Article

Studies on biodegradation and molecular characterization of 2,4-D Ethyl Ester and Pencycuron induced Cyanobacteria by using GC-MS and 16S rDNA sequencing

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Abstract

GC-MS study and molecular characterization by 16S rDNA amplification were carried out to evaluate differential effects of 2,4-D ethyl ester and pencycuron on *Anabaena fertilissima, Aulosira fertilissima* and *Westiellopsis prolifica*. Each organism has its own capacity to degrade both pesticides into various subgroups depending largely upon the main functional group of each individual pesticide. Hence, different subgroups like 2,4-D methyl ester, 2,4-D isobutyl ester, Isobutyric acid allyl ester, 3-Bromobutyric acid, 2,4-D butyl ester, Hydroxyurea, Trifluroacetic acid, 2-Methyl propyl ester, Acetic acid 2-propenyl ester and Acetic acid (2,3-dichlorophenoxy) were transformed from 2,4-D ethyl ester while Benzoxazole was the only compound generated from pencycuron treated *W. prolifica*. The results obtained by 16S rDNA sequencing confirmed that 16S rDNA region of *Anabaena fertilissima* was more affected by 2,4-D ethyl ester as there was no homology in the region of 39 basepairs, in addition, several mismatches and gaps were observed, whereas less difference in 16S rDNA was observed in case of *Aulosira fertilissima* and *W. prolific* on forth day. However, there was no significant change in the sequence of 16S rDNA pattern of all the three test organisms after 16-days of exposure to pencycuron treatment.

Keywords cyanobacteria; degradation; GC-MS; 16S rDNA sequencing; pencycuron; 2,4-D ethyl ester.

1 Introduction

The environment has been incessantly affected by the practices of agriculture, which include the extensive use of pesticides (Zhang and Pang, 2009; Zhang et al., 2011). The 2,4- dichlorophenoxyacetic acid (2,4-D) is a member of the chlorophenoxyacid herbicides and its amine and esters formulations have been used to control weeds in cereals, crops, sugar cane, fruit trees, fields and forest floor (Rodrigues et al., 1996). Pencycuron, a non-systemic protective fungicide for controlling sheath blight (*Rhizoctonia solani*) of rice (Sylvanie and Cornis, 1989), is widely used in agricultural production, particularly in Asia. Audus (1964) reported the disappearance of 2,4-D from soil within 3-4 weeks. Similarly, a reduction of 90% of 2,4-D from its original concentration in 2 weeks by a bacterial culture isolated from sewage was reported by Rosenberg and Alexander (1980). A number of 2,4-D degrading bacteria belonging to genera Pseudomonas, Streptomyces, Alcaligenes and Achromobacter have been isolated and identified (Sinton et al., 1986). Mass spectrometric

detection (MS), has inherent high selectivity or even simultaneous use of both ECD and MS detection, was carried out (Santos et al., 2000). However, information about the toxic effects of pencycuron on cyanobacteria is lacking. Kuck et al. (1988) were the first to report on the mode of action of pencycuron. Leroux et al. (1990) and Ueyama et al. (1990) presented additional information on the mode of action. However, up till now the biotransformation of pencycuron by species of cyanobacteria has not been explored.

Among prokaryotes, the photosynthetic oxygen-evolving cyanobacteria would be ideal for the treatment of effluents containing aromatic compounds since they would hasten the process of biodegradation through oxygenation and reduce BOD, unlike heterotrophic microorganisms. In addition, they possess advantages over other bacteria and green algae by their trophic independence for nitrogen as well as carbon (Carr and Whitton, 1982). There are reports on the cyanobacterial degradation of aromatic hydrocarbons (Narro et al., 1992) and xenobiotics (Kurtitz and Wolk, 1995).

Marine cyanobacteria oxidize naphthalene, biphenyl, and 1- and 2-methylnaphthalene to form 1-naphthol, 4-hydroxybiphenyl, and 1- and 2-hydroxymethylnaphthalene, respectively, as the major metabolites (Cerniglia et al., 1983). Jinqi and Houtian (1992) investigated the degradation of azo dyes by *Chlorella vulgaris* and *Chlorella pyrenoidosa* and found that certain dyes, such as Eriochrome blueSE and blackT, could be decolorized and actually used as carbon and nitrogen sources, but this was dependent on the chemical structure of the dyes. However, the role of cyanobacteria (blue-green algae) in biotransformation of chemicals has not been thoroughly investigated despite their wide distribution in aquatic ecosystems.

Prokaryote genomes are small and compact. This applies to cyanobacteria as well, in which known genome sizes vary between 1.6 Mbp (*Prochlorococcus marinus* MIT 9301) to 9.01Mbp (*Nostoc punctiforme* PCC 73102) (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). Insufficient genetic information on cyanobacteria is available. Cyanobacteria possess a single circular chromosome and may have one or more plasmids (Kaneko and Tabata, 1997). In cyanobacteria the size of the genome reflects the number of genes. By August 2008, 34 cyanobacterial genomes had been completely sequenced and many sequencing projects are currently in progress (http://bacteria.kazusa.or.jp/cyanobase/). Nirmal Kumar et al. (2011) examined the impact of 2,4-D ethyl ester and pencycuron in inducing DNA damage in three species of cyanobacteria-*Anabaena fertilissima*, *Aulosira fertilissima*, and *Westiellopsis prolifica* as evidenced by PCR-based assays: RAPD and 16S rRNA amplification. Actual genetic characterization, however, often rely on DNA sequencing, most commonly of the 16S rDNA gene (Comte et al., 2007). Priya et al. (2006) studied degradation of lignin model dye Poly R-478 and organophosphorous pesticide by *L. valderiana* BDU 140441 and azo dyes (orange G) by *L. valderiana* BDU 20041 signifying the genetic differences leading to the adaptability of strains to various environmental conditions. Asadi et al. (2011) investigated the influence of microwave radiation on *Phormidium* sp. Kutzing ISC31 (Oscillatoriales) and the result of PCR, when blasted with sequenced cyanobacteria in NCBI, showed 97% homology to the 16S rDNA.

Thus the objective of this study was to investigate the effect of 2,4-D ethyl ester and pencycuron on biodegradation or detoxicants and molecular characterization of *Anabaena fertilissima*, *Aulosira fertilissima* and *W. prolifica* by using GC-MS and 16S rDNA sequencing.

2 Materials and Methods

2.1 Cyanobacterial cultures

The axenic cultures of nitrogen-fixing cyanobacteria, viz., *Anabaena fertilissima*, *Aulosira fertilissima* and *W. prolifica* were obtained from the National Facility for Blue Green Algal Collections, IARI, New Delhi. The cyanobacteria were grown under controlled illumination of 40μEm-2s-1 at 27±1°C in a nitrogen-free BG_{11} liquid medium at pH 7.0±0.2 under aerobic and static conditions. All inoculations were carried out under

aseptic conditions and the cultures were periodically checked for any contamination. Only axenic cultures were used for experimental studies.

2.2 Pesticides

The pesticides chosen for the study were 2,4-D (38% EC 2,4-D ethyl ester) and Monceren (22.9% SC Pencycuron) obtained from Northern minerals limited, Haryana and Bayer CropScience limited, Mumbai respectively. Three concentrations for each pesticide were selected for the present investigation to analyze the response of *Anabaena fertilissima*, *Aulosira fertilissima* and *W. prolifica* after the determining LC_{50}. One LC_{50} concentration, another concentration is lower and the third concentration is higher to LC_{50} concentration were selected. Stock solution (200 µg ml^{-1}) of both the pesticide were prepared in sterilized double-distilled water and added aseptically to the culture medium to the final concentrations indicated for each treatment.

2.3 Analytical method for GC-MS

The crude methanolic extracts of three selected cyanobacterial species were subjected to GC-MS (Aseer et al., 2011). GC-MS analysis was performed using a Perkin Elmer AutoSystem XL GC apparatus attached to a PE-5MS fused silica capillary 5% diphenyl/95% dimethylpolysiloxane column (30 m x 50 m, 0.25 µm film thickness, Perkin Elmer). The column temperature was initially 80 ºC, held for 5 min, then ramped from 80-290 ºC at 10ºC/min. Helium (1.0 ml/min) was used as the carrier gas. Line and injector temperature were set at 250 ºC and 250 ºC, respectively.

Samples (1 µl) were injected in the split mode (1:40). MS conditions were run in EI+ through a Perkin Elmer TurboMass mass spectrometer as follows: ionization energy -70 eV; scan rate 1.6 scans/sec; interscan delay 0.01 sec; source temperature 250 ºC; mass range 30 to 650 m/z; solvent delay 3.00 min. Data were obtained by comparing spectra to those in the Wiley NIST/EPA/ NIH Mass Spectral Library 2005.

2.4 DNA extraction, PCR amplification and sequence analysis of 16S rDNA

The genomic DNA was extracted using the method described by Sambrook et al. (2001). The PCR reaction was performed with universal primers (16S Forward Primer: 5'-AGAGTRTGATCMTYGCTWAC-3' and 16S Reverse Primer: 5'-CGYTAMCTTWTTACGRCT-3') specific for the 16S rRNA gene (Iteman et al., 2000). PCR products were purified using gel extraction kit (Chromous Biotech Pvt. Ltd., Bangalore). After purification, PCR products of the 16S rDNA (1,500 bp PCR products were amplified) were directly sequenced at a commercial facility (Chromous Biotech Pvt. Ltd., Bangalore). The sequencing reaction was set up using Big Dye Terminator version 3.1 Cycle sequencing kit. The 10 µl of reaction mixture for sequencing PCR was made which consisted of 4.0 µl of big dye terminator ready reaction mix, 1.0 µl of Template (100 ng/µl), 2.0 µl of primer (10 pmol/λ) and 3.0 µl of milli Q water. The PCR conditions were as mentioned: initial denaturation (96°C for 1 min.) followed by 25 cycles of denaturation at 96°C for 10 sec, hybridization at 50°C for 5 sec and elongation at 60°C for 4 min. The amplified fragments were loaded onto Applied Biosystem Micro Amp Optical 96-Well Reaction plate of ABI 3500 XL Genetic Analyzer having POP_7 polymer and 50 cm Capillary Array. The analysis protocol followed was BDTv3-KB-Denovo_v 5.2 and the data was analyzed using Seq Scape_ v 5.2 software. The sequence data was analyzed by similarity search using the BLAST tool available at the website of the NCBI.

3 Results and Discussion

3.1 Biodegradation study using Gas Chromatography–Mass Spectrometry (GC-MS)

Ma and Chen (2005) suggested that different algal species have different sensitivity to herbicides, and responses vary widely depending on the species tested and the concentrations used. Differences in the uptake of this type of herbicides by microalgal cells result from a complex interaction between several metabolic factors (Weiner et al., 2004). Among different microalgae, cyanobacteria have been shown to be very effective

as accumulators and degraders of different kinds of environment pollutants, including herbicides (El-Bestawy et al., 2007).

Microbial degradation of 2,4-D ethyl ester and Pencycuron was observed for 4 days and 16 days. *Anabaena fertilissima* after 2,4-D exposure produced 2,4-D butyl ester at 60ppm after 4-days while Isobutyric acid allyl ester and 3-Bromobutyric acid were recorded at 30 and 60ppm respectively (Fig. 1) after 16-days. Similar observation was also made by Kurtitz and Wolk (1995) and Kuritz et al. (1997) that *Anabaena* sp. PCC 7120 shows degradation of lindane, yielding 2,3,4,5,6-pentachloro-1-cyclohexene, 1,2,3- and 1,2,4-trichlorobenzene. Cerniglia et al. (1980) reported that cyanobacteria can degrade man-made xenobiotics to produced α-naphthol from naphthalene by *Oscillatoria* sp. strain JCM. *Aulosira fertilissima* generated a new compound –Hydroxyurea at 80ppm after 4-days whereas Trifluroacetic acid, 2-Methyl propyl ester and Acetic acid 2-propenyl ester were recorded after 16-days in all the three treatments when compared to 2,4-D standard (Fig. 2). An induction of new detoxicants such as 2,4-D methyl ester and Acetic acid (2,3-dichlorophenoxy) were observed at the highest concentration (120ppm) of 2,4-D treated *W. prolifica* after 4 days and 16-days of exposure (Fig. 3). Jeong-Hun et al. (2001) showed that 2,4-D could be degraded both in liquid phase and in the sorbed state.

After 4-days and 16-days of incubation not a single unique compound was generated in the spectrum of all the treatments of Pencycuron in *Anabaena fertilissima* (Fig. 4) and *Aulosira fertilissima* (Fig.5) as compared to the standard. However, 200ppm treatment of pencycuron on *W. prolifica* produced the existence of one new compound- Benzoxazole after 4-days of incubation which was absent in results recorded after 16-days (Fig. 6). Evans et al. (1971) discussed the ability of certain microbes to degrade aromatic-ring-containing pesticides synthesized by man, that convert these strange compounds into simple aliphatic molecules, suitable for funneling into the respiratory cycles of the cell. Microbial halometabolites have been discussed by Petty (1961). Ando et al. (1970) have claimed that a soil *Penicillium* sp. biosynthesizes 2,4-dichlorophenol and some unidentified derivatives.

3.2 Effect of pesticide on 16S rDNA sequence

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. 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. It was observed that 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. 7). However, after 16-days of incubation, no major changes were observed in the nucleotide sequence of 16S rDNA of *Anabaena fertilissima* following the treatment of pencycuron (60 ppm). Out of 1276 nucleotides, 1268 were found to be identical (99% identity) with only 3 gaps (position 730, 782 and 1189). This suggested a meager action of pencycuron on the genome of *Anabaena fertilissima* (Fig. 8).

Considerable 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 which suggesting a 17% differences in the sequence after treatment and along-with this, 5% gaps were also registered (Fig. 9). DNA sequencing revealed that pencycuron (60 ppm; 16-days) did not affected 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 pencycuron treated cultures (Fig. 10).

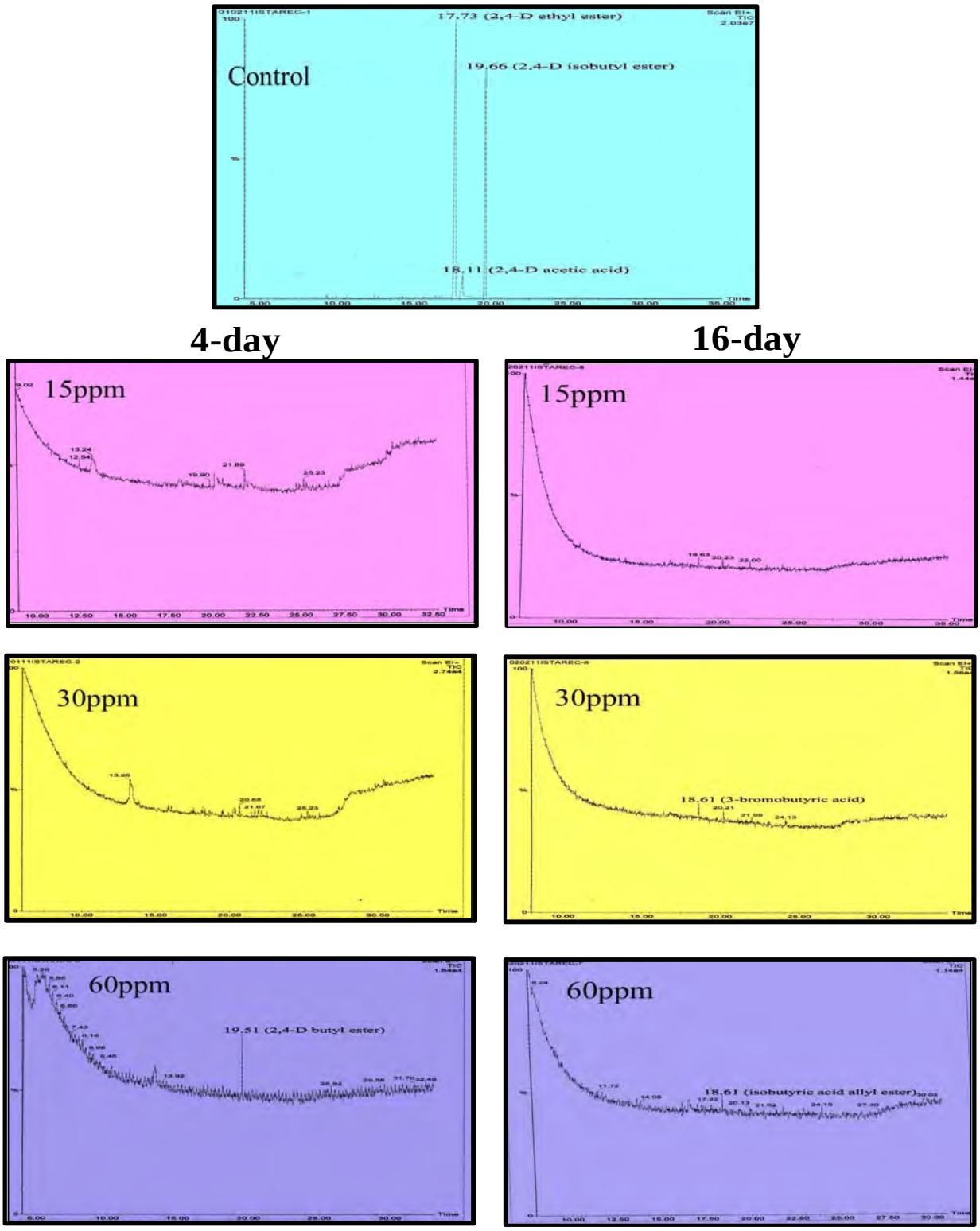

Fig. 1 GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated *Anabaena fertilissima*.

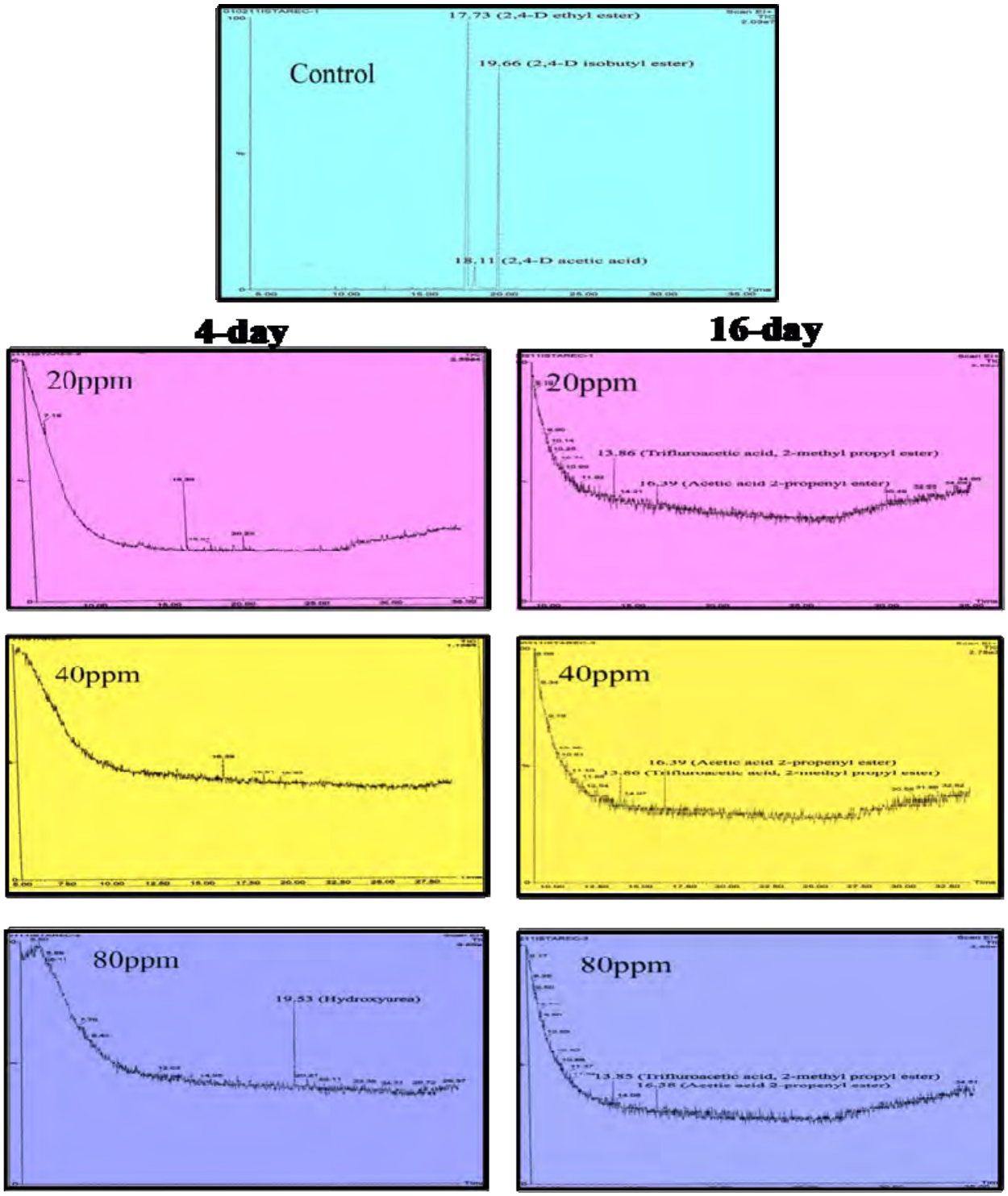

Fig. 2 GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated *Aulosira fertilissima*.

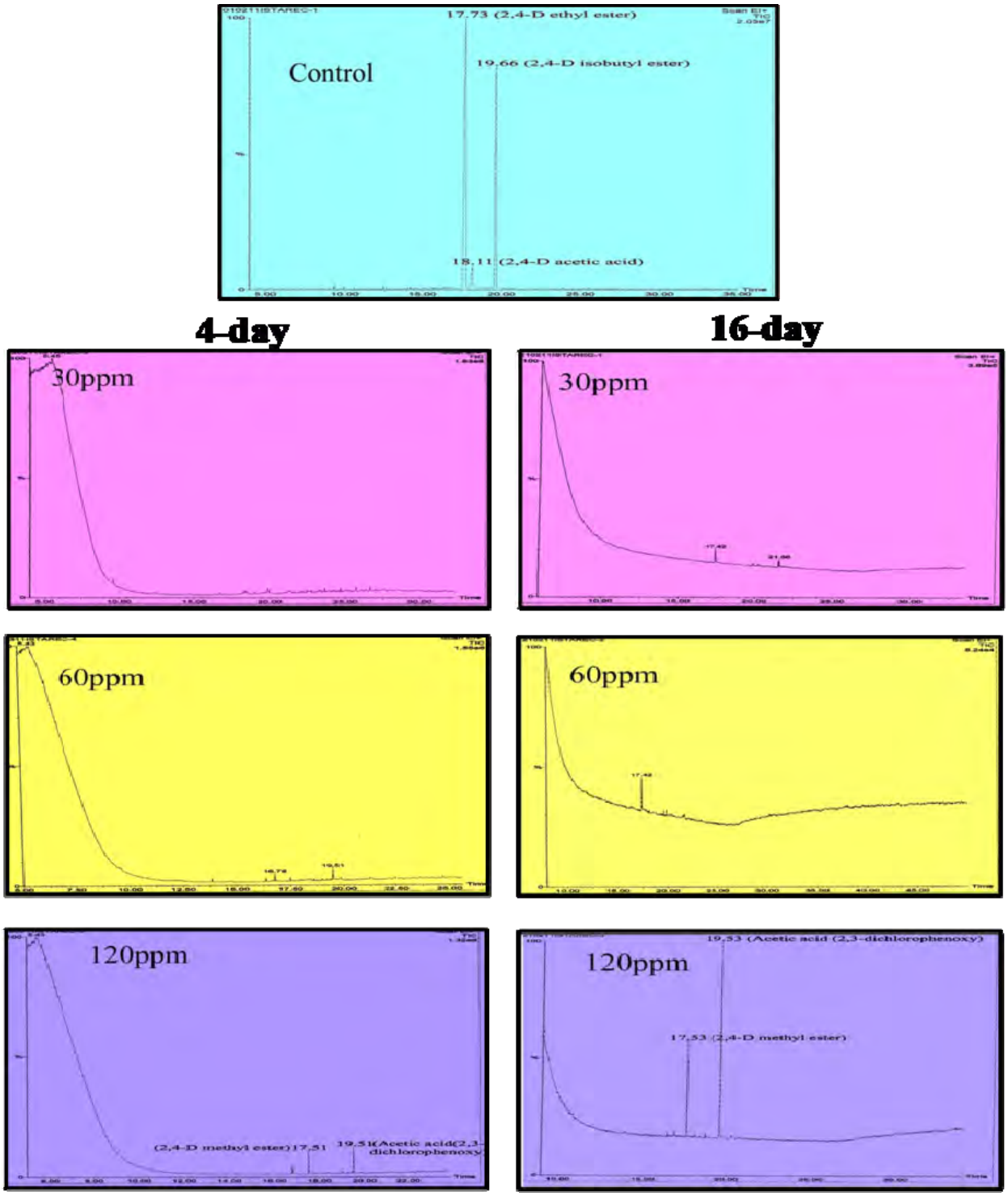

Fig. 3 GC-MS chromatogram of the crude extract of 2,4-D ethyl ester treated *Westiellopsis prolific*.

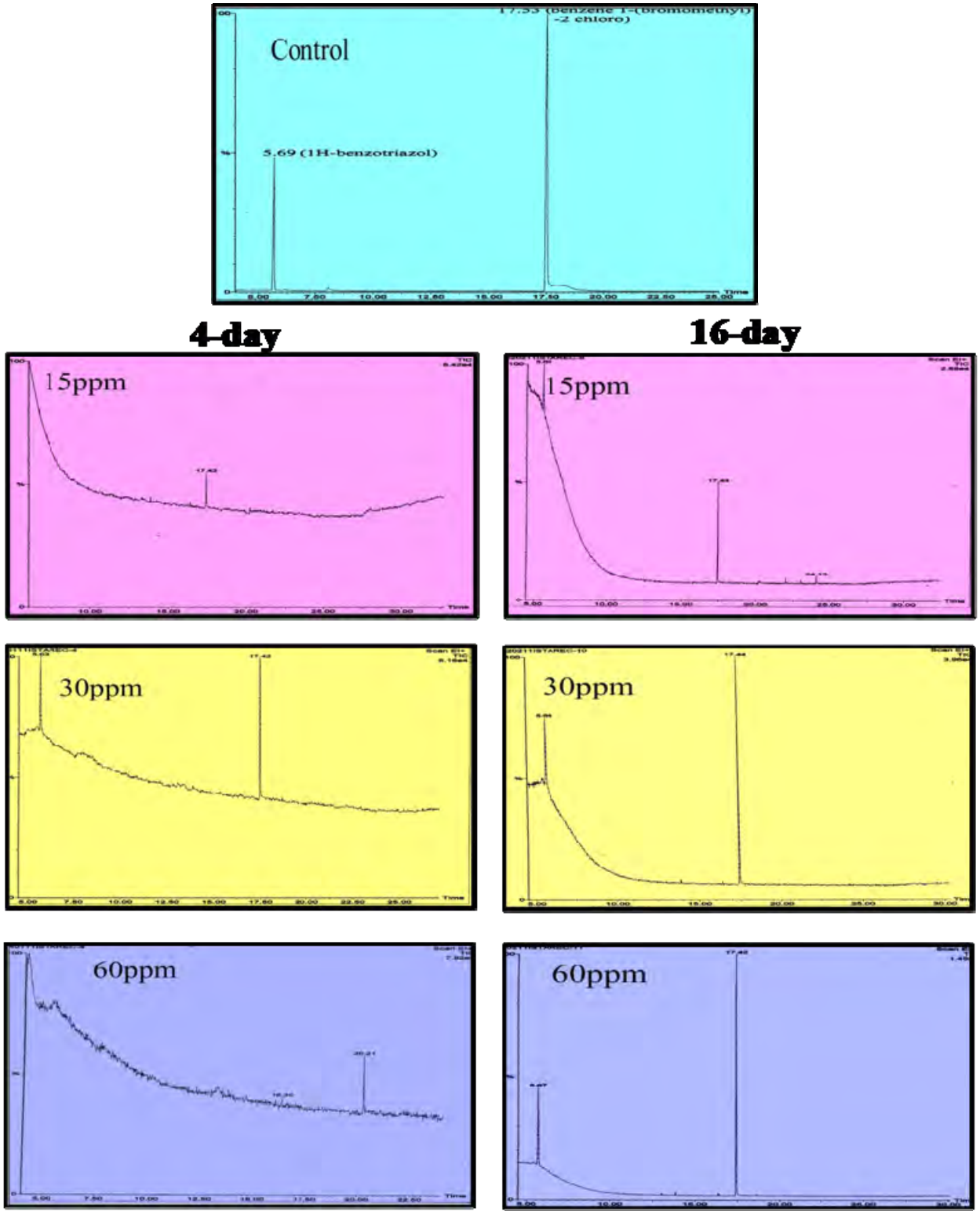

Fig. 4 GC-MS chromatogram of the crude extract of pencycuron treated *Anabaena fertilissima*.

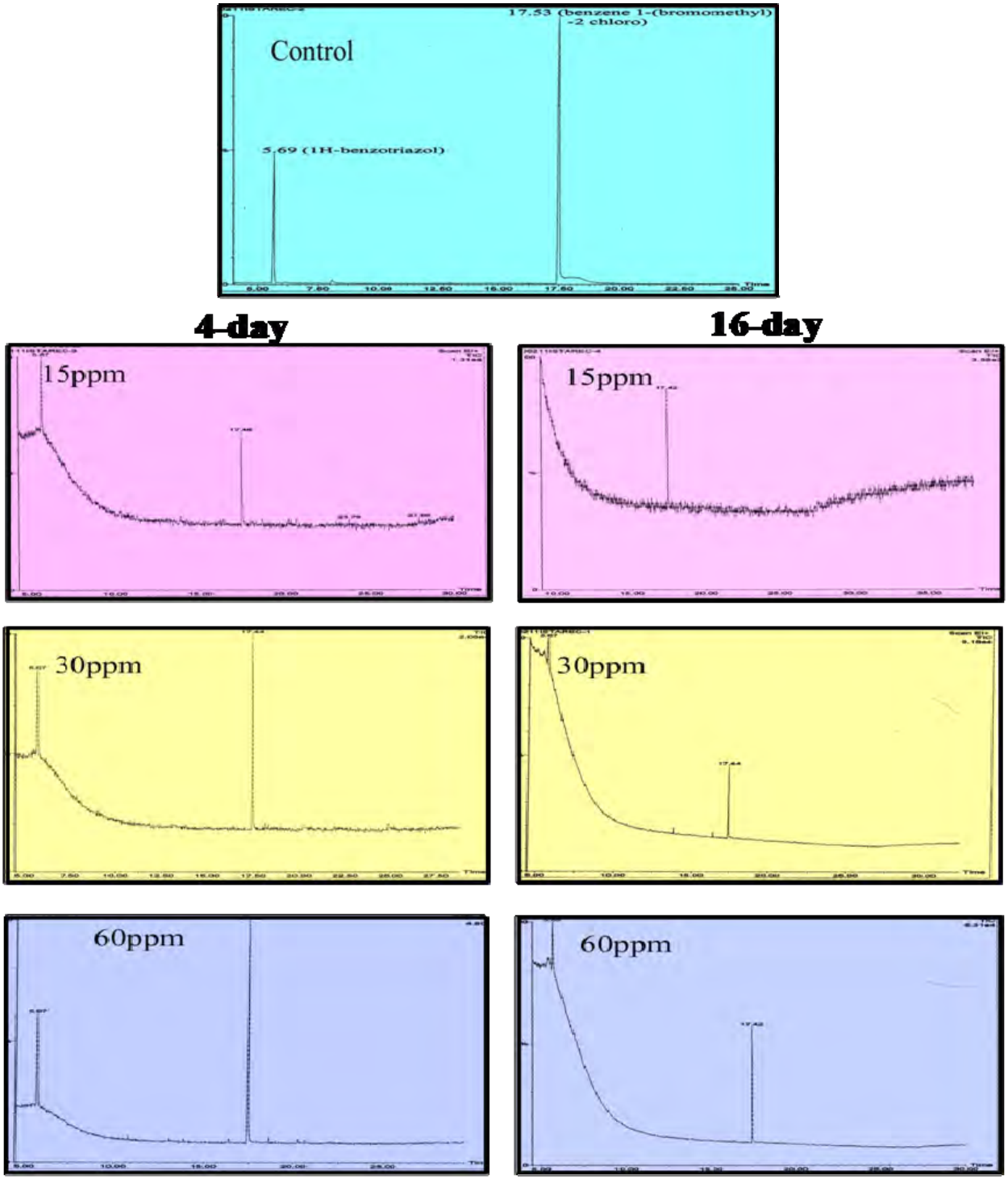

Fig. 5 GC-MS chromatogram of the crude extract of pencycuron treated *Aulosira fertilissima*.

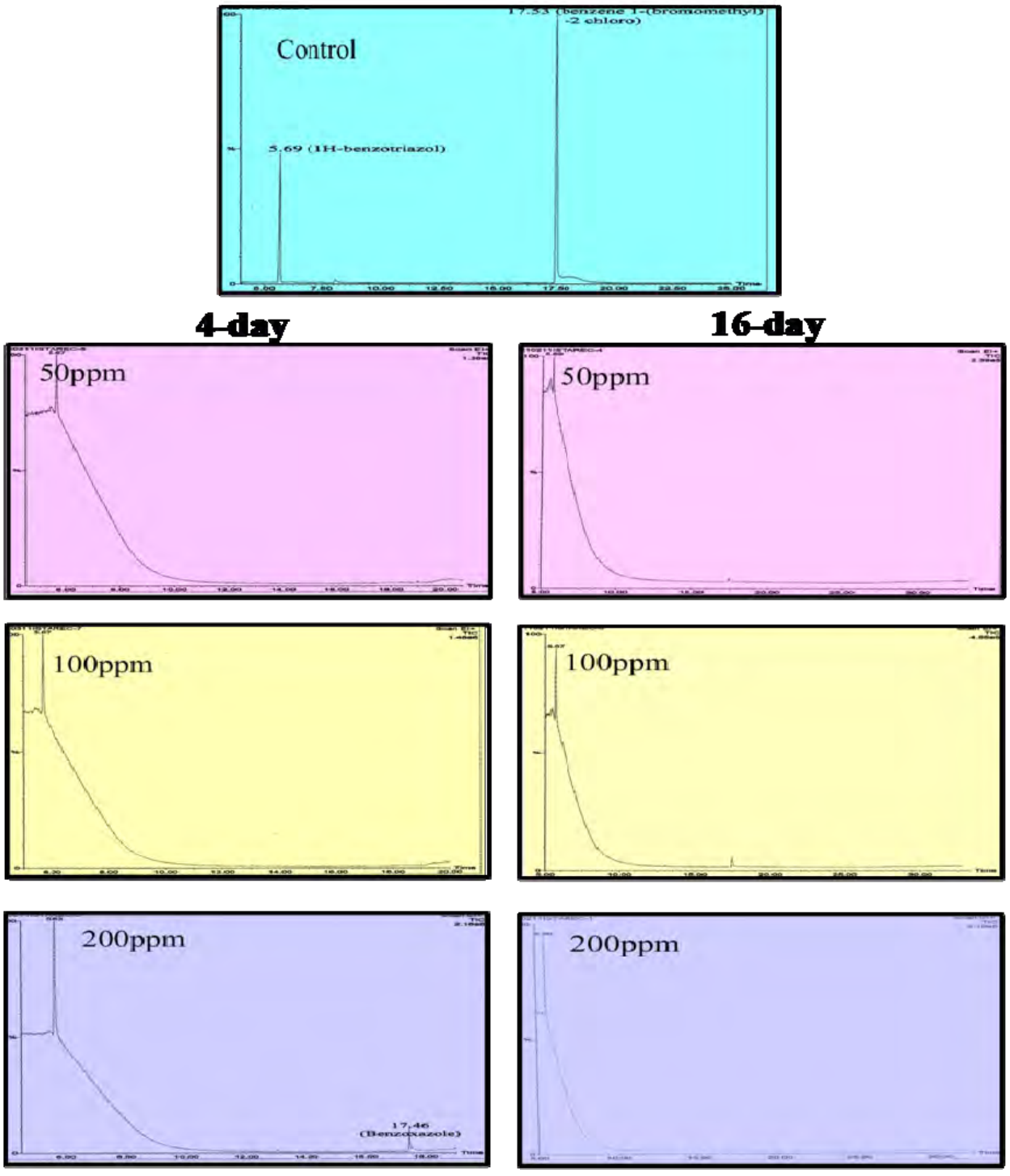

Fig. 6 GC-MS chromatogram of the crude extract of pencycuron treated *Westiellopsis prolific.*

Blast 2 sequences

An_Blast_Control_and_60ppm_2,4-D

Query ID	lcl|31255	**Subject ID**	31257
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	1159	**Subject Length**	1130
		Program	BLASTN 2.2.25+ ▸Citation

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 Identities = 191/203 (94%), Gaps = 0/203 (0%)
 Strand=Plus/Plus

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Query  61   AGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG  120
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Sbjct  121  TGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGG  180

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Sbjct  181  CCTTAGGGTTGTAAAGCTCTTTT  203


 Score =  806 bits (436),  Expect = 0.0
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 Strand=Plus/Plus

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Query  302   AGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGT  361
            |||| || |||||| |||||||| |||||||||||| | ||||||| || | |||||||
Sbjct  277   AGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGCGTTTTAAGT  336

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Query  420   TGTGGTAGAGGGAT-GCGGAATTTCTG-GTGTAGCA-GTGAAATGCGTAGAGA-TCAG-A  474
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Query  475   AGGAACATCC-GTGGCGAAGGCGG-CATCCTGGGCCAACACTGACACTGAGGCACGAAAG  532
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Query  533   CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTG-  591
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Query  646   CCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG  705
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Query  879   TTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACC  938
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Sbjct  969   TGCTACAATGGCGGTGACAGTGGGAGGCGAAGGAGCGATC-TGGAGCAAATCCCCA-AAA  1026

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Query  1114  ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA  1159
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Sbjct  1086  ATCGTGGATCAGCAT-GCCACGGTGAATACGTTCCCGGGCCTTGTA  1130
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Fig. 7 DNA sequence alignment of 16S rDNA sequence of control and 2,4 D ethyl ester (60ppm; 16-days) treated *Anabaena fertilissima* using BLAST.

Blast 2 sequences

An_Blast_Control_and_60ppm_Pen

Query ID	lcl|46801	**Subject ID**	46803
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	1273	**Subject Length**	1276
		Program	BLASTN 2.2.25+ ▷Citation

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 Strand=Plus/Plus

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Query  361   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT  420

Query  421   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  480

Query  481   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  540

Query  541   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  600

Query  601   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  660
```

```
Query  661   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  720

Query  721   TTGGCACCC-AGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAA  779
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   TTGGCACCCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAA  780

Query  780   GA-CTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAAT  838
             || ||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  781   GAACTGAAACTCAAAGGAATTGACGGGGGCCCGCCCAAGCGGTGGAGTATGTGGTTTAAT  840

Query  839   TCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGA  898
             | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  841   TAGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGA  900

Query  899   TTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGA  958
             |||||||||||||||||||||||||||||| |||||||| ||||||||||||||||||||
Sbjct  901   TTGGTGCCTTCGGGAACCGTGAGACAGGTGATGCATGGCGGTCGTCAGCTCGTGTCGTGA  960

Query  959   GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATG  1018
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATG  1020

Query  1019  GTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1078
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1021  GTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1080

Query  1079  CATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGC  1138
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1081  CATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGC  1140

Query  1139  AAACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTG-AGTCTGCAA  1197
             |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  1141  AAACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAA  1200

Query  1198  CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC  1257
             |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  1201  CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGATGCGGTGAATAC  1260

Query  1258  GTTCCCGGGCCTTGTA  1273
             ||||||||||||||||
Sbjct  1261  GTTCCCGGGCCTTGTA  1276
```

Fig. 8 DNA sequence alignment of 16S rDNA sequence of control and pencycuron (60ppm; 16-days) treated *Anabaena fertilissima* using BLAST.

Blast 2 sequences

Aul_Blast_Control_and_80ppm_2,4-D

Query ID	lcl|53777	**Subject ID**	53779
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	626	**Subject Length**	626
		Program	BLASTN 2.2.25+ ▶Citation

```
Score =  547 bits (296),  Expect = 5e-160
 Identities = 532/642 (83%), Gaps = 32/642 (5%)
 Strand=Plus/Plus

Query  1    ATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA  60
            ||||||||||||   |||| ||||  ||||||||| ||||||||||||||||||||||||
Sbjct  1    ATGACGGTACCTGCAGAAGAAGCTGCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT  60

Query  61   AGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGT  120
            |||  |||||||||  |||||||||||||||||||| ||||||||| ||| |  |||||
Sbjct  61   AGGCAGCAAGCGTTGTTCGGAATTACTGGGCGTAAAGAGTGCGTAGGCGGTTGACTAAGT  120

Query  121  CTGCTGTGAAAGC-CCTGGGCTCAACCTGGGA-ATTGCAG-TGGATACTGGATCACTAGA  177
             || ||||||| | ||  |||| || ||||||   ||| |  | | ||||| |  |||||
Sbjct  121  TTGGTGTGAAATCTCC-CGGCTTAA-CTGGGAGGGTGC-GCCGAAAACTGGTTGGCTAGA  177

Query  178  GTGTGGTAGAGGGAT-GCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGG  236
            |||||| |||||| | | |||||| |||||||||| |||||||||||||| ||||| |||
Sbjct  178  GTGTGGGAGAGGG-TAGTGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGG  236

Query  237  AACATCC-GTGGCGAAGGCGGCAT-CCTGGGCCAACACTGACACTGAGGCACGAAAGCGT  294
            |||| || |||| | || |||| | ||||| |||  |||||| ||||||||||||||||||
Sbjct  237  AACA-CCGGTGGTGTAGACGGC-TACCTGGACCATTACTGACGCTGAGGCACGAAAGCGT  294

Query  295  GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACT-GGAT  353
            |||||||||||||||||||||||||||||||||||||||||||| |||| |||| || |
Sbjct  295  GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACAATGCAAACTTGG-T  353

Query  354  GTTGGGTGCAAC-TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGA  412
            || | |  |  | ||||   |  ||  || |||||||||||||||| ||||||||||||
Sbjct  354  GT-GCGCCCTTCATTGGGTGC--GTGCCGTAGCTAACGCGTTAAGTTTGCCGCCTGGGGA  410


Query  413  GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA  472
            |||||||||||||| |||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  411  GTACGGTCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  470

Query  473  TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGA--A-C  529
            ||||||||||||||| |||||||||||||||||||| || || | |  | ||  | |
Sbjct  471  TGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCTGGGCTCGA-A-CGGCTGATCAAC  528

Query  530  TTTCC-AGAGATGGATTGGTGCCTTCGGGAACCGTG---AGAC-AGGTGCTGCATGGCTG  584
              ||  ||| ||  |  |||  || ||  ||  | |   || | ||||||||||||||||
Sbjct  529  GATCGTAGAAAT--AC-GGTTACTCCGC-AAGGGGGTTCAGTCGAGGTGCTGCATGGCTG  584

Query  585  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC  626
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  585  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC  626
```

Fig. 9 DNA sequence alignment of 16S rDNA sequence of control and 2,4 D ethyl ester (80ppm; 16-days) treated *Aulosira fertilissima* using BLAST.

Blast 2 sequences

AUL_Blast_Control_and_60ppm_Pen

Query ID	lcl|44905	**Subject ID**	44907
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	877	**Subject Length**	877
		Program	BLASTN 2.2.25+ ▸Citation

```
Score = 1620 bits (877),  Expect = 0.0
 Identities = 877/877 (100%), Gaps = 0/877 (0%)
 Strand=Plus/Plus

Query  1    ATGAGCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGAGCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGT  60

Query  61   AGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG  120

Query  121  GAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGG  180

Query  181  GTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAAT  240

Query  241  ACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCG  300

Query  301  CGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTG  360

Query  361  GTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGA  420


Query  421  TCACTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGAT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TCACTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGAT  480

Query  481  CAGAAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CAGAAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAA  540

Query  541  AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACT  600

Query  601  GGATGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGG  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  GGATGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGG  660
```

```
Query  661  GGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661  GGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA  720

Query  721  GTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAC  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721  GTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAC  780

Query  781  TTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTC  840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781  TTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTC  840

Query  841  AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC  877
            |||||||||||||||||||||||||||||||||||||
Sbjct  841  AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC  877
```

Fig. 10 DNA sequence alignment of 16S rDNA sequence of control and pencycuron (60ppm; 16-days) treated *Aulosira fertilissima* using BLAST.

Blast 2 sequences

West_Blast_Control_and_120ppm_2,4-D

Query ID	lcl|1941	**Subject ID**	1943
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	1149	**Subject Length**	1151
		Program	BLASTN 2.2.25+ ▷Citation

```
Score = 2025 bits (1096), Expect = 0.0
 Identities = 1133/1151 (98%), Gaps = 2/1151 (0%)
 Strand=Plus/Plus

Query  1     GGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTA  60
             |||||||||||||||||| ||||||||||||||||||||| ||||||||||||||||||
Sbjct  1     GGTGAGGAATACATCGGATTCTACCTTTTCGTGGGGGATAAAGTAGGGAAACTTACGCTA  60

Query  61    ATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGA  120
             ||||||||||||||||||||||    |||||||||||||||||||||||||||||| ||||
Sbjct  61    ATACCGCATACGACCTACGGGTCCTTGTGGGGGACCGCAAGGCCTCACGCGATTACATGA  120

Query  121   GCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCT  180
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   GCCGATGTCCGATTATCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCT  180

Query  181   GGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   GGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG  240

Query  241   CAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGA  300
             |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  241   CAGCAGTGGGGAATATTGGACAATGGGCGCAAACCTGATCCAGCCATACCGCGTGGGTGA  300

Query  301   AGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   AGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTC  360

Query  361   GGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT  420
             |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  361   GGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCACCAGCCGCGGT  420

Query  421   AATACGAAGGGTGCATGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGG  480
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421   AATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGG  480

Query  481   TTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGG-GAATTGCAGTGGATACTGGATCA  539
             |||||||||||||||||||||||||||||||||||  ||||||||||||||||||||||
Sbjct  481   TTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGCTAATTGCAGTGGATACTGGATCA  540

Query  540   CTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAG  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   CTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAG  600

Query  600   AAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGC  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601   AAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGC  660
```

```
Query  660   GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGA  719
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGA  720

Query  720   TGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGA  779
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   TGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGA  780

Query  780   GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA  839
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781   GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA  840

Query  840   TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACG-GAACTT  898
              ||||||||||||||||||||||||||||||||||||||||||||||||||  |  ||||||
Sbjct  841   TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCTTCTTGAACTT  900

Query  899   TCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAG  958
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  901   TCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAG  960

Query  959   CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGC  1018
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGC  1020

Query  1019  CAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGG  1078
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1021  CAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGG  1080

Query  1079  ATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGG  1138
              |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  1081  ATGACGTCAAGTCATCATGGCCCTTACTACCAGGGCTACACACGTACTACAATGGTGGGG  1140


Query  1139  ACAGAGGGCTG  1149
              ||||||||||| 
Sbjct  1141  ACAGAGGGCTG  1151
```

Fig. 11 DNA sequence alignment of 16S rDNA sequence of control and 2,4 D ethyl ester (120ppm; 16-days) treated *Westiellopsis prolifica* using BLAST.

Blast 2 sequences

West_Blast_Control_and_200ppm_Pen

Query ID	lcl|55877	**Subject ID**	55879
Description	None	**Description**	None
Molecule type	nucleic acid	**Molecule type**	nucleic acid
Query Length	1280	**Subject Length**	1280
		Program	BLASTN 2.2.25+ ▷Citation

```
Score = 2359 bits (1277), Expect = 0.0
 Identities = 1279/1280 (99%), Gaps = 0/1280 (0%)
 Strand=Plus/Plus

Query  1     GGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     GGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTA  60

Query  61    ATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    ATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGA  120

Query  121   GCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   GCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCT  180

Query  181   GGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   GGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG  240

Query  241   CAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241   CAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGA  300

Query  301   AGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   AGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTC  360

Query  361   GGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361   GGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT  420

Query  421   AATACGAAGGGTGCATGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGG  480
             |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421   AATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGG  480

Query  481   TTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCAC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481   TTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCAC  540

Query  541   TAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGA  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   TAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGA  600

Query  601   AGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601   AGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCG  660
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Query  661   TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGAT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGAT  720

Query  721   GTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAG  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   GTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAG  780

Query  781   TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTAT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781   TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTAT  840

Query  841   GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTC  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  841   GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTC  900

Query  901   CAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCT  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  901   CAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCT  960

Query  961   CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCA  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCA  1020

Query  1021  GCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGAT  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1021  GCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGAT  1080

Query  1081  GACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGAC  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1081  GACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGAC  1140

Query  1141  AGAGGGCTGCAAACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTG  1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1141  AGAGGGCTGCAAACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTG  1200

Query  1201  GAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTG  1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1201  GAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTG  1260

Query  1261  CGGTGAATACGTTCCCGGGC  1280
             ||||||||||||||||||||
Sbjct  1261  CGGTGAATACGTTCCCGGGC  1280
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Fig. 12 DNA sequence alignment of 16S rDNA sequence of control and pencycuron (200ppm; 16-days) treated *Westiellopsis prolifica* using BLAST.

Even at the end of experiment (16-days), very minor dissimilarities were observed in the 16S rDNA sequence of *W. prolifica* cultures when treated with 2,4-D ethyl ester at a concentration of 120 ppm, as compared to other two selected organisms, with respect to its untreated control. There were 1133 similarities out of total 1151 basepairs which denotes 98% identities and 2 gaps between the sequences of control and pesticide treated cultures (Fig. 11). On the other hand no changes were observed in the nucleotide sequence of 16S rDNA after 16-days treatment of *W. 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. 12).

Similarly Widenfalk et al. (2008) studied the effects of pesticides i.e. captan, glyphosate, isoproturon and pirimicarb at environmentally relevant and high concentrations on sediment microorganisms. In the case of 16S rRNA gene, distinct environmental conditions such as salinity and rocky shores rich in organic matter have contributed to the genetic relatedness leading to discernible ecological trends among the isolates as observed by Miller et al. (2007). In current study, 16S rDNA sequence of 2,4-D ethyl ester treated cyanobacterial showed 6% gap in *Anabaena fertilissima*, 5% gap in *Aulosira fertilissima* and no gap in *W. prolifica*. The gaps in the two sequences results from mismatching of purines and pyrimidines, depurination or mismatching of nitrogen bases, formation of dimeric products, single strand breaks and double strand breaks.

According to DeSantis et al. (2006) alignments are useful when gaps have been appropriately added to mark an inference of an insertion or deletion event where one sequence has a base while another sequence lacks a base at the corresponding position. However, after pencycuron treatment no gaps were found in the sequence alignment of three selected cyanobacterial species. Similarly, Mylvaganam and Dennis (1992) analyzed two nonadjacent ribosomal RNA operons, designated rrnA and rrnB, in *Haloarcula marismortui*. The 16S rRNA genes within these operons were 1472 nucleotides in length and there were no nucleotide gaps in the alignment of the two sequences; however, the two 16S sequences differ by nucleotide substitution at 74 positions which were randomly distributed. The experiments of Han and Hu (2007) suggested that 16S rRNA structures of the desiccation-tolerant *Nostoc* strains were more stable than that of planktonic *Nostocaceae* species. The adaptive strategies included replacement of GC with other types of base pairs in the DNA sequence. However, the stability and biophysical properties of macromolecules inside desiccated cells are still poorly understood.

4 Conclusion

This study revealed that various concentrations of 2,4-D ethyl ester and pencycuron could induce different physiological effects like alterations in degradation of pesticide and 16S rDNA sequence, of cyanobacteria. Through GC-MS profiles, it can be inferred that *Anabaena fertilissima* is prominent among other two test organisms in reacting with and transforming 2,4-D ethyl ester. While this was further confirmed by sequencing of 16S rDNA gene, since 2,4-D ethyl ester treated cultures showed greater changes in 16S rDNA region than pencycuron treated cultures.

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