Article

Toxicity analysis of pesticides on cyanobacterial species by 16S rDNA molecular characterization

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Abstract

Damaging effects of endosulfan on native structure of DNA, evident as a result of PCR based assay such as 16S rDNA amplification and sequencing, led to formation of gaps, mismatching of base pairs and dissimilarities in entire 16S rDNA sequences of treated cultures. Endosulfan was the most fatal to *Westiellopsis prolifica* of 16S rDNA region at 40ppm insecticide induced series of mispairing, and other lesions amounting up to 20% dissimilarity and 7% gaps. Whereas, 16S rDNA region of *Anabaena fertilissima* was comparatively less influenced with 18% dissimilarity and 7% gaps in response to 12ppm endosulfan, while 16S rDNA gene of *Aulosira fertilissima* was the least prone to changes with 17% dissimilarity, and 5% gaps under 60ppm endosulfan stress by the end of 16 days. On the other side, impact of fungicide tebuconazole after 16 days reflected identities up to 78% and 8% gaps for 30ppm treated *A. fertilissima*, while 60ppm treatment instilled 79% similarities with 10% gaps in *W. prolifica* and 83% identities with 5% gaps of *Aulosira fertilissima* after 16 days.

Keywords cyanobacteria species; endosulfan; tebuconazole; 16s rDNA molecular characterization.

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1 Introduction

Cyanobacteria are among the most known widespread, morphologically distinct and abundant prokaryotes. They are oxygenic photosynthetic autotrophs, originally considered as a class of algae, the blue-green algae possessing a unique ability in fixing atmospheric nitrogen (Holt et al., 1994). With an extraordinary biosynthetic potential and a repertoire of diverse metabolic activities, they are one of the dominant genera in various ecological habitats, especially in rice fields. *Anabaena fertilissima*, *Aulosira fertilissima and Westiellopsis prolifica*, photoautotrophic cyanobacteria constitute an important fraction of the N-fixing micro-flora of the paddy fields. Increased production of rice for meeting the food demand of the ever-growing population requires enormous use of fertilizers and pesticides, resulting in heavy contamination of paddy fields and the cyanobacteria inhabiting therein (Zhang and Zhang, 2007; Zhang et al., 2011; Enti-Brown, et al. 2012). Although pesticides are indispensable to the modern agricultural practice, however, these pesticides over the years have resulted in problems caused by their interactions with the biological systems in the environment and have deleterious effects on algae especially nitrogen fixing cyanobacteria by influencing growth, photosynthesis, nitrogen fixation, biochemical and molecular composition, and metabolic activities (Pankratz et al., 2003).

Polymerase Chain Reaction (PCR) technology has a significant impact in almost all the areas of molecular biology and the modification of this basic procedure has allowed a number of assays for detecting variation at the nucleotide level. A molecular approach based on 16S rDNA is useful in detecting bacterial community structural changes, because these genes are conserved and present in all bacteria (Moyer et al., 1994). Moreover, microbial community analyses using nucleic acids, such as 16S rDNA, can detect and identify community members with high specificity to the species and strain level, and can also detect and suggest phylogenetic relationships of uncultured organisms (Kerkhof et al., 2000). In addition, the impact of 2,4-D ethyl ester and pencycuron in inducing DNA damage in three species of cyanobacteria-*A. fertilissima*, *Aulosira fertilissima*, and *W. prolifica* as evidenced by PCR-based assays: RAPD and 16S rRNA amplification was examined (Nirmal Kumar et al., 2011a). Besides, an evaluation of pesticide-stress induced proteins in three cyanobacterial species- *A. fertilissima*, *Aulosira fertilissima*, and *W. prolifica* was also studied to observe protein profile changes and differentially expressed proteins under pesticide stress (Nirmal Kumar et al., 2011b). Current study was thus undertaken to investigate the various changes in 16S rDNA genomes of selected cyanobacterial species like *A. fertilissima*, *Aulosira fertilissima* and *W. prolifica* abundantly residing in Indian rice fields in response to different pesticide levels of endosulfan and tebuconazole.

2 Materials and Methods

2.1 Cyanobacteria strains, growth conditions and pesticide treatment

Axenic cultures of *A. fertilissima* Rao, *Aulosira fertilissima* Ghose and *W. prolifica* Janet were obtained from Centre for Conservation and Utilization of Blue-Green Algae, IARI, New Delhi and were grown photoautotrophically in nitrogen free BG_{11} medium (Rippka, 1979) within controlled temperatures (25±2°C) under 3000 lux light with the photoperiod of 14: 10 hours.

Endocel (35% EC, endosulfan manufactured by Excel Crop Care Ltd, Gujarat, India) and Folicur (25.9 % EC, tebuconazole manufactured by Bayer Crop Science, Mumbai) were used for the present study. Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepine-3-oxide) as a broad spectrum organochlorine insecticide to control aphids, beetles, foliar, etc and tebuconazole [1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol] as a systemic triazole fungicide against sheath blight of rice need special significance. Exponentially grown cyanobacterial cells were used throughout the experiment and organisms were subjected to various selected concentrations of the organochlorine insecticide and triazole fungicide based upon a set of experiments for determination of LC_{50}. LC_{50} values of the organisms for endosulfan and tebuconazole were determined based on chlorophyll-a of the cyanobacterial species and accordingly, two concentration like LC_{50} and higher concentrations of the pesticides were used in all further experiments up to a period of 16 days (Table 1). Axenic cultures and sterile conditions were maintained throughout the experimental period.

2.2 DNA extraction

The cultures were spinned down at 5000 rpm for 20min (20°C) and total genomic DNA was extracted from the cells using phenol–chloroform based method as previously described by (Adams, 1988) and precipitated from the supernatant by the addition of ice-cold absolute ethanol. Precipitated DNA was centrifuged at 15,000 rpm for 10 min at 4 ˚C, and the pellet was suspended in minimal volume of TE buffer (10mM Tris, 1mM EDTA; pH 7.6).

Table 1 LC_{50} values and pesticide treatments of the test organisms for endosulfan and tebuconazole.

Xenobiotic compound	**Organisms selected for study**	**LC_{50} values determined (ppm)**	**Treatments decided based upon LC_{50} (ppm)**
Endosulfan	*A. fertilissima*	6	6 12
	Aulosira fertilissima	30	30 60
	W. prolifica	20	20 40
Tebuconazole	*A. fertilissima*	15	15 30
	Aulosira fertilissima	30	30 60
	W. prolifica	30	30 60

Interference of RNA was eliminated by the treatment of DNase inactivated RNase at a final concentration of 10 µg/ml followed by an incubation of 1 h at 37˚C. A final precipitation step in the extraction of DNA was carried out by addition of 2.5 volumes of ice-cold ethanol and keeping the content at -20˚C overnight. After centrifugation the pellet was re-suspended in minimal volume of TE buffer.

2.3 Amplification of the 16S rDNA gene

PCR amplification was performed on purified DNA as per the method described by (Iteman et al., 2000). Amplification of the 16S rDNA gene was carried out by PCR using primers- 16s Forward Primer: 5'-AGAGTRTGATCMTYGCTWAC-3' and 16s Reverse Primer: 5'-CGYTAMCTTWTTACGRCT-3'.

The PCR mixture contained: 10µl 10X Taq DNA polymerase assay buffer, 400 ng each primer, 4 µl dNTP mix, 1 µl of Taq polymerase enzyme, 1 µl template DNA and make up the total reaction volume to 100 µl with Milli Q water. All the reagents were of Chromous make, Bangalore, India.

Total reaction volume was 100 µl after an initial cycle consisting of 5 min at 94°C, 35 cycles of amplification were started (30s at 94°C, 30s at 55°C and 2 min at 72°C). The termination cycle was 5 min at 72°C. PCR reactions were carried out in Thermal Cycler ABI2720 and PCR products obtained were migrated in agarose gel, extracted and purified using GEL EXTRACTION SPIN-50 kit (Chromous, Bangalore). The purified DNA so obtained was collected in the tube and used for sequencing reactions.

2.4 Sequencing of the 16S rDNA gene

Purified products were quantified using Big Dye Terminator version 3.1 , Cycle sequencing kit and run in ABI 3500 XL Genetic Analyzer with POP_7 polymer 50 cm Capillary Array. The sequencer was loaded with BDTv3-KB-Denovo_v 5.2 (analysis protocol) and Seq Scape_ v 5.2 (Data analysis software) and Applied Biosystem Micro Amp Optical 96-Well Reaction plate was used during the reaction.

3 Results

3.1 Changes in 16S rDNA gene sequences under endosulfan stress

3.1.1 *A. fertilissima*

It was noted that exposure of 6ppm endosulfan to the cells of *A. fertilissima* brought about significant changes in the 16S rDNA gene sequence of the cultures when compared to the control. Sequence similarity was carried out using BLAST. The programme confirmed 2% dissimilarity (1269/1290) and presence of 1% gap (9/1290) in sequences of 6ppm treated cultures, while 18% (954/1159) variation and gap of 7% (80/1159) was recorded when cultures were treated with 12ppm after 16 days. Differences in sequences as compared to control also reasoned the harmful effects of endosulfan on molecular functioning of the cells (Fig. 1a, b). Gaps created as a result of endosulfan stress are mostly elucidated in terms of formation of dimeric photoproducts, single strand breaks and double strand breaks, depurination or mismatching of nitrogen bases.

3.1.2 *Aulosira fertilissima*

16S rDNA gene sequencing of *Aulosira fertilissima* in response to endosulfan expressed concentration dependent variation in the gene sequence. While treatment by 30ppm endosulfan reflected up to 98% similarity (916/939) in gene sequence as compared to control, highest concentration of endosulfan (60ppm) displayed as much as 17% (798/956) alteration in genetic composition of 16S rDNA gene after 16 days (Fig. 2a, b). BLAST analysis further revealed that 30ppm treatment expressed gaps up to 2% (22/939) in *Aulosira fertilissima* while 60ppm endosulfan revealed gaps up to 5% (45/956) after 16 days. Gaps occurred as a result of endosulfan stress established the injurious effects of the insecticide upon the genetic constitution of the organism. endosulfan generated several lesions such as mismatching of the bases, dimer formation, strand breakage, lack of hydrogen bonds etc. resulting in vast differences in the 16s rDNA sequence as compared to the control.

3.1.3 *W. prolifica*

Treatment of *W. prolifica* with 20ppm endosulfan triggered a total difference of 19%, thus reflecting identity of 953/1170 and gap of 7% (85/1170) in16S rDNA gene sequence, whereas 40ppm endosulfan further enhanced the impact of endosulfan on gene sequence dissimilarity by 20% (933/1170) and 7% (86/1170) gap towards the end of 16 days (Fig. 3a, b). Gaps created in the 16S rDNA sequence led to visible dissimilarities in the nucleotide sequence as compared to control. The gaps so formed are characteristics of instabilities in sequences of the treated samples, resulted due to improper purine-pyrimidine bonding, removal of purine or pyrimidine base from a strand, purine-purine or pyrimidine-pyrimidine pairing, addition of purine in place of a pyrimidine or vice-versa.

3.2 Changes in 16S rDNA gene sequences under tebuconazole stress

3.2.1 *A. fertilissima*

Sequencing studies carried out using 16S rDNA gene amplification and sequence comparison using BLAST ascertained that 15ppm tebuconazole inflicted only a 1% difference, thus having identities of 99% (1271/1274) and 0% gap (1/1274) in 16S rDNA gene pattern of *A. fertilissima*, while a treatment of 30ppm brought about a difference as high as 21%, reflecting 79% similarity (938/1190) and 8% (96/1990) gap in the nucleotide composition of 16S rDNA gene sequence after 16 days (Fig. 4a, b). Gaps, induced could be a result of dimer formation, purine-purine interaction or pyrimidine-pyrimidine interaction and other lesions.

3.2.2 *Aulosira fertilissima*

Although 30ppm tebuconazole treatment did not bring about many changes in the gene pattern of *Aulosira fertilissima* even after 16 days, it was noted that 16S rDNA gene sequence varied by as much as 17% when treated by a concentration of 60ppm after 16 days. Hence, the entire identity thus amounted to 83% similarity (798/956). Moreover, a gap of 5% (45/956) was also recorded for treatment of 60ppm after 16 days (Fig. 5a, b).

Strand breakage under pesticide stress can contribute largely to incorrect arrangement of nucleotides in the 16s rDNA sequence leading to replacement of purines instead of pyrimidines and vice-versa.

3.2.3 *W. prolifica*

A difference of 19% leading to identities (948/1169) and 7% (85/1169) gap was observed in the nucleotide sequence of 16S rDNA gene when treated by 30ppm of tebuconazole after 16 days, swhile the difference further elevated to a total of 21% (935/1183) and a gap of 10% (116/1183) when treated with the maximum concentration of 60ppm after an interval of 16 days (Fig. 6a, b). The resultant gaps so formed may be a trait of mismatching of purines and pyrimidines, double strand or single strand breakage, or incorporation of incorrect nucleotide along with degradation of hydrogen bonds.

4 Discussion

Our findings clearly demonstrated that pesticide concentrations affecting cyanobacterial DNA and causing lethality to these microbes might be due to irreversible DNA damage. Friedberg et al., 2006 suggested that induction of DNA damage by a series of complex biochemical reactions as a result of chemicals, ionizing radiation, etc. tend to generate chain of single as well as double strand breaks and single strand gaps.

Among different types of damages, DNA double strand breaks (DSBs) are the most deleterious, since they affect both strands of DNA and can lead to loss of genetic material. DNA damage results in (i) misincorporation of bases during replication process, (ii) hydrolytic damage, which results in deamination of bases, depurination, and depyrimidination (Lindahl, 1993), (iii) oxidative damage (Valko et al., 2006; Halliwell and Gutteridge, 2007), and (iv) alkylating agents that may result in modified bases (Lindahl, 1993; Dizdaroglu, 1992). Moreover, Rastogi and Sinha (2011) stressed that a number of mutagenic and cytotoxic DNA lesions such as cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs), as well as DNA strand breaks so produced can alter the genome integrity and affect the normal life process of all organisms.

Lau et al. (2011) proposed that one of the most important protective defense mechanisms against such DNA damage could be the base excision repair (BER) pathway, which recognizes a wide range of DNA lesions.

These findings corroborate with our results thus explaining the occurrence of gaps, modified bases, dimer formation, mispairing of bases, depurination or depyrimidination etc. observed as a result of pesticide interaction with the cyanobacteria. Similarly, Viti et al. (2006) noted that chromate contamination affected the structure and diversity of the soil bacterial community. Bacterial strains isolated from the microcosm contaminated with highest concentration of chromate were identified by 16S rDNA gene sequencing. Kumar et al. (2004) substantiated damaging impact of ultraviolet-B radiation in causing damages to DNA of the cyanobacterium, *Anabaena* strain BT2 by PCR-based assays such as RAPD and rDNA amplification. While, template activity of UV-B-treated genomic DNA was drastically inhibited; there was no amplification in RAPD assay after prior exposure of DNA to UV-B for 60 min. While, only one band of approximately 400 bp was observed even after 60 min of exposure suggested that certain segment of DNA strand is resistant to UV-B effects, but amplification of rDNA was significantly inhibited following exposure of genomic DNA to UV-B. In addition, Nirmal Kumar et al. (2011) observed that RAPD profiles of pesticide treated species not only inhibited the template activity of genomic DNA but also led to the absence as well as generation of newer band patterns as compared to their respective controls. Moreover, rRNA amplification was also significantly inhibited following exposure of genomic DNA to 2,4-D ethyl ester and pencycuron.

Fig. 1(a) BLAST analysis between sequences of control and 6ppm endosulfan treated *Anabaena fertilissima* after 16 days.

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Identities = 1269/1290 (98%), Gaps = 9/1290 (1%)

Query  4     GGAATCTACCTTTTCGTGGG-GGATAA-CGTAGGGAAACTTACGCTAATACCGCATACGA  61

             ||||||||||||||||||||   ||||  | |||||||||||  ||||||||||||||||

Sbjct  21    GGAATCTACCTTTTCGTGGGTTTATAATAGAAGGGAAACTTAATCTAATACCGCATACGA  80

Query  62    CCTA-CGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGTCCGA  120

             |||| | || ||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  81    CCTACCCGGCGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGTCCGA  140

Query  121   TTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGA  180

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  141   TTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGA  200

Query  181   TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA  240

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  201   TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA  260

Query  241   ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCG  300

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  261   ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCG  320

Query  301   GGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGATGAC  360

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  321   GGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGATGAC  380

Query  361   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT  420

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Sbjct  381   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCACCAGCCGCGGTAATACGAAGGGT  440

Query  421   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  480

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Sbjct  441   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  500

Query  481   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  540

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  501   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  560
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Query  541   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  600
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Sbjct  561   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  620

Query  601   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  660
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Sbjct  621   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  680

Query  661   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  720
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Sbjct  681   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  740

Query  721   TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAG  780
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Sbjct  741   TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAG  800

Query  781   ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  801   ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTC  860

Query  841   GATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATT  900
             ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  861   GATGCAACGCGAAAAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATT  920

Query  901   GGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  921   GGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA  980

Query  961   TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGT  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  981   TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGT  1040

Query  1021  GGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCA  1080
             ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  1041  GGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAAGAAGGTGGGGATGACGTCAAGTCA  1100

Query  1081  TCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGCAA  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1101  TCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGCAA  1160
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Query  1141  ACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTG-AGTCTGCAACT  1199
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Sbjct  1161  -CCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACT  1219

Query  1200  CGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGG-TGAATACG  1258
             ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  1220  CGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGGTGAATACG  1279

Query  1259  TT-CCCGGGCCTTGTAACCACCATCCCGCC  1287
             || ||||||||||||||| || || |||||
Sbjct  1280  TTTCCCGGGCCTTGTAAC-AC-ATTCCGCC  1307
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Fig. 1(b) BLAST analysis between sequences of control and 12ppm endosulfan treated *Anabaena fertilissima* after 16 days.

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Identities = 954/1159 (82%), Gaps = 80/1159 (7%)

Query  119   GATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAGAG  178
             |||||||||||||| ||||||| |||| |||||||||||||||  |||||||||||||||
Sbjct  142   GATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAG  201

Query  179   GATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG  238
             |||||||||||||| ||| |||||||||||| ||| ||||||||||||||||||||||||
Sbjct  202   GATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGG  261

Query  239   GAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTT  298
             ||||||||||||||||||||||||||||||||||||| ||||||| |||| |||||||||
Sbjct  262   GAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTT  321

Query  299   CGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAAT-ACTCGGTGGGGAT  357
              |||||||||||| ||||    || |  |||     |   || ||| ||  |||    ||
Sbjct  322   AGGGTTGTAAAGCTCTTTCACCGG-A--GAA-----G---AT-AATGAC--GGT----AT  363

Query  358   GACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAG  417
               ||| |   || |||   || ||||||||||||||||||||||||||||||||||||||
Sbjct  364   C-CGG-AG--AA-GAA---GCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAG  415

Query  418   GGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGG-TGGTTTAAGTC  476
             || || ||||||  |||||||||||||||||||||||  |||||| || |  ||||||||
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Sbjct  416    GGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGATA-TTTAAGTC  474

Query  477    TGCTGTGAAAGCCCTGGGCTCAAC-CTGGGAATTGCAGTGGATACTGGATCA-CTAGAGT  534
               |  |||||| ||| | ||||||| |||| || |||  | |||||||| | | || ||||
Sbjct  475    AGGGGTGAAATCCCAGAGCTCAACTCTGG-AACTGCCTTTGATACTGGGT-ATCTTGAGT  532

Query  535    GTGGTAGAGGGATGCGGAATTTCTG-GTGTAGCAG-TGAAATGCGTAGAGAT-CAGAAGG  591
               ||| ||||| | | |||||| | | |||||| || |||||| |||||| || | || ||
Sbjct  533    ATGGAAGAGGTAAGTGGAATTCC-GAGTGTAG-AGGTGAAATTCGTAGATATTCGGA-GG  589

Query  592    AACATCC-GTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTG  650
              |||| || |||||||||||||| | |||| |||  |||||| ||||||  ||||||||||
Sbjct  590    AACA-CCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTG  648


Query  651    GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGG-AT-  708
              |||||||||||||||||||||||||||||||||||||| |||||||||  || ||  |
Sbjct  649    GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG--AA-TGTTAGC  705

Query  709    -GTTGGGT-GCA-ACTTGGCACCCAGTATCGAAGCTAACGCGTTAAG--TTCGCCGCCTG  763
               || |||  | | ||| |   |   ||  || ||||||||| ||||   ||| | |||||
Sbjct  706    CGTCGGGCAGTATACT-GTT-CG--GTGGCGCAGCTAACGCATTAAACATTC-C-GCCTG  759

Query  764    GGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGG  823
              |||||||||||||||||| | |||||||||||||||||||||||||||||||||||||||
Sbjct  760    GGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGG  819

Query  824    AGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAA  883
              || |||||||||||||||| |||||||| ||||||||||| | ||||||||| |  || |
Sbjct  820    AGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCTCTTGACATTCGGGGTA  879

Query  884    CTTTCCA--G-AGATGGAT-TGGTGCC-TTCGG--GAACCGTGAGACAGGTGCTGCATGG  936
               |   ||  | ||| | || |  | |  || ||  |  ||  || |||||||||||||||
Sbjct  880    -TGGGCATTGGAGACG-ATGTCCTTCAGTTAGGCTGGCCCCAGA-ACAGGTGCTGCATGG  936

Query  937    CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGT  996
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  937    CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGC  996

Query  997    CCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCG-GA  1055
```

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              |||||||||||||||  || |  |||| ||||||||| ||| |||||||| || ||| ||

Sbjct  997    CCTTAGTTGCCAGCATTTAGT--TGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGA  1054

Query  1056   GGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTA  1115

              |||||||||||||||||||||||| ||||||||||||||  | ||||||||||||| |||

Sbjct  1055   GGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTA  1114

Query  1116   CAATGGTGGGGACAGAGGGCTGCAA-ACCCGCGAGGGT-GAGCCAATC-CCAGAAACCCT  1172

              ||||||||| ||||| |||| || | ||  |||| | | |||| |||| ||| ||| ||

Sbjct  1115   CAATGGTGGTGACAGTGGGCAGCGAGACA-GCGATG-TCGAGCTAATCTCCA-AAAGCC-  1170

Query  1173   ATCTCAGTCCGGATTG-AGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCG  1231

              |||||||| ||||||| | |||||||||||| | |||||||| |||||||||||||||||

Sbjct  1171   ATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCG  1230

Query  1232   CAGATCA-GCATTGCTGCG  1249

              ||||||| || |||| |||

Sbjct  1231   CAGATCATGC-TTGCGGCG  1248
```

Fig. 2(a) BLAST analysis between sequences of control and 30ppm endosulfan treated *Aulosira fertilissima* after 16 days.

```
Identities = 916/939 (98%), Gaps = 22/939 (2%)

Query  29    GTCGAACGGCAGCACAGGAGAAGCTTGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGA  88

             |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||

Sbjct  22    GTCGAACGGCAGCACAGGAG-AGCTTGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGA  80

Query  89    ATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCA  148

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  81    ATACATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCA  140

Query  149   TACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGT  208

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  141   TACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGT  200

Query  209   CCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAG  268

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  201   CCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAG  260
```

```
Query  269  AGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  328

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  261  AGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  320

Query  329  GGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCC  388

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  321  GGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCC  380

Query  389  TTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGA  448

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  381  TTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGA  440

Query  449  TGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  508

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  441  TGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  500

Query  509  GGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTC  568

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  501  GGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTC  560

Query  569  TGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGT  628

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  561  TGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGT  620

Query  629  GGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACAT  688

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  621  GGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACAT  680

Query  689  CCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGC  748

            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  681  CCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGC  740

Query  749  AAACAGGATTAGATACCC-TGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGT  807

            |||||||||||||||||| |||||||||||||||||||||||||||||||||||||||||

Sbjct  741  AAACAGGATTAGATACCCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGT  800

Query  808  GCAACTT-GG-CACCCAGTATCGAAGCTAACGCGTTAAGTT-CGCCGCC-TGGGG-AGTA  862

            ||||||| || |||||||||||||||||||||||||||||| ||||||| ||||| ||||

Sbjct  801  GCAACTTTGGGCACCCAGTATCGAAGCTAACGCGTTAAGTTTCGCCGCCCTGGGGGAGTA  860
```

```
Query  863  CGG-TCG-CAAGACTGAAACTCAAAGG-AATTG-ACGGGGG-CCCGCACAAGCGGT-GG-  915
            ||| ||| ||||||||||||||||||| ||||| ||||||| |||||||| ||||| ||
Sbjct  861  CGGGTCGTCAAGACTGAAACTCAAAGGGAATTGGACGGGGGGCCCGCACA-GCGGTTGGG  919

Query  916  -AGTATGTGG-TTTAATTC-GAT-GC-AACG-CGAAGAA  948
             ||||||| | |||||||| ||| || |||| ||| |||
Sbjct  920  GAGTATGTTGATTTAATTCCGATTGCCAACGGCGA-GAA  957
```

Fig. 2(b) BLAST analysis between sequences of control and 60ppm endosulfan treated *Aulosira fertilissima* after 16 days.

```
Identities = 798/956 (83%), Gaps = 45/956 (5%)

Query  448   ATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA  507
             |||||||||||   |||| ||||  ||||||||| ||||||||||||||||||||||||
Sbjct  401   ATGACGGTACCTGCAGAAGAAGCTGCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT  460

Query  508   AGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGT  567
             |||  |||||||||  ||||||||||||||||||||| ||||||||| ||| |  |||||
Sbjct  461   AGGCAGCAAGCGTTGTTCGGAATTACTGGGCGTAAAGAGTGCGTAGGCGGTTGACTAAGT  520

Query  568   CTGCTGTGAAAGC-CCTGGGCTCAACCTGGGA-ATTGCAG-TGGATACTGGATCACTAGA  624
              || ||||||| | ||  |||| || ||||||   ||| |  | | ||||| |  |||||
Sbjct  521   TTGGTGTGAAATCTCC-CGGCTTAA-CTGGGAGGGTGC-GCCGAAAACTGGTTGGCTAGA  577

Query  625   GTGTGGTAGAGGGAT-GCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGG  683
             |||||| |||||| | | |||||| |||||||||| |||||||||||||| ||||| |||
Sbjct  578   GTGTGGGAGAGGG-TAGTGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGG  636

Query  684   AACATCC-GTGGCGAAGGCGGCAT-CCTGGGCCAACACTGACACTGAGGCACGAAAGCGT  741
             |||| || |||| | || |||| | ||||| |||  |||||| |||||||||||||||||
Sbjct  637   AACA-CCGGTGGTGTAGACGGC-TACCTGGACCATTACTGACGCTGAGGCACGAAAGCGT  694

Query  742   GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACT-GGAT  800
             ||||||||||||||||||||||||||||||||||||||||||||| |||| |||| || |
Sbjct  695   GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACAATGCAAACTTGG-T  753

Query  801   GTTGGGTGCAAC-TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGA  859
             || | |  |  | ||||   |  ||  || ||||||||||||||||| ||||||||||||
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```
Sbjct  754   GT-GCGCCCTTCATTGGGTGC--GTGCCGTAGCTAACGCGTTAAGTTTGCCGCCTGGGGA  810

Query  860   GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA  919

             ||||||||||||| |||||||||||||||||||||||||||||||||||||||||||| |

Sbjct  811   GTACGGTCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  870

Query  920   TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGA--A-C  976

             ||||||||||||||| ||||||||||||||||||||||| || || | |  | ||  | |

Sbjct  871   TGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCTGGGCTCGA-A-CGGCTGATCAAC  928

Query  977   TTTCC-AGAGATGGATTGGTGCCTTCGGGAACCGTG---AGAC-AGGTGCTGCATGGCTG  1031

               ||  ||| ||  |  |||  || ||  ||  | |   || | ||||||||||||||||

Sbjct  929   GATCGTAGAAAT--AC-GGTTACTCCGC-AAGGGGGTTCAGTCGAGGTGCTGCATGGCTG  984

Query  1032  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCT  1091

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  985   TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCT  1044

Query  1092  -TAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGA  1150

              | ||||||| ||  || |  |||| ||||| ||||||||||| ||||||||| ||||||

Sbjct  1045  GT-GTTGCCATCATTTAGT--TGGGCACTCTCAGGAGACCGCCAGTGACAAACTGGAGGA  1101

Query  1151  AGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAA  1210

             |||||||||||||||||||||||||||||| ||| | |||||||||||||||| ||||||

Sbjct  1102  AGGTGGGGATGACGTCAAGTCATCATGGCCTTTATGTCCAGGGCTACACACGTGCTACAA  1161

Query  1211  TGGTGGGGACAG-AGGGCTGCAAACCCGCGAGGGTGAGCCAATCC-CAGAAACCCTATCT  1268

             |||  || |||  | | | || |||||||||||| |||| ||||  || |||||   |||

Sbjct  1162  TGGCCGGTACAATACGAC-GCTAACCCGCGAGGGGGAGCAAATCGGCAAAAACCGG-TCT  1219

Query  1269  CAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAG-TCGGAATCGCTAGTAATCGCAG  1327

             |||| ||||| | || |||||||||| || | ||||| | ||||||||||||||| || |

Sbjct  1220  CAGTTCGGATCGCAGGCTGCAACTCGCCTGCGTGAAGCT-GGAATCGCTAGTAATGGC-G  1277

Query  1328  -ATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA  1382

              |||||||| || || ||||||||||||||||||||||||||||||||||||||||

Sbjct  1278  CATCAGCATGGC-GCCGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA  1332
```

Fig. 3(a) BLAST analysis between sequences of control and 20ppm endosulfan treated *Westiellopsis prolifica* after 16 days.

```
Identities = 953/1170 (81%), Gaps = 85/1170 (7%)

Query  68    AGATGAGCTCG-CGTCTGATTAGCTAGTTGGTGTGGTAA-GAGCGCACCAAGGCGACCAT  125

             |||||||| ||  ||| |||||||||||||| | ||||| | || |||||||||||| ||

Sbjct  116   AGATGAGC-CGATGTCCGATTAGCTAGTTGGCGGGGTAATG-GCCCACCAAGGCGACGAT  173

Query  126   CAGTAGCTGGTCTGAGAGGATGATCAGCCCCACTGGGACTGAGACACGGCCCAGACTCCT  185

             | ||||||||||||||||||||||||||| |||||| |||||||||||| ||||||||||

Sbjct  174   CGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCT  233

Query  186   ACGGGAGGCAGCAGTGGGGAATTTTCCG-CAATGGGCGAAAGCCTGACT-GAGCAATACC  243

             |||||||||||||||||||||| ||  | ||||||||| |||||||| |  ||| |||||

Sbjct  234   ACGGGAGGCAGCAGTGGGGAATATT-GGACAATGGGCGCAAGCCTGA-TCCAGCCATACC  291

Query  244   GCGTGAGG-GAGGAAGGCTCTT-GGGTTGTAAA-CCTCTTTTCTCAGG---G-AA-----  291

             ||||| || || |||||| ||| |||||||||| || ||||| |  ||   | ||

Sbjct  292   GCGTG-GGTGAAGAAGGC-CTTCGGGTTGTAAAGCC-CTTTTGTTGGGAAAGAAATCCTG  348

Query  292   T--AATAA-A----GT---GA--A-GGTACCTGAGGAATAAGCATCGGCTAACTCCGTGC  338

             |  | ||| |    ||   ||  | ||||||  | ||||||||| ||||||||| |||||

Sbjct  349   TCGATTAATACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGC  408

Query  339   CAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATC-CGGAATGATTGGGCGTAAAGCG  397

             ||||||||||||||||||| ||| |||| |||||| | |||||| | |||||||||||||

Sbjct  409   CAGCAGCCGCGGTAATACGAAGGGTGCATGCGTTA-CTCGGAATTACTGGGCGTAAAGCG  467

Query  398   TCCGCAGGTGGCACTGTAAGTCTGCTGTTAAAGAGCAAGGCTCAACCTTGTAAAGGCAGT  457

             | || ||||||   | |||||||||||| ||||  |  |||||||||| | ||  |||||

Sbjct  468   TGCGTAGGTGGTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGT  527

Query  458   GGAAACTACA-GAGCTAGAGTACGTTCG-GGGCAGAG-GGAATTCCTGGTGTAGCGGTGA  514

             ||| |||  |  | |||||||  | | | ||| |  | |||||| |||||||||| ||||

Sbjct  528   GGATACTGGATCA-CTAGAGTGTGGTAGAGGG-A-TGCGGAATTTCTGGTGTAGCAGTGA  584

Query  515   AATGCGTAGAGATCAGGAA-GAACA-CCGGTGGCGAAAGC-GCTCTGCTAGGCCGTA-AC  570

             ||||||||||||||| ||| ||||| || |||||||| || ||  | || ||||  | ||

Sbjct  585   AATGCGTAGAGATCA-GAAGGAACATCC-GTGGCGAAGGCGGC-ATCCTGGGCC-AACAC  640

Query  571   TGACACTGAGGGACGAAAGC-TAGGGGAGCGAATGGGATTAGATACCCCAGTAGTCCTA-  628
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             ||||||||||| |||||||| | ||||||| ||  |||||||||||||  ||||||| |
Sbjct  641   TGACACTGAGGCACGAAAGCGT-GGGGAGCAAACAGGATTAGATACCCTGGTAGTCC-AC  698

Query  629   GCCGTAAACGATG-GATACTAGGCGTGGCTTGTAT-CGACCCCG-AGCC-GTGCCGGAGC  684
             ||| ||||||||| || ||| ||  | | |||  | | ||   | | || ||  || |||
Sbjct  699   GCCCTAAACGATGCGA-ACT-GG-AT-G-TTGGGTGCAACTTGGCACCCAGTATCGAAGC  753

Query  685   CAACGCGTTAAGTATC-CCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATT  743
              |||||||||||| || ||||||||||||||||  ||||||  |||||||||||||||||
Sbjct  754   TAACGCGTTAAGT-TCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATT  812

Query  744   GACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCT  803
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  813   GACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCT  872

Query  804   TACCAAGACTTGACATGTCGCG-AATCTT-CTTGAAAGGGAA-GAGTGCCTTAGGGAGC-  859
             ||||  | ||||||||  | || || ||| |  || | |||  | ||||||| |||| |
Sbjct  873   TACCTGGTCTTGACATC-CACGGAA-CTTTCCAGAGATGGATTG-GTGCCTTCGGGAACC  929

Query  860   GCGAACACAG-TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC  918
             | ||  |||| || ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  930   GTGAG-ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC  988

Query  919   CGCAACGAGCGCAACCCTCGTTTTTAGTTGCCAGCAT-TAAAT--TGGGCACTCTAGAG-  974
             |||||||||||||||||| ||  |||||||||||||  ||| |  |||| |||||| ||
Sbjct  989   CGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAA-TGGTGGGAACTCTA-AGG  1046

Query  975   AGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTAC  1034
             |||| |||||||||||||||||||||||||||||||||||||||||| |||| |||||||
Sbjct  1047  AGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC  1106

Query  1035  GTCTTGGGCTACACACGTACTACAATGGTACGGACAGAGGGCAGCAAGCTAGCGATAGCA  1094
             | |  ||||||||||||||||||||||||  ||||||||||| |||| |  ||||  |
Sbjct  1107  GACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGCAAACCCGCGAGGGTG  1166

Query  1095  AGCAAATCCC-GTAAACCGTATCTCAGTTCAGATCGCAGTCTGCAACTCGCCTGCGTGAA  1153
             ||| |||||| | ||||| ||||||||| | ||| | ||||||||||||| || | ||||
Sbjct  1167  AGCCAATCCCAG-AAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAA  1225

Query  1154  GGAGGAATCGCTAGTAATTGCAG-TCAGCA  1182
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             |  ||||||||||||||| |||| ||||||

Sbjct  1226  GTCGGAATCGCTAGTAATCGCAGATCAGCA  1255
```

Fig. 3(b) BLAST analysis between sequences of control and 40ppm endosulfan treated *Westiellopsis prolifica* after 16 days.

```
Identities = 933/1170 (80%), Gaps = 86/1170 (7%)

Query  68    AGATGAGCTCG-CGTCTGATTAGCTAGTTGGTGTGGTAA-GAGCGCACCAAGGCGACCAT  125

             |||||||| ||  ||| |||||||||||||| | ||||| | || |||||||||||| ||

Sbjct  195   AGATGAGC-CGATGTCCGATTAGCTAGTTGGCGGGGTAATG-GCCCACCAAGGCGACGAT  252

Query  126   CAGTAGCTGGTCTGAGAGGATGATCAGCCCCACTGGGACTGAGACACGGCCCAGACTCCT  185

             | ||||||||||||||||||||||||||| |||||| |||||||||||| ||||||||||

Sbjct  253   CGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCT  312

Query  186   ACGGGAGGCAGCAGTGGGGAATTTTCCG-CAATGGGCGAAAGCCTGACT-GAGCAATACC  243

             |||||||||||||||||||||| ||  | ||||||||| |||||||| |  ||| |||||

Sbjct  313   ACGGGAGGCAGCAGTGGGGAATATT-GGACAATGGGCGCAAGCCTGA-TCCAGCCATACC  370

Query  244   GCGTGAGG-GAGGAAGGCTCTT-GGGTTGTAAA-CCTCTTTTCTCAGG---G-AA-----  291

             ||||| || || |||||| ||| |||||||||| || ||||| |  ||   | ||

Sbjct  371   GCGTG-GGTGAAGAAGGC-CTTCGGGTTGTAAAGCC-CTTTTGTTGGGAAAGAAATCCTG  427

Query  292   T--AATAA-A----GT---GA--A-GGTACCTGAGGAATAAGCATCGGCTAACTCCGTGC  338

             |  | ||| |    ||   ||  | ||||||  | ||||||||| ||||||||| |||||

Sbjct  428   TCGATTAATACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGC  487

Query  339   CAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATC-CGGAATGATTGGGCGTAAAGCG  397

             || |||||||||||||||| ||| ||||||||||| | |||||| | |||||||||||||

Sbjct  488   CACCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA-CTCGGAATTACTGGGCGTAAAGCG  546

Query  398   TCCGCAGGTGGCACTGTAAGTCTGCTGTTAAAGAGCAAGGCTCAACCTTGTAAAGGCAGT  457

             | || ||||||   | |||||||||||| ||||  |  |||||||||| | ||  |||||

Sbjct  547   TGCGTAGGTGGTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGT  606

Query  458   GGAAACTACA-GAGCTAGAGTACGTTCG-GGGCAGAG-GGAATTCCTGGTGTAGCGGTGA  514

             ||| |||  |  | |||||||  | | | ||| |  | |||||| |||||||||| ||||

Sbjct  607   GGATACTGGATCA-CTAGAGTGTGGTAGAGGG-A-TGCGGAATTTCTGGTGTAGCAGTGA  663
```

```
Query  515   AATGCGTAGAGATCAGGAA-GAACA-CCGGTGGCGAAAGC-GCTCTGCTAGGCCGTA-AC  570

             ||||||||||||||| ||| ||||| || |||||||| || ||  | || ||||  | ||

Sbjct  664   AATGCGTAGAGATCA-GAACGAACATCC-GTGGCGAATGCGGC-ATCCTGGGCC-AACAC  719

Query  571   TGACACTGAGGGACGAAAGC-TAGGGGAGCGAATGGGATTAGATACCCCAGTAGTCCTA-  628

             ||||||||||| |||||||| | | ||||| ||  |||||| ||||||  ||||||| |

Sbjct  720   TGACACTGAGGCACGAAAGCGT-GTGGAGCAAACAGGATTACATACCCTGGTAGTCC-AC  777

Query  629   GCCGTAAACGATG-GATACTAGGCGTGGCTTGTAT-CGACCCCG-AGCC-GTGCCGGAGC  684

             ||| ||||||||| || ||| ||   | | |||  | | ||   | | || ||  || ||

Sbjct  778   GCCTTAAACGATGTGA-ACT-GG-AT-G-TTGGGTGCAACTTGGCACCCAGTATCGAAGA  832

Query  685   CAACGCGTTAAGTATC-CCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATT  743

              |||||  ||||| || ||||||||||||||||  ||||||  |||||||||||||||||

Sbjct  833   TAACGCCATAAGT-TCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATT  891

Query  744   GACGGGGG-CCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACC  802

             || ||||| ||||| |||||||||||||| ||||||||||| |||| |||||||||| ||

Sbjct  892   GA-GGGGGCCCCGCCCAAGCGGTGGAGTAGGTGGTTTAATTAGATGAAACGCGAAGACCC  950

Query  803   TTACCAAGACTTGACATGTCGCGAATC-TTCTTGAAAGGGA-AGAGTGCCTTAGGGAGCG  860

             || ||  | |||||| |  | || | | |||  || | |||  | || |||| |||| |

Sbjct  951   TTCCCGGGTCTTGACCT-CCACGGACCTTTCCAGAGATGGATTG-GTTCCTTTGGGAAC-  1007

Query  861   CG-AACACA-GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC  918

             ||  | ||| ||| |||||||| || ||||||||||||||||||||||||||||||||||

Sbjct  1008  CGTCAGACAGGTGATGCATGGCGGTTGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC  1067

Query  919   CGCAACGAGCGCAACCCTCGTTTTTAGTTGCCAGCA-TTAAAT--TGGGCACTCT-AGAG  974

             |||||||||||||||||| ||  |||||||||||||  | |||  |||| ||||| || |

Sbjct  1068  CGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGT-AATGGTGGGAACTCTAAG-G  1125

Query  975   AGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTAC  1034

             |||| |||||||||||||||||||||||||||||||||||||||||| |||| |||||||

Sbjct  1126  AGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC  1185

Query  1035  GTCTTGGGCTACACACGTACTACAATGGTACGGACAGAGGGCAGCAAGCTAGCGATAGCA  1094

             | |  |||||||||||||| |||||||||  ||||||||||| |||| |  ||||  |

Sbjct  1186  GCCCAGGGCTACACACGTA-TACAATGGTGGGGACAGAGGGCTGCAACCCCGCGAGGGTG  1244
```

```
Query  1095  AGCAAATCCC-GTAAACCGTATCTCAGTTCAGATCGCAGTCTGCAACTCGCCTGCGTGAA  1153

             ||| |||||| | ||||| ||||||||| | ||| | ||| ||||||| | || | ||||

Sbjct  1245  AGCCAATCCCAG-AAACCCTATCTCAGTCCGGATTGGAGTTTGCAACTTGACTCCATGAA  1303

Query  1154  GGAGGAATCGCTAGTAATTGCAG-TCAGCA  1182

             |  ||||||||||||||| |||| ||||||

Sbjct  1304  GTCGGAATCGCTAGTAATCGCAGATCAGCA  1333
```

Fig. 4(a) BLAST analysis between two sequences of untreated and 15ppm tebuconazole treated *Anabaena fertilissima* after 16 days.

```
Identities = 1271/1274 (99%), Gaps = 1/1274 (0%)

Query  1     ATCGGAATCTACCTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACG  60

             ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||

Sbjct  91    ATCGGAATCTACCTTTTCGTGGGGGATAACGTACGGAAACTTACGCTAATACCGCATACG  150

Query  61    ACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGTCCGA  120

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  151   ACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTAGATGAGCCGATGTCCGA  210

Query  121   TTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGA  180

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  211   TTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGA  270

Query  181   TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA  240

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  271   TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA  330

Query  241   ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCG  300

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  331   ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCG  390

Query  301   GGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGATGAC  360

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  391   GGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAATACTCGGTGGGGATGAC  450

Query  361   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT  420

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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```
Sbjct  451   GGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGT  510

Query  421   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  480

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  511   GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCT  57

Query  481   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  540

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  571   GTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTA  630

Query  541   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  600

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  631   GAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGGAACATCCGT  690

Query  601   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  660

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  691   GGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAAC  750

Query  661   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  720

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  751   AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAAC  810

Query  721   TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAG  780

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  811   TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAG  870

Query  781   ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTC  840

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  871   ACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTC  930

Query  841   GATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATT  900

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  931   GATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATT  990

Query  901   GGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA  960

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  991   GGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA  1050

Query  961   TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGT  1020

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  1051  TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGT  1110

Query  1021  GGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCA  1080

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1111  GGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCA  1170

Query  1081  TCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGCAA  1140

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1171  TCATGGCCCTTACGACCAGGGCTACACACGTACTACAATGGTGGGGACAGAGGGCTGCAA  1230

Query  1141  ACCCGCGAGGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTG-AGTCTGCAACT  1199

             ||||||||||||||||||||||| |||||||||||||||||||||||| |||||||||||

Sbjct  1231  ACCCGCGAGGGTGAGCCAATCCCGGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACT  1290

Query  1200  CGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGT  1259

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1291  CGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGT  1350

Query  1260  TCCCGGGCCTTGTA  1273

             ||||||||||||||

Sbjct  1351  TCCCGGGCCTTGTA  1364
```

Fig. 4(b) BLAST analysis between two sequences of untreated and 30 ppm tebuconazole treated *Anabaena fertilissima* after 16 days.

```
Identities = 938/1190 (79%), Gaps = 96/1190 (8%)

Query  115   GTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTGGTCTG  174

             ||| ||||| |||||||| | ||||||||| |||||||||||||||| ||||||||||||

Sbjct  1265  GTCTGATTAACTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCAGTAGCTGGTCTG  1206

Query  175   AGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG  234

             | |||||||||| ||||| ||| ||||| |||||| ||  ||||||||||||||||||||

Sbjct  1205  AAAGGATGATCAACCACATTGGGACTGAAACACGG-CCCCACTCCTACGGGAGGCAGCAG  1147

Query  235   TGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGG  294

             |||||||||||||||||||||||||||||||||||  |||| ||||||| |||| |||||

Sbjct  1146  TGGGGAATATTGGACAATGGGCGCAAGCCTGATCCC-CCATGCCGCGTGAGTGATGAAGG  1088

Query  295   CCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCTGTCGATTAAT-ACTCGGTGG  353
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             ||||  ||||||||||| |||||||  ||   |||     |   || ||| ||| | |

Sbjct  1087  CCTTAAGGTTGTAAAGCTCTTTTGTCCGG---GAA-----G---AT-AATGACT-G-T--  1044

Query  354   GGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATAC  413

               |   ||| |   |  |||||||| ||||||||||||||||||||||||||||||||||

Sbjct  1043  --AC--CGG-A---A--GAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATAC  994

Query  414   GAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGT-TTA  472

              ||||| || |||||| |||||||| ||||||||||||| | ||||| | ||   | |||

Sbjct  993   CAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGCGCGTAAGCGGAC-TCTTA  935

Query  473   AGTCTGCTGTGAAAGCCCTGGGCTCAACC-TGGGAATTGCAGTGGATACTGG-A-TCACT  529

             |||| |  |||||||||| |||||||||| ||| ||||||  |  ||||||| | ||  |

Sbjct  934   AGTCCGGGGTGAAAGCCCAGGGCTCAACCCTGG-AATTGCCTTCTATACTGGGAGTCT-T  877

Query  530   AGAGTGT-GGTAGAGGGATGC-GGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGAT-CA  586

              |||| | || ||| |  ||  |||| | |  |||||  |||||||| | || | || |

Sbjct  876   -GAGT-TCGGAAGAAGT-TGGTGGAACTGCCAGTGTAAAAGTGAAATTCCTAAATATTC-  821

Query  587   G-AAGGAACATCC-GTGGCGAAGGCGGC-ATCCTGGGCCAACACTGACACTGAGGCACGA  643

             | ||| |||| || |||||||||||||| | | ||| || | |||||| |||| || |||

Sbjct  820   GCAAG-AACA-CCAGTGGCGAAGGCGGCCAAC-TGGTCCGATACTGACGCTGAAGCGCGA  764

Query  644   AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAAC  703

             ||||||||||||||| ||||||||||||||||||||||||||||| |||||||||  ||

Sbjct  763   AAGCGTGGGGAGCAA-CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG--AA-  708

Query  704   TGG-AT--GTTGGGTGCAACTTGGCACC-CAGTATCGAAGCTAACGCGTTAAG--TTCGC  757

             ||  |   ||||||||||  | | |||  ||||  || ||||||||| |||||  ||| |

Sbjct  707   TGCCAGCCGTTGGGTGCA--T-G-CACTTCAGTGGCGCAGCTAACGCTTTAAGCATTC-C  653

Query  758   CGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG  817

              ||||||| ||||| ||||||||| | |||||||||| ||| ||||||||||||| ||||

Sbjct  652   -GCCTGGG-AGTACCGTCGCAAGATTAAAACTCAAAGAAATGGACGGGGGCCCGCCCAAG  595

Query  818   CGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTT-GACATC  876

             |||||||  ||||||||||||| || ||||||||  || |||| || |  ||| |||||

Sbjct  594   CGGTGGACCATGTGGTTTAATTGGAAGCAACGCGCGGACCCTTCCCAGC-CTTTGACATG  536

Query  877   -CACGGA---ACTTTCCAGAGATGGA-TTGGTGCCTT-CGG--GAACCGTGAG-ACAGGT  927
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              | |||    || | ||||||| ||  ||  | | || |||  |  ||| ||  ||||||

Sbjct  535   TC-CGGTTTGACAT-CCAGAGA-GGTCTTTCTTCATTTCGGCTGG-CCG-GAACACAGGT  481

Query  928   GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  987

             ||||||||||||||||||||| || |||||||||||||||||||||||||||||||||||

Sbjct  480   GCTGCATGGCTGTCGTCAGCTGGTTTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  421

Query  988   AACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGAC  1047

             |||||| | |||||||||||  ||  | | | |||||||| || || ||| ||||||||

Sbjct  420   AACCCTCGCCCTTAGTTGCCTTCAT-TCA-GTTGGGAACTTTAGGGGGACTGCCGGTGAT  363


Query  1048  AAACCG-GAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACA  1106

             || ||| | |||||| ||||| |  |||| ||| ||||| ||||| ||    ||||||||

Sbjct  362   AAGCCGCGGGGAAGGGGGGGAGGGTGTCATGTCCTCATGCCCCTTGCGGGGGGGGCTACA  303

Query  1107  CACGTACTACAATGGTGGGGACAGA-GGGCTGC-AAACCCGCGAGGGT-GAGCCAATCCC  1163

             ||||| ||||||||| || |||| | |||| |  |||   | ||   | |||  ||||||

Sbjct  302   CACGTGCTACAATGGCGGTGACA-ATGGGCAGAGAAAGG-GAGACC-TCGAGAAAATCCC  246

Query  1164  AGAAACCCTATCTCAGTCCGGATTGAGTCTG--CAACTCGACTCCATGAAGTCGGAATCG  1221

             | ||| ||  ||||||| | ||| |  ||||  ||||| || | |||||||  |||||||

Sbjct  245   AAAAAGCCG-TCTCAGTTCAGATCGT-TCTGTGCAACTGGAGTACATGAAGGGGGAATCG  188

Query  1222  CTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTG  1271

             || |||||||  ||||||||| ||  ||||||||||||||||||||||||

Sbjct  187   CTTGTAATCGTGGATCAGCAT-GCCACGGTGAATACGTTCCCGGGCCTTG  139
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Fig. 5(a) BLAST analysis between two sequences of untreated and 30 ppm tebuconazole treated *Aulosira fertilissima* after 16 days.

```
Identities = 1446/1446 (100%), Gaps = 0/1446 (0%)

Query  1     TAGGAGCCCGGCCAAAAAACCTTGCAAGGTCGAACGGCAGCACAGGAGAAGCTTGCTCTC  60

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1     TAGGAGCCCGGCCAAAAAACCTTGCAAGGTCGAACGGCAGCACAGGAGAAGCTTGCTCTC  60

Query  61    TGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATA  120

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  61     TGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATA  120

Query  121    ACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAA  180

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  121    ACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAA  180

Query  181    GGCCTCACGCGATTAGATGAGCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCAC  240

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  181    GGCCTCACGCGATTAGATGAGCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCAC  240

Query  241    CAAGGCGACGATCGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACG  300

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  241    CAAGGCGACGATCGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACG  300

Query  301    GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATC  360

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  301    GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATC  360

Query  361    CAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGA  420

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  361    CAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGA  420

Query  421    AATCCTGTCGATTAATACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAAC  480

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  421    AATCCTGTCGATTAATACTCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAAC  480

Query  481    TTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGT  540

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  481    TTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGT  540

Query  541    AAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAAT  600

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  541    AAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCTCAACCTGGGAAT  600

Query  601    TGCAGTGGATACTGGATCACTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAG  660

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  601    TGCAGTGGATACTGGATCACTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAG  660

Query  661    TGAAATGCGTAGAGATCAGAAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACT  720

              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  661   TGAAATGCGTAGAGATCAGAAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACT  720

Query  721   GACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC  780

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  721   GACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC  780

Query  781   CTAAACGATGCGAACTGGATGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGT  840

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  781   CTAAACGATGCGAACTGGATGTTGGGTGCAACTTGGCACCCAGTATCGAAGCTAACGCGT  840

Query  841   TAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC  900

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  841   TAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC  900

Query  901   CCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTC  960

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  901   CCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTC  960

Query  961   TTGACATCCACGGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTG  1020

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  961   TTGACATCCACGGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTG  1020

Query  1021  CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1080

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1021  CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1080

Query  1081  ACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACA  1140

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1081  ACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACA  1140

Query  1141  AACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACA  1200

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1141  AACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACA  1200

Query  1201  CGTACTACAATGGTGGGGACAGAGGGCTGCAAACCCGCGAGGGTGAGCCAATCCCAGAAA  1260

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1201  CGTACTACAATGGTGGGGACAGAGGGCTGCAAACCCGCGAGGGTGAGCCAATCCCAGAAA  1260

Query  1261  CCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA  1320

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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```
Sbjct  1261  CCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA  1320

Query  1321  ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA  1380

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1321  ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA  1380

Query  1381  CACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGGAGGCCCGGTTTG  1440

             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1381  CACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGGAGGCCCGGTTTG  1440

Query  1441  CCCCAG  1446

             ||||||

Sbjct  1441  CCCCAG  1446
```

Fig. 5(b) BLAST analysis between two sequences of untreated and 60 ppm tebuconazole treated *Aulosira fertilissima* after 16 days.

```
Identities = 798/956 (83%), Gaps = 45/956 (5%)

Query  448   ATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA  507

             |||||||||||   |||| ||||  ||||||||| ||||||||||||||||||||||||

Sbjct  397   ATGACGGTACCTGCAGAAGAAGCTGCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT  456

Query  508   AGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGT  567

             |||  |||||||||  ||||||||||||||||||||| ||||||||| ||| |  |||||

Sbjct  457   AGGCAGCAAGCGTTGTTCGGAATTACTGGGCGTAAAGAGTGCGTAGGCGGTTGACTAAGT  516

Query  568   CTGCTGTGAAAGC-CCTGGGCTCAACCTGGGA-ATTGCAG-TGGATACTGGATCACTAGA  624

              || ||||||| | ||  |||| || ||||||   ||| |  | | ||||| |  |||||

Sbjct  517   TTGGTGTGAAATCTCC-CGGCTTAA-CTGGGAGGGTGC-GCCGAAAACTGGTTGGCTAGA  573

Query  625   GTGTGGTAGAGGGAT-GCGGAATTTCTGGTGTAGCAGTGAAATGCGTAGAGATCAGAAGG  683

             |||||| |||||| | | |||||| |||||||||| |||||||||||||| ||||| |||

Sbjct  574   GTGTGGGAGAGGG-TAGTGGAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAGG  632

Query  684   AACATCC-GTGGCGAAGGCGGCAT-CCTGGGCCAACACTGACACTGAGGCACGAAAGCGT  741

             |||| || |||| | || |||| | ||||| |||  |||||| |||||||||||||||||

Sbjct  633   AACA-CCGGTGGTGTAGACGGC-TACCTGGACCATTACTGACGCTGAGGCACGAAAGCGT  690

Query  742   GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACT-GGAT  800
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             ||||||||||||||||||||||||||||||||||||||||||||| |||| |||| || |
Sbjct  691   GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACAATGCAAACTTGG-T  749
Query  801   GTTGGGTGCAAC-TTGGCACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGA  859
             || | |  |  | ||||   |  ||  || ||||||||||||||||| ||||||||||||
Sbjct  750   GT-GCGCCCTTCATTGGGTGC--GTGCCGTAGCTAACGCGTTAAGTTTGCCGCCTGGGGA  806
Query  860   GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA  919
             ||||||||||||| |||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  807   GTACGGTCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  866
Query  920   TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGA--A-C  976
             ||||||||||||||| ||||||||||||||||||||||| || || | |  | ||  | |
Sbjct  867   TGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCTGGGCTCGA-A-CGGCTGATCAAC  924
Query  977   TTTCC-AGAGATGGATTGGTGCCTTCGGGAACCGTG---AGAC-AGGTGCTGCATGGCTG  1031
               ||  ||| ||  |  |||  || ||  ||  | |   || | ||||||||||||||||
Sbjct  925   GATCGTAGAAAT--AC-GGTTACTCCGC-AAGGGGGTTCAGTCGAGGTGCTGCATGGCTG  980
Query  1032  TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCT  1091
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  981   TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCT  1040
Query  1092  -TAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGA  1150
              | ||||||| ||  || |  |||| ||||| ||||||||||| ||||||||| ||||||
Sbjct  1041  GT-GTTGCCATCATTTAGT--TGGGCACTCTCAGGAGACCGCCAGTGACAAACTGGAGGA  1097
Query  1151  AGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAA  1210
             |||||||||||||||||||||||||||||| ||| | |||||||||||||||| ||||||
Sbjct  1098  AGGTGGGGATGACGTCAAGTCATCATGGCCTTTATGTCCAGGGCTACACACGTGCTACAA  1157
Query  1211  TGGTGGGGACAG-AGGGCTGCAAACCCGCGAGGGTGAGCCAATCC-CAGAAACCCTATCT  1268
             |||  || |||  | | | || |||||||||||| |||| ||||  || |||||   |||
Sbjct  1158  TGGCCGGTACAATACGAC-GCTAACCCGCGAGGGGGAGCAAATCGGCAAAAACCGG-TCT  1215
Query  1269  CAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAG-TCGGAATCGCTAGTAATCGCAG  1327
             |||| ||||| | || |||||||||| || | ||||| | ||||||||||||||| || |
Sbjct  1216  CAGTTCGGATCGCAGGCTGCAACTCGCCTGCGTGAAGCT-GGAATCGCTAGTAATGGC-G  1273
Query  1328  -ATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA  1382
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              |||||||| || || ||||||||||||||||||||||||||||||||||||||||

Sbjct  1274  CATCAGCATGGC-GCCGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA  1328
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Fig. 6(a) BLAST analysis between two sequences of untreated and 30 ppm tebuconazole treated *Westiellopsis prolifica* after 16 days.

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Identities = 948/1169 (81%), Gaps = 85/1169 (7%)

Query  70    ATGAGCTCG-CGTCTGATTAGCTAGTTGGTGTGGT-AAGAGCGCACCAAGGCGACCATCA  127

             |||||| ||  ||| |||||||||||||| | ||| ||| || |||||||||||| |||

Sbjct  221   ATGAGC-CGATGTCGGATTAGCTAGTTGGCGGGGTAAAG-GCCCACCAAGGCGACGATCC  278

Query  128   GTAGCTGGTCTGAG-AGGATGAT-CAGCCCCACTGGGACTGAGACACGGCCCAGACTCCT  185

             ||||||||||||||    |  ||  |||| |||||| |||||||||||| ||||||||||

Sbjct  279   GTAGCTGGTCTGAGTTTTAAAATGGAGCCACACTGGAACTGAGACACGGTCCAGACTCCT  338

Query  186   ACGGGAGGCAGCAGTGGGGAATTTTCCG-CAATGGGCGAAAGCCTGACT-GAGCAATACC  243

             |||||||||||||| ||||||| ||  | ||||||||| |||||||| |  ||| |||||

Sbjct  339   ACGGGAGGCAGCAGCGGGGAATATT-GGACAATGGGCGCAAGCCTGA-TCCAGCCATACC  396

Query  244   GCGTGAGG-GAGGAAGGCTCTT-GGGTTGTAAA-CCTCTTTTCTCAGG---G-AAT--A-  293

             ||||| || || |||||| ||| |||||||||| || ||||| |  ||   | |||  |

Sbjct  397   GCGTG-GGTGAAGAAGGC-CTTCGGGTTGTAAAGCC-CTTTTGTTGGGAAAGAAATCCAG  453

Query  294   ----ATAA-A----G-T--GA--A-GGTACCTGAGGAATAAGCATCGGCTAACTCCGTGC  338

                  ||| |    | |  ||  | ||||||  | ||||||||| ||||||||| |||||

Sbjct  454   CTGGTTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGC  513

Query  339   CAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATC-CGGAATGATTGGGCGTAAAGCG  397

             ||||||||||||||||||| ||| ||||||||||| | |||||| | |||||||||||||

Sbjct  514   CAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA-CTCGGAATTACTGGGCGTAAAGCG  572

Query  398   TCCGCAGGTGGCAC-TGTAAGTCTGCTGTTAAAGAGCAAGGCTCAACCTTGTAAAGGCAG  456

             | || ||||||  | | |||||| | ||| ||||  |  |||||||||| | ||  ||||

Sbjct  573   TGCGTAGGTGG-TCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAG  631

Query  457   TGGAAACTACAGAG-CTAGAGTACGTTCG-GGGCAGAGGGAATTCCTGGTGTAGCGGTGA  514

             |||| |||   | | ||||| |  | | | ||| ||  ||||||||||||||||| ||||

Sbjct  632   TGGATACT-GGGCGACTAGAATGTGGTAGAGGGTAG-CGGAATTCCTGGTGTAGCAGTGA  689
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Query  515   AATGCGTAGAGATCAGGAAGAACA-CCGGTGGCGAAAGC-GCTCTGCTAGG-CCGTAAC-  570
             |||||||||||||||||| ||||| ||  ||||||| || |||   || || ||  |||
Sbjct  690   AATGCGTAGAGATCAGGAGGAACATCC-ATGGCGAAGGCAGCT-ACCT-GGACC--AACA  744

Query  571   -TGACACTGAGGGACGAAAGC-TAGGGGAGCGAATGGGATTAGATACCCCAGTAGTCCTA  628
              ||||||||||| |||||||| | ||||||| ||  |||||||||||||  ||||||| |
Sbjct  745   TTGACACTGAGGCACGAAAGCGT-GGGGAGCAAACAGGATTAGATACCCTGGTAGTCC-A  802

Query  629   -GCCGTAAACGATG-GATACTAGGCGTGGCTTGTAT-CGACCCCG-A-GCCGTGCCGGAG  683
              ||| ||||||||| || ||| ||  | | |||  | | |    | | || ||  || ||
Sbjct  803   CGCCCTAAACGATGCGA-ACT-GG-AT-G-TTGGGTGCAATTTGGCACGCAGTATCGAAG  857

Query  684   CCAACGCGTTAAGTATC-CCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAAT  742
             | |||||||||||| || ||||||||||||||||  ||||||  ||||||||||||||||
Sbjct  858   CTAACGCGTTAAGT-TCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAAT  916

Query  743   TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACC  802
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  917   TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACC  976

Query  803   TTACCAAGACTTGACATGTCGCGAATCTT-CTTGAAAGGGAA-GAGTGCCTTAGGGAGCG  860
             |||||  | |||||||||||| ||| ||| |  || | |||  | ||||||| |||| |
Sbjct  977   TTACCTGGCCTTGACATGTCGAGAA-CTTTCCAGAGATGGATTG-GTGCCTTCGGGAACT  1034

Query  861   CGAACACAG-TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  919
             ||||||||| || |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1035  CGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  1094

Query  920   GCAACGAGCGCAACCCTCGTTTTTAGTTGCCAGCAT-TAAAT--TGGGCACTCTAGAG-A  975
             ||||||||||||||||| ||  |||||||||||||  ||| |  |||| |||||| || |
Sbjct  1095  GCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAA-TGGTGGGAACTCTA-AGGA  1152

Query  976   GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTACG  1035
             ||| |||||||||||||||||||||||||||||||||||||||||| |||| ||||||||
Sbjct  1153  GACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACG  1212

Query  1036  TCTTGGGCTACACACGTACTACAATGGTACGGACAGAGGGCAGCAAGCTAGCGATAGCAA  1095
              |  ||||||||||||||||||||||||| ||||||||||| ||||||  ||||  | ||
Sbjct  1213  GCCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAA  1272
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Query  1096  GCAAATCCC-GTAAACCGTATCTCAGTTCAGATCGCAGTCTGCAACTCGCCTGCGTGAAG  1154

             || |||||| | ||||| ||||||||| | ||| | ||||||||||||| || | || ||

Sbjct  1273  GCCAATCCCAG-AAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGGAG  1331

Query  1155  GAGGAATCGCTAGTAATTGCAG-TCAGCA  1182

               ||||||||||||||| |||| ||||||

Sbjct  1332  TCGGAATCGCTAGTAATCGCAGATCAGCA  1360
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Fig. 6(b) BLAST analysis between two sequences of untreated and 60 ppm tebuconazole treated *Westiellopsis prolifica* after 16 days.

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Identities = 935/1183 (79%), Gaps = 116/1183 (10%)

Query  68    AGATGAG-CTCGCGTCTGATTAGCTAGTTGGTGTGGTAA-GAGCGCACCAAGGCGACCAT  125

             ||||||| || | ||| |||||||||||||||| ||||| | || |||||||||||| ||

Sbjct  179   AGATGAGCCTAG-GTCGGATTAGCTAGTTGGTGGGGTAATG-GCTCACCAAGGCGACGAT  236

Query  126   CAGTAGCTGGTCTGAGAGGATGATCAGCCCCACTGGGACTGAGACACGGCCCAGACTCCT  185

             | ||| ||||||||||||||||||||| | |||||| |||||||||||| ||||||||||

Sbjct  237   CCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCT  296

Query  186   ACGGGAGGCAGCAGTGGGGAATTTTCCG-CAATGGGCGAAAGCCTGACT-GAGCAATACC  243

             |||||||||||||||||||||| ||  | |||||||||||||||||| |  ||| || ||

Sbjct  297   ACGGGAGGCAGCAGTGGGGAATATT-GGACAATGGGCGAAAGCCTGA-TCCAGCCATGCC  354

Query  244   GCGTGAGGGAGGAAGGCTCTT-GGGTTGTAAA-C-CTCTT---TT----CTCAGGG-AAT  292

             ||||| | || ||||| |||| || ||||||| | || ||   ||       |||| | |

Sbjct  355   GCGTGTGTGAAGAAGG-TCTTCGGATTGTAAAGCACT-TTAAGTTGGGAGGAAGGGCAGT  412

Query  293   AA--TAA-A-------G---TGAAGGTACCTGA-GGAATAAGCATCGGCTAACTCCGTGC  338

             ||  ||| |       |   ||| |||| ||  ||||||||| |||||||||| ||||

Sbjct  413   AAGCTAATACCTTGCTGTTTTGACGTTACC-GACAGAATAAGCACCGGCTAACTCTGTGC  471

Query  339   CAGCAGCCGCGGTAATACGGAGGATGCAAGCGTT-ATCCGGAATGATTGGGCGTAAAGCG  397

             |||||||||||||||||| |||| |||||||||| || |||||| | |||||||||||||

Sbjct  472   CAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAAT-CGGAATTACTGGGCGTAAAGCG  530

Query  398   TC-CGCAGGTGGCACTG-TAAGTCT-GCTGTTAAAGAGCAAGGCTCAACCTTGTAAAGGC  454

              | || ||||||  | | ||||| | | ||| ||||  |  |||||||||| | ||  ||
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Sbjct  531   -CGCGTAGGTGGTTC-GTTAAGT-TGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGC  587

Query  455   AGT-GGAAACT-ACAGAGCTAGAGTACGTTCG-GGGCAGAG-GGAA-TTCCTGGTGTAGC  509

             | |   |||||  | ||||||||||||| | | |||  | | |||| ||||| |||||||

Sbjct  588   A-TCCAAAACTGGC-GAGCTAGAGTACGGTAGAGGG-TG-GTGGAATTTCCT-GTGTAGC  642

Query  510   GGTGAAATGCGTAGAGATCAGGAA-GAACACCGGTGGCGAAAGCG-CTCTGCTAGG-CCG  566

             ||||||||||||||| || ||||| ||||||| |||||||| ||| | |  || || | |

Sbjct  643   GGTGAAATGCGTAGATAT-AGGAAGGAACACCAGTGGCGAAGGCGAC-CACCT-GGACTG  699

Query  567   TA-ACTGACACTGAGG-GACGAAAGC-TAGGGGAGCGAATGGGATTAGATACCCCAGTAG  623

              | ||||||||||||| |  |||||| | ||||||| ||  |||||||||| ||  ||||

Sbjct  700   -ATACTGACACTGAGGTG-TGAAAGCGT-GGGGAGCAAACAGGATTAGATATCCTGGTAG  756

Query  624   TCCTA-GCCGTAAACGATG-GATACTAGGCG-TGG----CTTGTATCGACCCCGAGCCGT  676

             ||| | |||||||||||||  | | ||| || |||    |||| |  ||     |   ||

Sbjct  757   TCC-ACGCCGTAAACGATGTCA-AGTAGCCGTTGGAATCCTTG-A--GA-TTTTA---GT  807

Query  677   GCCGGAGCCAACGCGTTAAGTAT-CCCGCCTGGGGAGTAC-GCACGCAA-GTGTGAAACT  733

             | || ||  ||||| |||||| |  | || || ||||||| || ||||| || | | |||

Sbjct  808   GGCGCAGATAACGCATTAAGT-TGACTGCGTGTGGAGTACGGC-CGCAAGGT-TTATACT  864

Query  734   CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACG  793

             |||| ||||||||||||||||||||||||||||||| ||||||||||||| || ||||||

Sbjct  865   CAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTAGAAGCAACG  924

Query  794   CGAAGAACCTTACCAAGACTTGACATGTC-GCGAATC-TTCTTGAAAGGGA-AGAGTGCC  850

              |||||||||||||| | ||||||||| | | ||| | |||  || | |||  | |||||

Sbjct  925   AGAAGAACCTTACCAGGCCTTGACATG-CAGAGAA-CTTTCCAGAGATGGATTG-GTGCC  981

Query  851   TTAGGGAGCGC-GAACACA-GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG  908

             || |||| | | | ||||| ||| ||||||||||||||||||||||||| ||||||||||

Sbjct  982   TTCGGGAACTCTG-ACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTTGAGATGTTG  1040

Query  909   GGTTAAGTCCCGCAACGAGCGCAACCCTCGTTTTTAGTTGCCAGCA--TTAAATTGGGCA  966

             |||||||||||| ||||||||||||||| || ||| ||| ||||||  |||   ||||||

Sbjct  1041  GGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTTCGTTACCAGCACGTTATGGTGGGCA  1100

Query  967   CTCT-AGAGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCAT  1025

             |||| || |||||||||||||||||||||||||||||||||||||||||||||||| |||
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Sbjct  1101  CTCTAAG-GAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCAT  1159

Query  1026  GCCCCTTACGTCTTGGGCTACACACGTACTACAATGGTACGG-ACAGAGGGCAGC-AAGC  1083

             | || ||||| | ||||||||||||||  ||||||||| ||| ||||||||  || ||||

Sbjct  1160  GGCCTTTACGGCCTGGGCTACACACGTGTTACAATGGT-CGGTACAGAGGGTTGCCAAGC  1218

Query  1084  TAGCGA--TAGCAAGCA-AATCCCGTAAA-CCGTATC-TCAGTTCAGATCGCAGTCTGCA  1138

               ||||  | | | | | |||| |  ||| ||| ||| | ||| | ||| ||||| ||||

Sbjct  1219  C-GCGAGGTGG-A-G-ATAATCTCACAAAACCG-ATCGT-AGTCCGGATGGCAGTTTGCA  1272

Query  1139  ACTCGCCTGCGTGAAGGAGGAATCGCTAGTAATTGC-AGTCAG  1180

             ||||| ||||||||||  ||||||||||||||| || | ||||

Sbjct  1273  ACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAG  1315
```

5 Conclusion

The present investigation clearly revealed that DNA profiles appropriate for the inference of toxicity assessment of pesticides on cyanobacterial strains. Results of present study indicated the formation of gaps in pesticide treated 16S rDNA gene sequences of selected organisms. Resultant gaps so formed as a result of endosulfan and tebuconazole stress may be a trait of mismatching of purines and pyrimidines, degradation of hydrogen bonds, double strand or single strand breakage, or incorporation of incorrect nucleotide as well as formation of dimeric photoproducts. 16S rDNA sequencing also conferred that while endosulfan was the most toxic towards *W. prolifica* followed by *A. fertilissima* and *Aulosira fertilissima*, on the other hand, tebuconazole inferred highest toxicity towards *Aulosira fertilissima> W. prolifica> A. fertilissima.*

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References

Adams DG. 1988. Isolation and restriction analysis of DNA from heterocysts and vegetative cells of cyanobacteria. Journal of General Microbiology, 134: 2943-2949

Dizdaroglu M. 1992. Oxidative damage to DNA in mammalian chromatin. Mutation Research, 275(3-6): 331-342

Enti-Brown S, et al. 2012. Quality control analysis of imported fertilizers used in Ghana: the macronutrients perspective. Proceedings of the International Academy of Ecology and Environmental Sciences, 2(1): 27-40

Friedberg EC, Walker GC, Siede W, et al. 2006. DNA Repair and Mutagenesis. ASM Press, Washington DC, USA

Halliwell B, Gutteridge J. 2007. Free Radicals in Biology and Medicine. Oxford University Press, Oxford, UK

Holt JG, Krieg NR, Sneath PHA, 1994. Group 11. Oxygenic phototrophic bacteria. In: Bergey's Manual of Determinative Bacteriology (9th edn) (Hensyl WR, ed). 377-425, Williams &Wilkins, Baltimore, USA

Iteman I, Rippka R, Tandeau de Marsac N, et al. 2000. Comparison of conserved structural and regulatory domains within divergent 16S rRNA-23 S rRNA spacer sequences of cyanobacteria. Microbiology, 146: 1275-1286

Kerkhof L, Santoro M, Garland J. 2000. Response of soybean rhizosphere communities to human hygiene water addition as determined by community-level physiological profiling (CLPP) and terminal restriction fragment length polymorphism (TRFLP) analysis. FEMS Microbiology Letters, 184: 95-101

Kumar A, Tyagi MB, Jha PN. 2004. Evidences showing ultraviolet-B radiation induced damage of DNA in cyanobacteria and its detection by PCR assay. Biochemical and Biophysical Research Communications, 318: 1025-1030

Lau SKP, Fan RYY, Ho TCC, et al. 2011. Environmental adaptability and stress tolerance of *Laribacter hongkongensis*: a genome-wide analysis. Cell and Bioscience, 1: 22

Lindahl T. 1993. Instability and decay of the primary structure of DNA. Nature, 362(6422): 709-715

Moyer C, Dobbs F, Karl D. 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Applied and Environmental Microbiology, 60: 871-879

Nirmal Kumar JI, Rita NK, Manmeet Kaur A, et al. 2011a. Evidences showing 2, 4-D ethyl ester and pencycuron-induced DNA damage in cyanobacteria and detection by PCR. Toxicological and Environmental Chemistry, 93(5): 974-984

Nirmal Kumar JI, Anubhuti B, Manmeet Kaur A, et al. 2011b. An evaluation of pesticide stress induced proteins in three cyanobacterial species-*Anabaena fertilissima*, *Aulosira fertilissima* and *Westiellopsis prolifica* using SDS-PAGE. Advances in Environmental Biology, 5(4): 739-745

Pankratz FB, Doebel C, Farenhorst A, et al. 2003. Interactions between algae (*Selenastrum capricornutum*) and pesticides: implications for managing constructed wetlands for pesticides removal. Journal of Environmental Science and Health B, 38: 147-155

Rastogi RP, Sinha RP. 2011. Solar ultraviolet radiation-induced DNA damage and protection/repair strategies in cyanobacteria. International Journal of Pharma and Bio Sciences, 2(2): 271-288

Rippka R, Deruelles J, Waterbury JB, et al. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 111: 1-61

Valko M, Rhodes CJ, Moncol J, et al. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160(1): 1-40

Viti C, Mini A, Ranalli G, et al. 2006. Response of microbial communities to different doses of chromate in soil microcosms. Applied Soil Ecology, 34(2-3): 125-139

Zhang WJ, Jiang FB, Ou JF. 2011. Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences, 1(2): 125-144

Zhang WJ, Zhang XY. 2007. A forecast analysis on fertilizers consumption worldwide. Environmental Monitoring and Assessment, 133: 427-434