and 56% from an initial level of 13%, 19% and 25% (P<0.001, ANOVA) followed by 18%, 24% and 37% on day 8 (P<0.001, ANOVA) and by 21%, 37% and 45% on day 12 (P<0.001, ANOVA), at 15 ppm, 30 ppm and 60 ppm treatments, respectively (Appendix PE1 B; Fig.4.30 (b)).

(C) *Westiellopsis prolifica*:

In *Westiellopsis prolifica* as compared to 50 ppm (8.5 to 6.8 μg of NO\(_2\)/20ml/min) and 100 ppm (8.5 to 5.7 μg of NO\(_2\)/20ml/min), the 200 ppm (8.5 to 4.5 μg of NO\(_2\)/20ml/min) of pencycuron had shown most deleterious effects (P<0.001, ANOVA) on NR activity after 16-days of exposure (Appendix PE1 C). The highest level of inhibition in NR activity observed at 200 ppm dose was 34%, 36%, 41% and 47% after 4, 8, 12 and 16-days, respectively. On the other hand, the reduction at 100 ppm dose (28% after 4-days, 29% after 8-days, 31% after 12-days and 33% after 16-days) was higher than 50 ppm dose (7% after 4-days, 13% after 8-days, 18% after 12-days and 20% after 16-days) of treatment (Fig.4.30 (c)).
4.2.3.2 Glutamine Synthetase:

(A) *Anabaena fertilissima:*

The 4th day results relative to control (5.2 to 5.8 μg of γ-glutamyl hydroxamate/20ml/min), demonstrated difference in terms of inhibition in GS activity (P<0.001, ANOVA) by 15% at 15 ppm, 35% at 30 ppm and 42% at 60 ppm treatment which was pursued by 18% at 15 ppm, 36% at 30 ppm and 57% at 60 ppm treatment after 8-days (P<0.001, ANOVA) of exposure. Meanwhile, the parallel experiments for 12 and 16-days exhibited marked inhibition (P<0.001, ANOVA) by 23% at 15 ppm, 37% at 30 ppm and 65% at 60 ppm and 24% at 15 ppm, 41% at 30 ppm and 66% at 60 ppm treatment, respectively (Appendix PE2 A; Fig.4.30 (a)).

(B) *Aulosira fertilissima:*

The effect of pencycuron was, however, more pronounced at the end of experiment on 16-days (P<0.001, ANOVA), since all the three concentrations of pencycuron markedly depressed the GS activity of the cyanobacterium from 4 to 2.6 μg of γ-glutamyl hydroxamate/20ml/min in a time-dose dependent manner (Appendix PE2 B). Prompt action of pencycuron on GS was observed soon after 4-days (13% at 15 ppm, 22% at 30 ppm and 35% at 60 ppm), inducing an increase in level of reduction by 17% at 15 ppm dose, 25% at 30 ppm dose and 45% at 60 ppm dose after 8-days followed by 12-days (18% at 15 ppm, 30% at 30 ppm and 52% at 60 ppm) and 16-days (19% at 15 ppm, 34% at 30 ppm and 53% at 60 ppm) (Fig.4.3 (b)).

(C) *Westiellopsis prolifica:*

The GS activity from initial point of incubation till the end of experiment varied from 5.4 to 6.2 μg of γ-glutamyl hydroxamate/20ml/min in control, 5 to 4.9 μg of γ-glutamyl hydroxamate/20ml/min at 50 ppm, 4.8 to 4.4 μg of γ-glutamyl hydroxamate/20ml/min at 100 ppm and 4 to 3.8 μg of γ-glutamyl hydroxamate/20ml/min at 200 ppm. Maximal decrease in GS activity measured at 200 ppm concentration of pencycuron exposure was 36% after 16-days, 34% after 12-days, 34% after 8-days and 26% after 4-days being highly significant (P<0.001, ANOVA) to control values (Appendix PE2 C; Fig.4.3 (c)).
4.2.3.3 Succinate Dehydrogenase:

(A) *Anabaena fertilissima*:

The effect of even lower concentration of pencycuron on the SDH activity relative to control (0.17 to 0.24 μg of TTC reduced/20ml/min) was not favorable, since the range of enzyme activity reduced was from 0.14 to 0.18 μg of TTC reduced/20ml/min at 15 ppm dose, 0.12 to 0.15 μg of TTC reduced/20ml/min at 30 ppm dose and 0.09 to 0.10 μg of TTC reduced/20ml/min at 60 ppm dose (Appendix PE3 A). The highest concentration (60 ppm), however showed the drastic shift in a regular pattern (P<0.001, ANOVA), since maximum reduction by 47%, 47%, 54% and 58% was achieved on 4th, 8th, 12th and 16th day of culture, respectively (Fig.4.30 (a)).

(B) *Aulosira fertilissima*:

A wide variation in SDH activity from 0.29 to 0.16 μg of TTC reduced/20ml/min was observed in response to pencycuron treatment after 16-days. Except for a partial decrease of 9% at 15 ppm, 22% at 30 ppm and 35% at 60 ppm treatment after 4-days (P<0.001, ANOVA), essentially the enzyme activity of cultures exposed to pencycuron treatments for 8-days (P<0.001, ANOVA; 11% at 15 ppm, 23% at 30 ppm and 38% at 60 ppm), 12-days (P<0.001, ANOVA; 15% at 15 ppm, 31% at 30 ppm and 42% at 60 ppm) and 16-days (P<0.001, ANOVA; 17% at 15 ppm, 31% at 30 ppm and 45% at 60 ppm) were progressively affected (Appendix PE3 B; Fig.4.30 (b)).

(C) *Westiellopsis prolifica*:

The values of SDH activity ranged from 0.22 to 0.34 μg of TTC reduced/20ml/min. After 4-days of exposure, the pencycuron treated cultures exhibited an initial period of slow inhibition (P<0.001, ANOVA) of enzyme activity by only 4% at 50 ppm, 18% at 100 ppm and 36% at 200 ppm treatment which was succeeded by a period of chronological depression (P<0.001, ANOVA) after 8-days (8% at 50 ppm, 19% at 100 ppm and 38% at 200 ppm), 12-days (10% at 50 ppm, 21% at 100 ppm and 40% at 200 ppm) and 16-days (12% at 50 ppm, 20% at 100 ppm and 41% at 200 ppm) (Appendix PE3 C; Fig.4.30 (c)).
Figure 4.30  Effect of different concentrations of pencycuron on nitrate reductase, glutamine synthetase and succinate dehydrogenase activities of cyanobacteria.
4.2.4.1 Analysis of Functional groups using Fourier Transform Infrared Spectroscopy (FTIR):

(A) *Anabaena fertilissima*:

The IR spectrum of pencycuron treated biomass showed the omission of a single peak (1703 cm⁻¹) after 4-days of exposure (Fig.4.31). The absorption wavelength at 1703 cm⁻¹ could be assigned to the C=O stretching vibration of α, β unsaturated aldehydes, ketones. Comparing the IR spectrum of three pencycuron treatments, addition of aromatic amines (C-N stretch) at 30 ppm, while carboxylic acids (O-H stretch) and 1°, 2° amines (N-H wag) at 60 ppm dose of the absorption wavelengths of 1313, 3109 and 870 cm⁻¹ respectively were observed. After 16-days, two new groups which were common in 30 ppm and 60 ppm treatment with a frequency of 1337 cm⁻¹ (aromatic amines (C-N stretch)) and 1284 cm⁻¹ (nitro compounds (N-O symmetric stretch)) were recorded, while in addition to earlier mentioned groups, alkynes (-C≡C- stretch) with wavelength of 2106 cm⁻¹ were also noticed at 30 ppm concentration of pencycuron treatment (Fig.4.32).

(B) *Aulosira fertilissima*:

Moreover, in *Aulosira fertilissima*, pencycuron treatment induced nitro compounds (N-O symmetric stretch) of 1348 cm⁻¹ at 15 ppm dose; alcohols, carboxylic acids, esters, ethers of 1313-1244 cm⁻¹ (C-O stretch) at 30 ppm and 60 ppm treatment and an exceptional presence of carboxylic acids (O-H bend) of 929 cm⁻¹ at 60 ppm was revealed at the end of 4-days (Fig. 4.33). While the results recorded after 16th day showed 1° amines (N-H bend at 1601 cm⁻¹), nitro compounds (N-O symmetric stretch at 1347 cm⁻¹) and aliphatic amines (C-N stretch at 1241 cm⁻¹) at 30 ppm and 60 ppm of pencycuron (Fig.4.34).

(C) *Westiellopsis prolifica*:

After 4-day exposure of pencycuron, new peaks when compared to control with wavelengths of 1245 cm⁻¹, 1348 cm⁻¹, and 1597- 1446 cm⁻¹ representing aliphatic amines (C-N stretch), nitro compounds (N-O symmetric stretch) and aromatics (C-C stretch (in- ring)) were generated respectively (Fig. 4.35). In all the three concentrations two new functional groups were produced with a wavelength range of 1448-1499 cm⁻¹ (aromatics (C-C stretch (in- ring))) and 1311-1346 cm⁻¹ (nitro compounds (N-O symmetric stretch)) after 16-days of incubation. Further at the highest concentration other two new groups i.e. 1° amines (N-H bend), carboxylic acid (O-H bend) and 1°, 2° amines (N-H wag) were found with wavelength of 1628 cm⁻¹, 929 cm⁻¹ and 868 cm⁻¹ respectively (Fig. 4.36).
Figure 4.31 FTIR chromatogram of pencycuron treated *Anabaena fertilissima* after 4-days of incubation.
Figure 4.32  FTIR chromatogram of pencycuron treated *Anabaena fertilissima* after 16-days of incubation.
Figure 4.33  FTIR chromatogram of pencycuron treated *Aulosira fertilissima* after 4-days of incubation.
Figure 4.34  FTIR chromatogram of pencycuron treated *Aulosira fertilissima* after 16-days of incubation.
Figure 4.35 FTIR chromatogram of pencycuron treated *Westiellopsis prolifica* after 4-days of incubation.
Results

Figure 4.36 FTIR chromatogram of pencycuron treated *Westiellopsis prolifica* after 16-days of incubation.

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An investigation of differential effects of 2,4-D and Pencycuron on three species of Cyanobacteria in-vitro – A Biochemical and Molecular approach
4.2.4.2: Biotransformation studies using Gas Chromatography–Mass Spectrometry (GC-MS):

(A) *Anabaena fertilissima*:

Chromatographic and spectroscopic analysis of the crude methanolic extracts of pencycuron treated *Anabaena fertilissima* was carried out in order to record new by-products produced during biotransformation process. The experiment conducted in the spectrum of all the treatments of pencycuron in *Anabaena fertilissima* at the end of 4-days and 16-days of incubation resulted in not a single unique compound as compared to the standard i.e. 100 ppm of pencycuron (Fig. 4.37).

B) *Aulosira fertilissima*:

The 4-day treatment of pencycuron to cyanobacterium did not yield any metabolite/by-product formation in either of the given treatments. Similar observation was recorded even at the end of the experiment (16-days) as the pencycuron treated *Aulosira fertilissima* had not generated a single unique compound when compared with the standard (Fig. 4.38).

(C) *Westiellopsis prolifica*:

The 200 ppm treatment of pencycuron on *Westiellopsis prolifica* indicated the existence of one principle compound i.e. benzoxazole after 4-days of incubation which was absent in results recorded after 16-days (Fig. 4.39). While throughout the experimental period no metabolite was formed at 50 ppm and 100 ppm pencycuron treatment.
Figure 4.37 GC-MS chromatogram of the crude extract of pencycuron treated *Anabaena fertilissima*.
Figure 4.38  GC-MS chromatogram of the crude extract of pencycuron treated *Aulosira fertilissima*.
Figure 4.39 GC-MS chromatogram of the crude extract of pencycuron treated *Westiellopsis prolifica.*
4.2.4.3 Protein profiling using SDS-PAGE:

(A) *Anabaena fertilissima:*

A total of 9 bands having molecular weights of 65 kDa, 61 kDa, 50 kDa, 48 kDa, 45 kDa, 28 kDa, 23 kDa, 24 kDa and 21 kDa were observed in control after 4-days of incubation. It is evident from the results after 4-days that there was no difference in the number of the protein bands in *Anabaena fertilissima* treated with 15 ppm, 30 ppm and 60 ppm pencycuron but band intensities were less in comparison with control cultures (Fig. 4.40 (a)). When culture was grown on 15 ppm pencycuron after 16-days 61 kDa, 28 kDa, 24 kDa, 23 kDa and 21 kDa proteins were not detected. Also, synthesis of a new set of proteins of approximately 43 kDa and 41 kDa was observed after 16-days of incubation at 30 ppm pencycuron. However, only three protein bands were visible at 60 ppm treatment after 16-days of exposure to pencycuron from which intensity of protein band of molecular weight 50 kDa was decreased as a result of pencycuron treatment (Fig. 4.40 (b)).

(B) *Aulosira fertilissima:*

The protein profiles of untreated *Aulosira fertilissima* after 4 and 16-days showed 7 protein bands of 65 kDa, 60 kDa, 50 kDa, 48 kDa, 29 kDa, 18 kDa and 16 kDa. However 4-day treatment of pencycuron to *Aulosira fertilissima* resulted in low levels of proteins of molecular weight 18 kDa and 16 kDa at 15 ppm and 60 ppm. However, expression of protein bands of size 65 kDa and 60 kDa was reduced, which were otherwise, intense at 60 ppm when compared to control and other treatments after 4-days of incubation. The intensity of one very prominent band at around 29 kDa was observed to decrease after 16-days of pencycuron exposure in all three concentrations. After 16-days in 15 ppm, 30 ppm and 60 ppm pencycuron, the protein bands of molecular weight 18 kDa and 16 kDa almost disappeared while the bands of 48 kDa and 43 kDa disappeared at only 15ppm of dose. Some of the bands that disappeared at 15 ppm of pencycuron treatment were observed to reappear at higher concentrations of pencycuron i.e. 30 ppm and 60 ppm (Fig.4.40 (d)).
(C) *Westiellopsis prolifica*:

After an interval of 4 and 16-days about 4 protein bands of 62 kDa, 59 kDa, 30.4 kDa and 14 kDa were recorded in control as well as in all the treated samples of *Westiellopsis prolifica*. The results after 4-days exposure showed that protein pattern were not severely affected, furthermore the intensity of protein bands (62 kDa, 59 kDa, 30.4 kDa and 14 kDa) were decreased within 16-days of 100 and 200 ppm pencycuron treatment (Fig.4.40 (e) and (f)). Thus exposure of pencycuron to *Westiellopsis prolifica* failed to generate any significant effect on the protein profiling patterns of the organism. These observations suggest that the decrease in the amount of protein bands is time and concentration dependent along with the tolerance of the tested organism.
Figure 4.40 Effects of pencurion on polypeptide pattern of total proteins. Lanes C, 1, 2 and 3 represent proteins extracted from control, 15ppm, 30ppm and 60ppm treated Anabaena fertilissima (a & b); control, 20ppm, 40ppm and 80ppm treated Aulosira fertilissima (c & d); control, 30ppm, 60ppm and 120ppm treated Westiellopsis prolifica (e & f), respectively.
4.2.4.4 DNA Profiling using RAPD:

(A) *Anabaena fertilissima:*

The primer OPAH-02 produced 5 amplified DNA fragments after 4-days and after 16-days of molecular size ranging from 1750 bp to 400 bp. After 4-days of incubation with pencycuron, DNA bands at 30 ppm and 60 ppm dose were slightly changed since 1500 bp band was absent and the intensity of 1300 bp band was fainted (Fig. 4.41(a)). The high concentration (60 ppm) treatment was not suited after 16-days of exposure as the disappearance of 1500 bp, 1300 bp, 700 bp, 500 bp and 200 bp of DNA fragments was observed, but 30 ppm displayed some overlap with control as only one band of 1500 bp was absent and was thus significantly different from high concentration treatment cyanobacterium (Fig. 4.41(b)).

(B) *Aulosira fertilissima:*

The primer OPG-04 specific for amplification of *Aulosira fertilissima* generated approximately 5 RAPD profiles (1900 bp to 900 bp) after 4-days and 6 profiles (1700 bp to 300 bp) after 16-days in untreated control culture. Moreover the amplified DNA concentration of *Aulosira fertilissima* was not affected by the pencycuron treatment after 4-days of exposure when compared with control (Fig. 4.41 (c)). Treatment with 60 ppm pencycuron for 16-days resulted in almost complete loss of different DNA fragments (1800 bp, 1500 bp, 1000 bp, 800 bp, 700 bp and 300 bp) thereby suggesting severe inhibitory effect on DNA; however, one DNA fragment of approximately 1000 bp was recorded irrespective of duration of pencycuron treatment to *Aulosira fertilissima* (Fig. 4.41 (d)).

(C) *Westiellopsis prolifica:*

In *Westiellopsis prolifica*, 7 amplified fragments were recorded in control cultures after 4-days (10,000-100 bp) and after 16-days (2000-700bp) for primer OPB-09. At the end of 4-days, new bands of 2500 bp, 1400 bp, 1300 bp, 950 bp, 900 bp, 600 bp and 500 bp were recorded at 100 ppm and 200 ppm dose of pencycuron (Fig. 4.41 (e)). However, after 16-days of incubation the intensity of two bands (1179 bp and 965 bp) were severely decreased at 50 ppm pencycuron and recovered at 200 ppm (Fig. 4.41 (f))
Figure 4.41 RAPD profile of pencycuron treated cyanobacterial cultures. Lane 1, 2, 3 and 4 represent control, 15ppm, 30ppm and 60ppm treated *Anabaena fertilissima* (a & b); control, 20ppm, 40ppm and 80ppm treated *Aulosira fertilissima* (c & d); control, 30ppm, 60ppm and 120ppm treated *Westiellopsis prolifica* (e & f), respectively. Lane M: Standard molecular weight DNA markers.
4.2.4.5 Sequencing of 16S rDNA Gene:

(A) *Anabaena fertilissima:*

After 16-days of incubation, no major changes were observed in the nucleotide sequence of 16S rDNA following the treatment of *Anabaena fertilissima* with pencycuron (60 ppm). Out of 1276 nucleotides, 1268 were found to be identical (99% identity) with only 3 gaps (position 730, 782 and 1189). The mismatches observed were only the purine-pyrimidine mismatches at 5 places while no purine-purine or pyrimidine-pyrimidine mismatch was observed. This suggested a feeble action of pencycuron on the genome of *Anabaena fertilissima* (Fig. 4.42).
**Figure 4.42** DNA sequence alignment of 16S rDNA sequence of control and pencycuron (60ppm; 16-days) treated *Anabaena fertilissima* using BLAST.
(B) *Aulosira fertilissima:*

DNA sequencing revealed that pencuron (60 ppm; 16-days) did not affect the 16S rDNA region in the genome of *Aulosira fertilissima,* since there were 100% identities and no gaps between the sequences of control i.e. untreated and pencuron treated cultures (Fig.4.43). The DNA sequence was observed to be exactly similar to control.
Figure 4.43 DNA sequence alignment of 16S rDNA sequence of control and pencycuron (60ppm; 16-days) treated Aulosira fertilissima using BLAST.

(C) *Westiellopsis prolifica*:

Almost no changes were observed in the nucleotide sequence of 16S rDNA after 16-days treatment of *Westiellopsis prolifica* with pencycuron (200 ppm). Out of 1280 nucleotides, 1279 were found to be exactly identical (99.9% identity) with zero gaps in between (Fig. 4.44). The only mismatch obtained was a purine-pyrimidine mismatch at position 436 where thymine in the control was replaced by an adenine in the treated cyanobacteria’s 16S rDNA sequence.

**Figure 4.44** DNA sequence alignment of 16S rDNA sequence of control and pencycuron (60ppm; 16-days) treated Aulosira fertilissima using BLAST.
Results

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Figure 4.44 DNA sequence alignment of 16S rDNA sequence of control and pencycuron (200 ppm; 16-days) treated Westiellopsis prolifica using BLAST.

4.3 Statistical analysis:

Test of significance (Student ‘t’ test) of all the parameters estimated in response to different concentrations of 2,4-D ethyl ester and pencycuron in Anabaena fertilissima, Aulosira fertilissima and Westiellopsis prolifica was studied (Appendix 1 to Appendix 12). A positive correlation was found between all the parameters except phenols since phenols showed negative correlation with all the parameters of Anabaena fertilissima when treated with 2,4-D ethyl ester (r = -0.842 to 0.972) and pencycuron (r = -0.231 to 0.982) (Appendix A and Appendix B). In Aulosira fertilissima carotenoids, phycoyanin, phycoerythrin, allophycocyanin, carbohydrates, amino acids, glutamine synthetase and succinate dehydrogenase exhibited a negative correlation with phenols and a positive correlation between each other and with other parameters after 2,4-D ethyl ester (r = -0.615 to 0.974) treatment (Appendix C). Similarly in pencycuron (r = -0.511 to 0.986) treated Aulosira fertilissima chlorophyll a, carotenoids, phycoyanin, phycoerythrin, carbohydrates and glutamine synthetase exhibited a negative correlation with phenols and a positive correlation between each other and with other parameters (Appendix D). However, under 2,4-D ethyl ester (r = -0.474 to 0.990) exposure Westiellopsis prolifica demonstrated positive correlation of phenols with proteins while negative correlation with all other tested parameters (Appendix E). The correlation matrix (r = -0.574 to 0.975) of Westiellopsis prolifica after pencycuron treatment revealed negative correlation of phenol with chlorophyll a, phycoerythrin, allophycocyanin, amino acids and glutamine synthetase (Appendix F).