MOLECULAR IDENTIFICATION OF *ASPERGILLUS FLAVUS* AND ITS ABILITY FOR MALATHION BIODEGRADATION

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Pesticides are considered a vital part in agriculture worldwide. In spite of the fact that they enhanced the quality and yield of the agricultural production however they do have certain serious effects on the environment. This study was carried out for isolation and molecular identification of microorganisms from water naturally contaminated with pesticides. Moreover, also to evaluate the ability of the identified isolate for growth in the presence of malathion. Furthermore, the ability of identified isolate for potential degradation of malathion residue in aqueous media was evaluated. In this study based on morphological and molecular data, the selected isolate was identified as *A. flavus* with respect to its relation to the data from gene bank. Also the lowest nucleotide diversity value between the tested isolate and *A. flavus*, support the identification of selected isolate as *A. flavus.* The identified isolate has grown successfully in media supplemented with malathion much faster than without it. Hundred percent of malathion initial concentration was degraded within 36 days of incubation with *A. flavus*. Bioremediation of malathion residues in water using *A. flavus* isolate are promising and considered first reported.

Key words: Water. Malathion. Biodegradation. 18S rRNA genes.

INTRODUCTION

Use of pesticides in the agricultural activities is increasing with growing populations to fulfill the demand of food and currently 4 million tons of pesticides are used annually to world crops for pest control (USFDA 2015). Huge amount of pesticides accumulated in the environment as a result of their intensive use, subsequently the uptake and accumulation of these toxic pesticides in the food chain and drinking water induced substantial health hazard for the current and future generations. Malathion is an insecticide that is broadly utilized all over the world in light of its proficiency for controlling insects. It is evaluated that more than 13,500 tons of malathion are utilized every year in the USA as indicated by U.S. EPA (Tchounwou *et al*., 2015). Malathion has been most appropriate for the control of sucking insects on field crops, fruits, vegetable, livestock, etc. (Barlas 1996). Malathion is a highly toxic compound and is recorded by the United States

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Environmental Protection Agency (USEPA) depending of its toxicity in category Group 2A (US Department of Health and Human Services1999; Zheng and Hwang 2006). It is classified as carcinogenic to humans and animals. Its high-level exposure will affect nerve fibers and is neurotoxic in animals and immunity of higher vertebrates (US Department of Health and Human Services1999; Gurushankara *et al*., 2007; Budischak *et al*., 2009; Kumar *et al*., 2010; International Agency for Research on Cancer 2015). Malathion irreversibly inhibits the acetylcholinesterase enzyme that hydrolyzes acetylcholine (Jebali *et al*., 2006). Several international agencies such as Food and Drug Administration (FDA) and USEPA have allowed a maximum malathion concentration of a residue on specific crops used as foods (US Department of Health and Human Services 1999). The National Institute of Occupational Safety and Health (NIOSH) also recommends that malathion ambient air concentration of 250 $mg \cdot m^{-3}$ be considered as highly hazardous to human health and life. Moreover, malathion reacts with other chemicals in the presence of sunlight in the atmosphere to produce 40 times more toxic compound malaoxon but breaks down very quickly (US Department of Health and Human Services1999; International Agency For Research on Cancer 2015).

There are a lot of remediation methods for malathion and other pesticides such as electrochemical oxidation, Fenton oxidation, solvent extraction, chemical oxidation and adsorption (Chian *et al*., 1975; Pal and Vanjara 2001; Noradoun *et al*., 2005; Mulligan *et al*., 2011; Ganesapillai and Simha 2015). These remediation methods have their own disadvantages such as sludge generation, the formation of toxic metabolites, high cost, consume more energy and also these techniques can not be used to remove a wide range of pesticides. Therefore, the destruction of the malathion by biological methods is a promising method because of its low investment and low cost operations, and it is considered environmentally friendly. It is believed that microorganisms play an important role in the elimination and detoxification of pesticides from the environment. However, it is very important to isolate and identify microorganisms capable to degrade pesticides from natural water contaminated with pesticides. Moreover, morphological identification of microbial isolates could be insufficient for distinguishing certain species belonging to numerous sections. DNA sequences have been developed, included analysis of rDNA as a fast, reliable method and sensitive for detecting genetic relationships and diversity among microorganisms (Zhang *et al*., 2004). Nuclear rDNA are good targets for the phylogenetic analysis in fungi identifications (Munusamy *et al*., 2010). Some studies included analysis of rDNA for differentiation of *A. flavus* and *A. parasiticus* (El Khoury *et al*., 2011) and *A. niger* (Sabreen *et al*., 2015). In this report we isolate and molecularly identify *Aspergillus flavus* from water contaminated with pesticides for potential biodegradation of malathion in aqueous media, the ability of isolated fungus to grow in the presence of malathion as a source of carbon and energy was estimated, the efficiency to identify the isolate in bioremediation of malathion contaminated water was evaluated.

MATERIALS AND METHODS

Malathion with purity of 99.5% was obtained from the Central Laboratory for Pesticides, Agriculture Research Centre, Giza, Egypt. Other chemicals with high purity were obtained from Biojet for Fine Chemicals, Alexandria, Egypt. Mineral salt medium (M9) (MSM, pH 7.2) was prepared by adding 1.5 gm K_2HPO_4, 0.5 gm KH_2PO_4, 1.0 gm $(NH_4)_2SO_4$, 0.5 gm NaCl, 0.2 gm $MgSO_4$ and 0.02 gm $FeSO_4$ into 1000 mL of distilled water (Liu *et al*., 2012), then supplemented with the tested insecticide as the sole carbon source just before inoculation. The fungus was grown on potato dextrose agar medium (PDA).

FUNGAL ISOLATION AND MORPHOLOGICAL IDENTIFICATION

Enrichment culture of microorganisms capable to degrade the malathion was established from water samples which were collected from Metobess (El-Hokss drainage) in summer of 7/2016), at Kafr El-Sheikh Governorate, polluted by persistent organic pesticides (POPs) (Derbalah *et al*., 2016). Twenty (20) µL of water sample were added to 50 mL sterilized mineral salt medium in a flask (100 mL) containing 20 ppm of the tested insecticide as a sole source of carbon (Hassan 1999) and phosphorus (Subramanian *et al*., 1994) and incubated at 30°C and shaken at 150 rpm for 21 days. After that 10 mL of culture was transformed into fresh 50 mL MSL medium containing same concentration of the tested insecticide. This procedure was repeated four times. Dilution series were prepared after final time from enrichment culture in a flask containing 50 mL MSL medium up to $1{:}10^{-6}$ and then 100 µL of it was spread on plates MSL/insecticide (20 ppm) by using drigalsky triangle. The plates were sealed by polyethylene bags and incubated at 30°C for 7 days monitored for appearance of colonies. Single colonies growing on these diluted plates were isolated by picking the colonies using a sterile inoculation needle and were further purified by addition of ampicilline 800 mg/L to complex medium for fungal isolate (PDA) medium was used for fungal isolate). Identification of the fungal isolate was done at the Mycology Research and Survey of Plant Diseases Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt following the description given by McClenny (2005) and Diba *et al.* (2007).

Molecular identification

DNA was extracted using the CTAB method described by Doyle and Doyle (1990). Primers were designed using Primer3web ver., 4 according to the alimented sequences from GenBank. Partial sequence with size of 824pb for small-subunit rDNA (18S *rRNA* genes) was amplified, useing primer 5'-AGAAACGGCTACCACATCCA-3' for forward and 5'-TCTGGACCTGGTGAGTTTCC-3' for reverse. PCR molecular identification using 18S rRNA region of selected isolate was performed in a total volume of 50 µL containing 25-50 ng DNA, 50 mM each primer, 0.5 U/µL *Taq* DNA polymerase and 0.2 mM of each dNTPs. The amplification conditions were

performed as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 45 sec and extension at 72°C for 1 min as well as final extension at 72°C for 5 min. The PCR product was separated by 1.5% agarose gel electrophoresis in a 1X TAE, and stained with Red safe. The amplified products were sequenced by using the aforementioned primers. Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied BioSystems). The partial sequence was adjusted and alignments using BioEdit (Hall 1999). To determine relative phylogenetic positions of studied isolate with other related sequences in Genebank BLAST https://blast.ncbi.nlm.nih.gov/Blast.cgi was used. Neighbor-joining (NJ) trees and parsimony trees were constructed including gaps (Saitou and Nei 1987) using program adapted by Thomson *et al.* (1997). Nucleotide diversity was performed using the DnaSP program (Rozas *et al*., 2003).

Determination of microbial growth

The growth of the tested fungal isolate (A1) was measured as optical density (O.D) at wavelength of 405 nm (Antachopoulos *et al*., 2006, 2007) using a Spectrophotometer. Water samples contaminated with malathion in the presence of fungal isolate were collected at 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 days to assay the growth of the tested fungal isolate. The growth of fungal isolate was calculated as shown in the following equation: $X\ (gm\ L^{-1}) = 0.4033_{OD405} - 0.0057$ ($r^2 = 0.988$).

Bioremediation of the tested insecticide

The identified microbial isolate *A. flavas* (A1) were cultured onto MSL spiked with the tested insecticide (malathion), separately for 7 days and then the growing colonies were washed with three mL sterilized of MSL medium. The cell suspension of 10^8cfu/mL (cfu: colony forming unit) was used to inoculate 100 mL MSL medium containing 5 ppm of the tested insecticide. The cultures were incubated at 30ºC, pH (7) (Derbalah *et al*., 2008) and 150 rpm as optimum conditions for the growth of the tested microbial isolate for 36 days. Samples were collected at 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 days for monitoring the parent compound degradation of the tested insecticide. Control flasks of equal volume of MSL medium and the tested insecticide without the selected microbial isolate were run in parallel at all intervals to assess a biotic loss (Derbalah *et al*., 2008; Derbalah and Ismail 2013). The collected water samples of tested insecticide were filtered using syringe filtered (Derbalah and Ismail 2013) followed by HPLC analysis. A Shimadzu SCL-10AVP, Version 5.22 High performance liquid chromatography with reversed phase C_{18} column (Nova pack) and UV/visible detector was employed for malathion determination. The mobile phase was acetonitrile: water; (70:30) performed under isocratic elution with a flow rate of 1 mL $min^{-1.}$ UV/visible detector was fixed at 230 nm (Islam *et al*., 2009). In order to determine the degradation rate, plots of Ln concentration against time were made. The degradation rate constant

(slope) k was calculated from the following first order equation: $C_t=C_0e^{kt}$, where $(C)_t$ represents the concentration of the insecticide at time t, $(C)_0$ represents the initial concentration and k is the degradation rate constant. When the concentration falls to 50% of its initial concentration, the half-life ($t_{1/2}$) was estimated as shown in the following equation, $t_{1/2}$ = 0.693/K (Monkiedje and Spiteller 2005).

RESULTS AND DISCUSSION

The fungal isolate was identified as *Aspergillus* sp in Mycology Research and Survey of Plant diseases Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt following the description given by McClenny (2005) and Diba *et al*. (2007). The method to identify *Aspergillus* at the species level was developed using the 18S rRNA genes. The partial sequence with 824 bp was amplified, sequenced, and compared with other sequences in GeneBank. This gene was subsequently sequenced and phylogenetic relationships were assigned. Based on BLST, the selected isolate was closely related to various species of *Aspergillus* and is 98% identical with *A. favus* (Fig. 1). The tested isolate was partially aligned with 766 pb and clustered with *Aspergillus sp.* Based on phylogenetic tree, it was identical to *A. flavus* with 98% with only ten nucleotide substitutions found at positions of 585,551, 481, 351, 343, 151,100, 96, 81 and 50 (Fig. 2). Based on DNA sequence analysis, nucleotide diversity (π) between selected isolate and *A. flavus* (KM870530) was 0.013 with 10 singleton variable sites. On the other hand, it was 0.018 with 14 singleton variable sites between selected isolate and *A. fumigatus* (MG015949), while between selected isolate and *A. niger* (KM516789) it was 0.031 with 24 singleton variable sites.

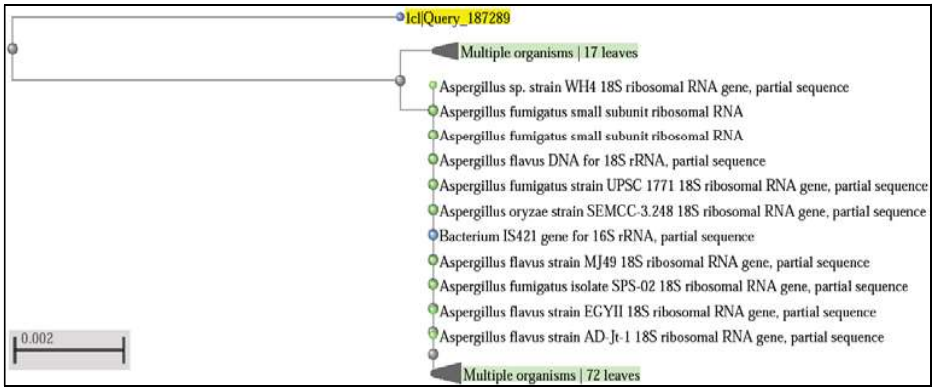

Fig. 1. Phylogenetic tree of the nucleotide sequences of the PCR product of 18S rRNA gene amplified from the DNA of *Asperigillus* isolate and universal fungi from BLAST.

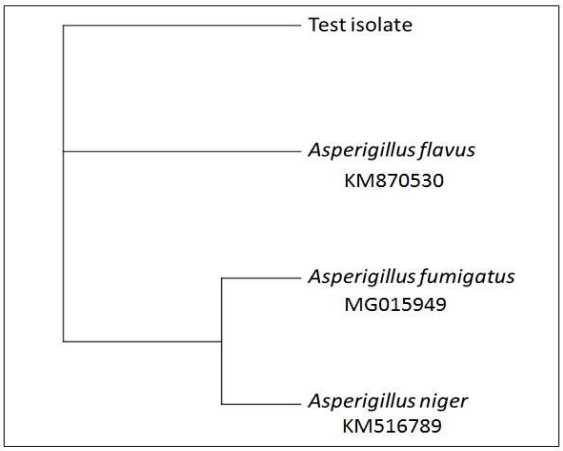

Fig. 2. Phylogenetic tree based on NJ method of tested *Asperigillus* isolate with other species in Gnen Bank.

THE GROWTH OF THE TESTED MICROBIAL ISOLATES

The growth of *A. flavas* in the presence of malathion was higher than its growth in the presence of medium without malathion. For *A. flavus* the biomass production was increased gradually in the presence of malathion after zero time of incubation and the highest biomass production was recorded at 32 days of incubation time with malathion (Fig. 3). After that the biomass production decreased once again till the end of incubation time.

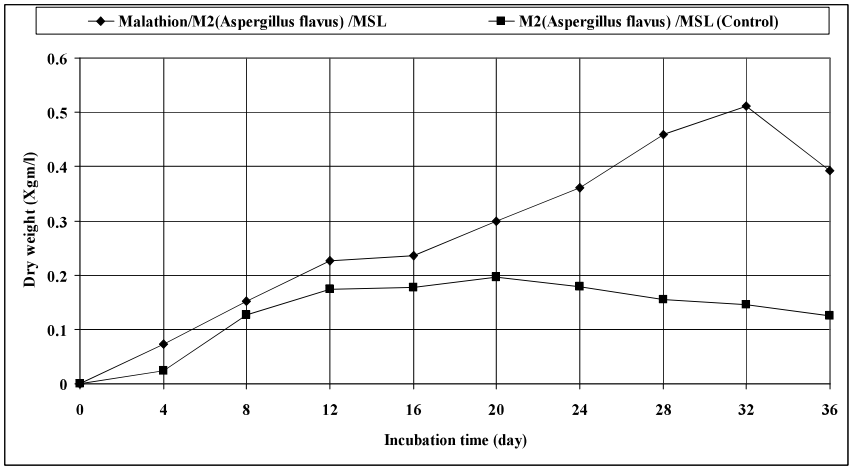

Fig. 3. Microbial growth of *A. flavus* isolate incubated with and without malathion in aqueous media.

BIOREMEDIATION OF THE TESTED INSECTICIDE IN AQUEOUS MEDIA

The ability of *A. flavus* isolates for biodegradation of malathion was illustrated in Table 1 and Fig. 4. *A. flavus* showed high potential in the biodegradation of the tested insecticide and about 100% of malathion were degraded within the 36 days of incubation. On the other hand, the degradation percentage of the tested insecticide was lower than 1.2 % at the end of incubation time in control or non-inoculated samples as shown in Fig. 4. As shown in Table 1, the degradation rate constant and half-life values of the tested insecticide by the tested microbial isolate and half-life value of malathion were 15.678 days in water treated with *A. flavus*.

Table 1
Degradation rate constant and half-life values of malathion under *A. flavus*

Degradation kinetics	Values (mean + SE)
Degradation rate constant (day $^{-1}$)	0.0442 ± 0.0001
Half-life ($t_{1/2}$) (day)	15.678 ± 0.011
R^2	0.99 ± 0.01

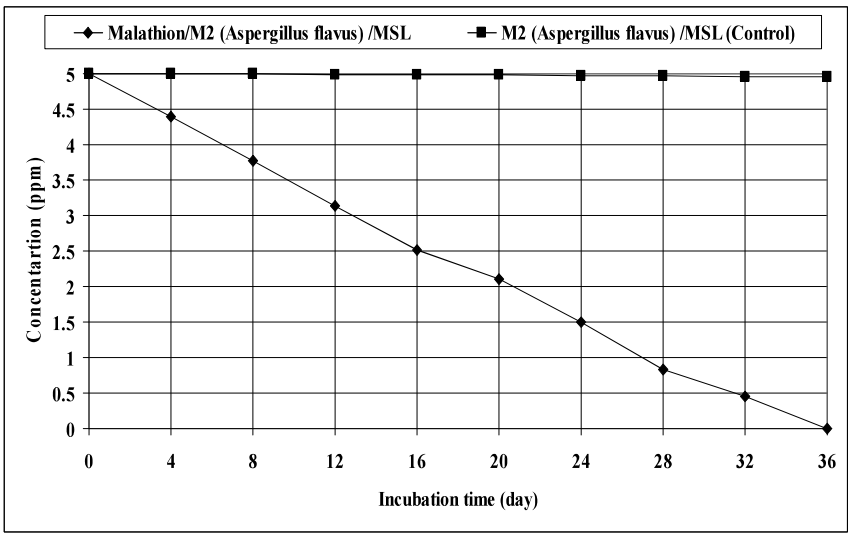

Fig. 4. Bioremediation of malathion at concentration level 5 ppm by *A. flavus isolate* in aqueous media relative to control.

In this study based on the morphological and physiological characters the isolated fungus was identified as *Aspergillus* sp. However, based on molecular identification using 18 S rRNA sequencing the isolate was classified as *A. flavus.* Also the lowest nucleotide diversity value between the tested isolate and *A. flavus* support the identification of selected isolate as *A. flavus.* The morphological

definition of microbial isolates is not highly recommended due to intraspecific similarities and differences between each other (Rodrigues *et al*., 2007). Moreover, *Aspergillus sp.* taxonomy is complex, with similar morphological and biochemical characteristics which make its morphological identification unreliable as a result of intraspecific similarities (Rodrigues *et al*., 2009; Gontia-Mishra *et al*., 2013). Therefore, molecular characterization seems to be an authoritative tool in the identification fungal isolates based on the sequencing of the 18 S rRNA genes (Hunt *et al*., 2004). Lee *et al*. (1998) reported that rDNA regions such as 18S and phylogenetic analysis have been used as targets for microbial isolates identification because they generally display sequence variation between species, but only slight variation within the same species (Lee *et al*., 1998). It could be concluded that the use of DNA sequences gives a better picture of *Aspergillus* identification.

Study of microbial growth rates is very important for extrapolation of the potential colonization capacity in the real water as it provides a good indication of the speed at which a microbe is able to colonize and transverse a substrate. Growth rates may also indicate which species may be dominant over a particular substrate; fast growing species have an advantage over slower species as they can reach and utilize resources before their competitors (Martin *et al*., 2009). The identified isolate in this study was able to grow well in culture medium in the presence of malathion, it is also able to survive and adapt in difficult environmental conditions (polluted areas). Also, the isolation of the identified microbe from water contaminated pesticides contributes for its higher growth in the presence of malathion than in the absence of it. In this manner, distinguished microbial detach are competent for malathion biodegradation and it affirms that the secluded microorganism utilized malathion as wellspring of carbon for its development. Thus this microbial isolate, having the ability to destroy the malathion biologically, confirms that it uses malathion as a source of carbon for its growth.

The results in this study indicated that the identified isolate showed potential effect in malathion biodegradation in aqueous media and the degradation rate was proportional to the growth rate of the identified isolate. This agrees with findings of Massoud *et al*. (2007) who reported that the growth response of malathion degrading isolates (represented in mycelia dry weight) was increased gradually by increasing malathion degradation percentages. Moreover, the degradation of malathion by *Asperigillus* sp. (EM8) and *Penicillium* sp. (EMT) has been reported by Hassan (1999). The results also indicated that the degradation of the malathion at the end of incubation time in control samples was negligible. This indicated that the abiotic losses of the tested insecticide are negligible. This implied that the quote of the tested insecticide decay due to temperature effect and photodecomposition and volatilization is very slight or completely absent (Derbalah *et al*., 2008). The biodegradation process of the malathion by the identified fungal isolate may be attributed to the secretion of enzymes such as cutinase enzyme (Kim *et al*., 2005) from fungal strain which is capable of degrading malathion (Karigar and Rao 2011).

CONCLUSION

The tested microbe was isolated and molecularly identified as *A. flavus.* The identified isolate showed much promise in the complete degradation and detoxification of malathion in contaminated water. To the best of our knowledge here we reported that *A. flavus* is a promising microorganism for malathion biodegradation. The designed primer could be used as a target of the most highly conserved regions of the 18S rRNA gene within *Aspergillus sp.*

REFERENCES

1. Antachopoulos C., Meletiadis J., Roilides E., 2006, Rapid susceptibility testing of medically important zygomycetes by XTT assay. *J Clinical Microbiol*, **44**: 553–60.
2. Antachopoulos C., Meletiadis J., Sein T., Roilides E., Walsh, T.J., 2007, Use of high inoculum for early metabolic signaling and rapid susceptibility testing of Aspergillus species. *J of Antimicrob Chemo*, **59**:230–237.
3. Barlas N.E., 1996, Toxicological assessment of biodegraded malathion in albino mice. *Bull Environ Contam Toxicol*, **57** (5): 705–712.
4. Budischak S.A., Belden L.K., William A.H., 2009, Relative toxicity of malathion to trematode-infected and noninfected *Rana palustris* tadpoles. *Arch Environ Contam Toxicol*, **56** (1): 123–128.
5. Derbalah A., Ismail A., 2013, Remediation technologies of diazinon and malathion residues in aquatic system. *Environ Protect Engin*, **39**(3):135–147.
6. Derbalah A.S., Ismail A.A., Shaheen S.M., 2016, The presence of organophosphorus pesticides in wastewater and its remediation technologies. *Environ Engin and Manag J*, **15**(8):1777–1787.
7. Derbalah A.S., Massoud A.H., Belal E.B., 2008, Biodegrability of famoxadone by various microbial isolates in aquatic system. *Land Contaminat Reclamat*, **16**:13–23.
8. Diba K., Kordbacheh P., Mirhendi S.H., Rezaie S., Mahmoudi M., 2007, Identification of Aspergillus species using morphological characteristics. *Pakist J Med Sci*, **23**(6): 867–872.
9. Doyle J.J. and Doyle J.L., 1990, A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Focus*, **12**:13–15.
10. El Khoury A., Atoui A., Rizk T., Lteif R., Kallassy M., Lebrihi A., 2011, Differentiation between *Aspergillus flavus* and *Aspergillus parasiticus* from Pure Culture and Aflatoxin-Contaminated Grapes Using PCR-RFLP Analysis of aflR-aflJ Intergenic Spacer. *J Food Sci*, **76**:247–253.
11. Ganesapillai M. and Simha P., 2015, The rationale for alternative fertilization: equilibrium isotherm, kinetics and mass transfer analysis for urea-nitrogen adsorption from cow urine. *Resour Efficient Technol*, **1**: 90–97.
12. Gontia-Mishra I., Dhanshree D., Niraj T., Khushboo B., Keerti T., Sharad T., 2013, Isolation, morphological and molecular characterization of phytate-hydrolysing fungi by 18S rDNA sequence analysis. *Brazil J of Microbiol*, **44**(1):317–323.
13. Gurushankara H.P., Krishnamurthy S.V., Vasudev V., 2007, Effect of malathion on survival, growth, and food consumption of Indian cricket frog (*Limnonectus limnocharis*) tadpoles. *Arch Environ Contam Toxicol*, **52**(2): 251–256.
14. Hall T.A., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp*, **41**:95–98.
15. Hassan H.A., 1999, Fungal utilization of organophosphorus pesticides and their degradation by *Aspergillus flavus* and *A. sydowii* in soil. *Folia Micrbiol*, **44**(1):77–84.
16. Hunt J., Boddy L., Randerson P.F., Rogers H.J. 2004. An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. *Microb Ecol*, **47**:385–395.

17. International Agency for Research on Cancer (IARC), 2015, Evaluation of five organophosphate insecticides and herbicides (WHO), 20 March, Monographs 112.
18. Islam S., Afrin N., Hossain M.S., Nahar N., Mosihuzzaman M., Mamun M.I.R., 2009, Analysis of some pesticide residues in cauliflower by high performance liquid chromatography. *American J of Environ Sci*, **5**(3):325–329.
19. Jebali J., Banni M., Guerbej H., Almeida E.A., Bannaoui A., Boussetta H., 2006, Effects of malathion and cadmium on acetylcholinesterase activity and metallothionein levels in the fish *Seriola dumerilli*. *Fish Physiol Biochem*, **32**(1): 93–98.
20. Kim Y.H., Ahn J.Y., Moon S.H., Lee J. 2005, Biodegradation and detoxification of organophosphate insecticide malathion by *Fusarium oxysporum* f. sp. *pisi* cutinase. *Chemo*, **60**:(10) 1349–1355.
21. Kumar R., Nagpure N.S., Kushwaha B., Srivastava S.K., Lakra W.S., 2010, Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay, *Arch Environ Contam Toxicol*, **58**(1): 123–130.
22. Lamb D.C., Lei L., Warrilow A.G., Lepesheva G.I., Mullins J.G., Waterman M.R. 2009. The first virally encoded cytochrome P450. *J of Virol*, **83**(16):8266–8269.
23. Lee C.H., Helweg-Larsen J., Tang X., Jin S., Li B., Bartlett M.S., Lu J.J., Lundgren B., Lundgren J.D., Olsson M., Lucas S.B., Roux P., Cargnel A., Atzori C., Matos O., Smith J.W., 1998, Update on *Pneumocystis carinii* f. sp. *hominis* typing based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. *J Clin Microbiol*, **36**:734–741.
24. Liu Z., Chen X., Shi Y., 2012, Bacterial degradation of chlorpyrifos by *Bacillus cereus*. *Adva Mater Res*, **356**(360):676–680.
25. Martin M.M.B., Perez J.A.S., Lopez J.L.C., Oller I., Rodriguez S.M., 2009, Degradation of a four pesticides mixture by combined photo-Fenton and biological oxidation. *Wat Res*, **43**(3):653–660.
26. Massoud A.H., Derbalah A.S., Belal A.B., El-Fakharany I.I., 2007, Bioremediation of malathion in aquatic system by different microbial isolates. *J Pest Cont Environ Sci*, **15**(2):13–28.
27. McClenny N., 2005, Laboratory detection and identification of Aspergillus species by microscopic observation and culture: the traditional approach. *J Medical & Vet Mycol*, **43**:125–128.
28. Monkiedje A., Spiteller M., 2005, Degradation of metalaxyl and mefenoxam and effects on the microbiological properties of tropical and temperate soils. *Inter J Environ Res Public Health*, **2**:272–285.
29. Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Eng Geol*, **60**(1):193–207.
30. Munusamy M., Ayyathurai V., Muthusamy K., Subramanian M., Vaikuntavasan P., Rethinasamy V., 2010, Analysis of molecular variability among isolates of *Aspergillus flavus* by PCR-RFLP of its regions of rDNA. *J plant Protect Res*, **50**(4):446–451.
31. Noradoun C.E., Mekmaysy C.S., Hutcheson R.M., Cheng I.F., 2005, Detoxification of malathion a chemical warfare agent analog using oxygen activation at room temperature and pressure, *Green Chem*, **7**(6):426–430.
32. Pal O.R. and Vanjara A.K., 2001, Removal of malathion and butachlor from aqueous solution by clays and organoclays, *Sep Purif Technol*, **24**(1):167–172.
33. Rodrigues P., Soares C., Kozakiewicz Z., Paterson R., Lima N., Venancio A., 2007, Identification and characterization of *Aspergillus flavus* and aflatoxins. In: Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Edited by Méndez-Villas A. Badajoz: Formatex; 527–534.
34. Rozas J., Sanchez-DelBarrio J.C., Messeguer X., Rozas R., 2003, DnaSp, DNA polymorphism analyses by the coalescent and other methods. *Bioinformat*, **19**:2496–2497.
35. Sabreen A.A.K., Al-Jaryawi H.A., Al-Marzoqi A.H., 2015, Phenotypic and Genotyping Study of *Aspergillus Niger*: Molecular Detection of Calmodulin, 18srRNA and Pepsin like Protease Genes Based on Multiplex PCR. *Food Sci and Qual Manag*, **35**:1–7.
36. Sogin M.L., Morrison H.G., Huber J.A., Mark Welch D., Huse S.M., Neal P.R., Jesus M., Arrieta. , J.M., Herndl G.J., 2006, Microbial diversity in the deep sea and the underexplored rare biosphere. *Proc Nat Acad Sci USA*, **103**:12115–12120.

37. Subramanian G., Sekar S., Sampoomam S., 1994, Biodegradation and utilization of organophosphorus pesticides by cyanobacteria. *Inter Biodeterorat and Biodegradat*, **33**(2):129–143.
38. Tchounwou P.B., Patlolla A.K., Yedjou C.G., Moore P.D., 2015, Environmental exposure and health effects associated with malathion toxicity. *Toxicity and Hazard of Agrochem*, **71**.
39. Thomson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G., 1997, The CLUSTAL_X windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools. *Nucleic Acids Res*, **25**:4876–4882.
40. US Food and Drug Administration Center of Drug 2015, Evaluation and Research Using batch and fixed bed-reactors. *Appl Catal A*, **333**: 122–130.
41. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry 1999, Toxicological profile for asbestos, Update, Agency for Toxic Substances and Disease Registry.
42. Zhang Z.G., Zhang J.Y., Zheng X.B., Yang Y.W., Ko W.H., 2004, Molecular distinctions between *Phytophthora capsici* and *P. tropicalis* based on ITS sequences of ribosomal DNA. *J Phytopathol*, **152**(6):358–364.
43. Zheng Y., Hwang H.M., 2006, Effects of temperature and microorganisms on malathion transformation in river water. *Bull Environ Contam Toxicol*, **76**(4):712–719.