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ORIGINAL PAPER

Isolation and molecular identification of *Aspergillus flavus* and the study of its potential for malathion biodegradation in water

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Abstract
In spite of the fact that pesticides enhanced the quality and yield of the agricultural production however do have certain serious effects on the environment. This study was carried out for isolation and molecular identification of microorganisms from water for malathion biodegradation in aquatic system. PCR analysis was used for identification of the isolated fungus. The growth kinetics of *A. flavus* in the presence of malathion under different environmental factors (pH, temperature and malathion concentration) were evaluated. Furthermore, the degradation kinetics of malathion by *A. flavus* in aqueous media under different environmental factors was evaluated. The isolated microorganism was identified as *A. flavus* with respect to it relation to the data from the gene bank and the lowest nucleotide diversity value between the tested isolate and *A. flavus*. The identified isolate grew successfully in a 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*. The temperature of 30 °C, pH value of 7 and malathion initial concentration of 5 mg/l were the optimum conditions of *A. flavus* for growth and degradation of malathion. Bioremediation of malathion residues in water using *A. flavus* isolate are promising and considered the first report.

Keywords Water · Malathion · Biodegradation · Molecular identification · 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 ton of pesticides are used annually to world crops for pest control (Goda et al. 2010; USFDA 2015). A huge amount of pesticides accumulated in the environment as a result of intensive using of these pesticides, 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 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. (Rettich 1979; Chambers 1992; Barlas 1996). Malathion is a highly toxic compound and is recorded by the United State Environmental Protection Agency (USEPA) depend on its toxicity in category Group 2A (US Department of Health and Human Services 1999; Zheng and Hwang 2006). Its high-level exposure will affect nerve fibers and is neurotoxic in animals and immunity of higher vertebrates (US Department of Health and Human Services 1999; Gurushankara et al. 2007; Budischak et al. 2009; Kumar et al. 2010; International Agency for Research on Cancer 2015). 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). 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 Services 1999; Durkin 2008; Inter-Organization Programme for the Sound Management of Chemicals and World Health Organization WHO 2010; International Agency For

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Research on Cancer 2015). Therefore remediation of pesticide residues in water considered a source of major concern.

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 cannot be used to remove a wide range of pesticides. Therefore, the destruction of the malathion by biologic 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 biodegradation and detoxification of organophosphorus insecticides from the environment (DaSilva et al. 2013, 2014, 2015; Alvarenga et al. 2015, 2018).

However, it is very important to isolate and identify microorganisms that 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 section numerous. However, 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). Moreover, using nuclear rDNA, considered a good target 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).

Control and improvement of bioremediation processes is a complex system of many factors. The success of bioremediation depends not only on the high decomposition capacity but also on the stability of active microorganisms under various conditions, such as changes in pH, temperature and concentration of pesticides (Derbalah et al. 2008). Therefore, it is necessary to study the effects of different environmental factors on the growth potential of tested organisms (Derbalah et al. 2008).

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 *Aspergillus flavus* to grow in the presence of malathion as a source of carbon and energy was estimated, the efficiency of *Aspergillus flavus* for bioremediation of malathion in water was evaluated and finally the effect of different environmental factors (pH, temperature and malathion initial concentration) on the ability of *Aspergillus flavus* for growth and degradation potential of malathion was also investigated.

Materials and methods

Chemicals

Malathion with a purity of 99.5% was obtained from Central laboratory for Pesticides, Agriculture Research Centre, Giza, Egypt. Disodium hydrogen orthophosphate (Na_2HPO_4) with 99% purity, potassium dihydrogen orthophosphate KH_2PO_4 with 99% purity, ammonium sulfates $(NH_4)_2SO_4$ with 98.5% purity, sodium chloride (NaCl) with 98% purity, magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$) with 98% purity, calcium chloride ($CaCl_2{\cdot}2H_2O$) with 98% purity, agar with 98% purity were obtained from El-Nasr Pharmaceutical Chemical Company, Abu Zaabal, Cairo, Egypt. Ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) with 99% purity and dextrose ($C_6H_{12}O_6$) with 98% were obtained from Biojet for Fine Chemicals, Alexandria, Egypt.

Growth media

Mineral salt medium (M9) (MSM, pH 7.2) was prepared by adding 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0.5 g NaCl, 0.2 gm $MgSO_4$ and 0.02 g $FeSO_4$ into 1000 ml of distilled water (Liu et al. 2012a). Then the pH was adjusted to 7.2 using sodium hydroxide solution and the medium autoclaved (121 °C for 15 min) then supplemented with the tested insecticide as the sole carbon source just before inoculation (Chaudhry and Ali 1988). The fungus was grown on potato dextrose agar medium (PDA) containing potato (peeled) 200 g, dextrose (20 g) and agar (15 g) were pot in distilled water (1000 ml) having pH of 5 based on the method described by Vincent (1970). Then was autoclaved at 121 °C for 20 min (Maheswari and Ramya 2013).

Isolation

Enrichment culture of microorganism capable to degraded the malathion was established from water samples which collected from Metobess (El-Hokss drainage) Summer of 7/2016), at Kafr El-Sheikh Governorate, which polluted by persistent organic pesticides (POPs) (Derbalah et al. 2016). Twenty (20) µl of water sample was added to 50 ml sterilized mineral salt medium in 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 shake at 150 rpm for 21 days. After that, 10 ml of culture was transformed into a fresh 50 ml MSL medium containing the same concentration 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 drigalisky triangle. The plates were sealed by polyethylene bags and incubated at 30 °C for 7 days monitored for the appearance of colonies. Single colonies growing on these diluted plates were isolated by picking the colonies using sterile inoculation needle and were further purified by addition of ampicillin 800 mg/l to complex medium for fungal isolate (PDA) medium was used for fungal isolate).

Morphological identification of the isolated microorganism

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). Macroscopic (colony diameter, exudates and colony reverse) and microscopic (conidiophores, vesicle, metulae, phialides, and conidia) characteristics were used to identify the characters of the fungal isolate. Microscopic characteristics slides were prepared by staining it with cotton blue and mounted in lactophenol. Naked eye and at low magnification power of microscope was used the first morphological examination of the tested isolate. After that further investigation was done by measuring the dimensions of the microscopic structures, photographing the microscopic structures and using relevant literature as reference (McClenny 2005).

Identification using 18S rRNA gene analysis

DNA isolation and primer design

Fungal mycelium or spores were cultured on potato dextrose broth medium. The flasks were incubated at 30 °C for 2–3 days with shaking. The fungal mycelium was used for DNA isolation. DNA was extracted using a CTAB method described by Doyle and Doyle (1990). Primers were designed using Primer3web ver., 4 (https://primer3.ut.ee/) according to the alimented sequences from GenBank accession No., KM870530, HQ393872, MG015949, KM516789, and KF175513. Partial sequence with a size of 824pb for small-subunit rDNA (18S *rRNA* genes) was amplified. The used primer sequences was 5′-AGAAACGGCTACCACATCCA-3′ for forward and 5′-TCTGGACCTGGTGAGTTTCC-3′ for reverse.

PCR amplification and 18S rRNA sequence determination

PCR molecular identification using 18S rRNA region of the 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 follow: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 45 s and extension at 72 °C for 1 min as well as a 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. PCR amplification of the 18S rRNA region yielded a 824 bp band. The amplified products were sequenced by using the aforementioned primers. Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied Bio Systems).

Data analysis and phylogenetic analysis

Sequence DNA of partial 18S rRNA 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). Multiple alignments of the sequences were carried out against corresponding nucleotide sequences retrieved from GenBank.

Optimization the growth conditions of *A. flavus*

The growth kinetics of the identified microbial isolate using MSL medium in the presence and absence of malathion (5 mg/l) was examined under different environmental factors (temperature, pH, and malathion initial concentration). Samples 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 absorbance value for each sample was measured as optical density (O.D) at a wavelength of 405 nm (Antachopoulos et al. 2006, 2007) using a Spectrophotometer. Then, the growth of *A. flavus* was calculated using Eq. 1. This equation was obtained from the relationship between absorbance and dry weight of serial known concentrations of fungal spore suspension (10^2 to 10^6 cfu/ml) after growing for 7 days. Dry weight of serial known concentrations of the fungus was obtained by filtration and drying each known concentration (fungal suspension) in the oven at 70 °C overnight, and weighting the dried fungal biomass.

$$X\left(\text{gm L}^{-1}\right) = 0.4033_{\text{OD405}} - 0.0057, \left(r^2 = 0.988\right) \quad (1)$$

where, 0.4033 is the slope value of the straight line, OD_{405} is the absorbance value at 405 nm wavelength, 0.0057 is the value of the truncated part of the y-axis, X (gm L^{-1})

is the biomass value for fungal isolate and $r^2 = 0.988$ is the correlation coefficient value of relation.

To determine the effect of pH on the growth of identified isolate, experiments were carried out as mentioned above at pH values of 5, 7 and 9 at 30 °C and malathion initial concentration level of 5 mg/l. To determine the effect of temperature on the growth of *A. flavus*, experiments were carried out as mentioned above under different temperature (20, 25 and 30 °C) at pH value of 7 and malathion initial concentration of 5 mg/l. To determine the effect of malathion initial concentration on the growth of *A. flavus*, experiments were carried out as mentioned above at concentration of 1, 2, 5 and 10 mg/l at pH value of 7 and temperature degree of 30 °C.

Degradation kinetics of malathion

These experiments were carried out to determine the degradation kinetics of malathion by *A. flavus* under different environmental factors (temperature, pH, and malathion initial concentration). To determine the effect of malathion initial concentration on degradation of malathion by *A. flavus,* experiments were carried out using 100 ml MSL medium supplemented with malathion at different concentrations (1, 2, 5 and 10 mg/l) at pH value of 7 and temperature degree of 30 °C. MSL medium was inoculated by 1 ml from fungal suspension with a concentration of 10^4 cfu/ml and the mixture was shacked on a rotary shaker for 8 h every day. Then 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 an equal volume of MSL medium and the tested insecticide without the selected microbial isolate were run in parallel at all intervals to asses abiotic loss (Derbalah et al. 2008; Derbalah and Ismail 2013). The collected water samples were filtered using syringe filtered (Derbalah and Ismail 2013) followed by HPLC analysis. To determine the effect of pH on degradation of malathion by *A. flavus,* experiments using MSL medium with pH values of 5, 7 and 9 at 30 °C and malathion initial concentration level of 5 mg/l was carried out as mentioned before. To determine the effect of temperature on the degradation of malathion by *A.flavus*, experiment using MSL medium with pH of 7 was incubated at 20, 25 and 30 °C and with malathion initial concentration level of 5 mg/l was carried out as mentioned before.

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).

Calculation of degradation rate and half-life time

In order to determine the degradation rate, plots of Ln concentration against time were made. The degradation rate constant (slope), k was calculated from Eq. 2

$$C_t = C_0 e^{-kt} \quad (2)$$

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 Eq. 3 (Monkiedje and Spiteller 2005).

$$t_{1/2} = 0.693/\,K \quad (3)$$

Data analysis

For analysis of variance (ANOVA)of obtained data, XLSTAT PRO statistical analysis software (Addinsoft) was used. Fisher's least significant difference (LSD) test was used to separate the mean of each treatment. All analyses were performed at a significance value of $P \leq 0.05$.

Results

Identification of the tested isolate

Morphological identification

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).

Molecular identificaion

The method to identify Aspergillus at the species level was developed using the 18S rRNA genes (Fig. 1). 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 has 98% identical with *A. flavus* (Fig. 2). The tested isolate was partially alimented with 766 pb and clustered with *Aspergillus* sp. Based on the phylogenetic tree, it was identical to *A. flavus* with 98% with only ten nucleotide substitutions were found at positions of 585,551, 481, 351, 343,

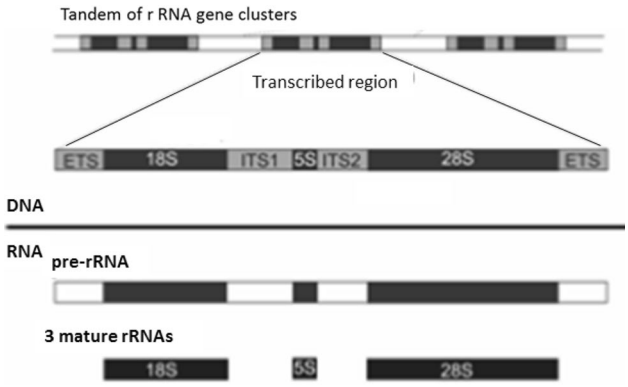

Fig. 1 Diagram illustration of 18S rDNA region with ITS regions

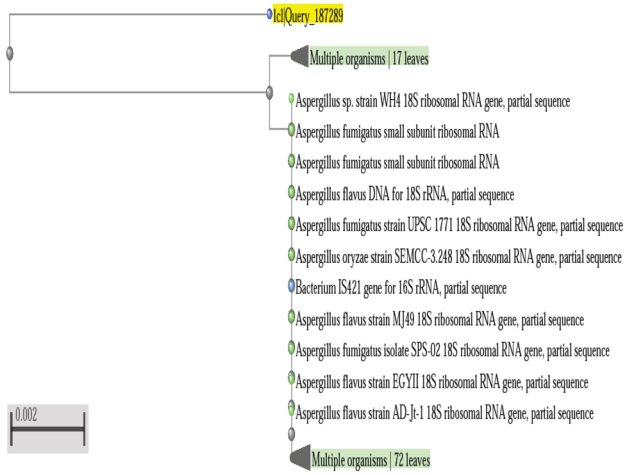

Fig. 2 Phylogenetic tree of the nucleotide sequences of the PCR product of 18S rRNA gene amplified from the DNA of *Asperigillus* isolate and universal fungi from BLAST

151,100, 96, 81 and 50 (Fig. 3). 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.

In this study, one microbe was isolated from water contaminated with pesticides by enrichment culture and purified in a specific medium. The Isolation of microbes by means of standard culturing for biotechnological purposes is actually impressive, and their applications vary from industrial procedures of the food, and pharmaceutical, to growing areas such as bioremediation of water contaminated with pesticides.

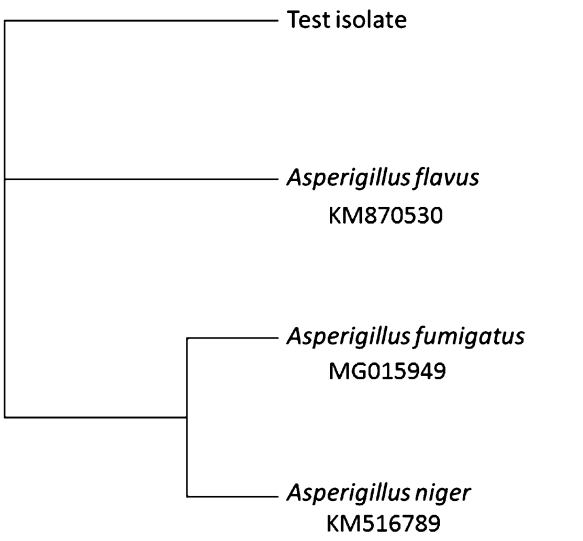

Fig. 3 Phylogenetic tree based on NJ method of tested *Asperigillus* isolate with other *Asperigillus* sp. in GneBank

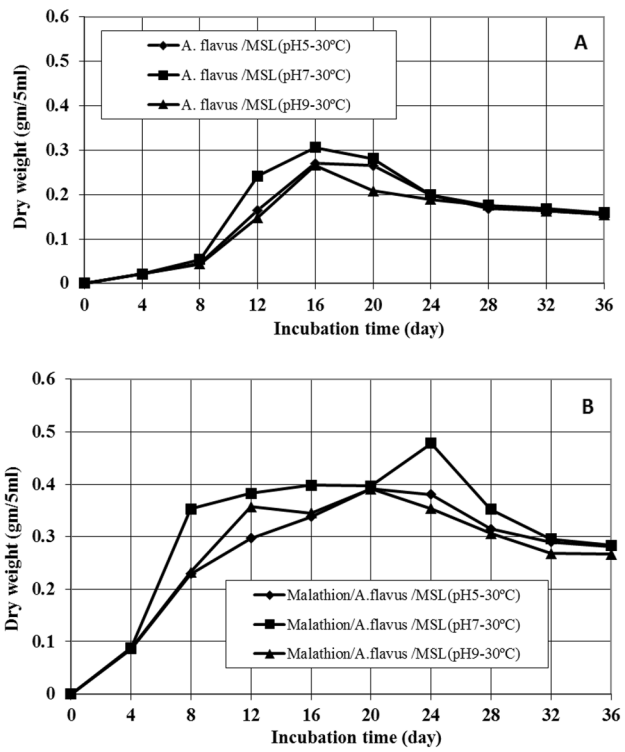

Fig. 4 Microbial growth of *A. flavus* in MSL medium with (**b**) and without malathion (**a**) under a different pH values in aqueous media

Optimization the growth conditions of *A. flavus*

The growth of *A. flavus* in the presence of malathion was higher than its growth in the presence of medium without malathion. The biomass production of *A. flavus* 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 (Figs. 4–6). After that, the biomass production decreased once again till the end of incubation time. Regarding the pH impact on the growth of *A. flavus*, the fungus grew at a quite wide range of pH from 5 to 9 (Fig. 4) and the optimum pH was 7 for fungal isolate since the maximum growth for *A. flavus* was recorded at pH 7 (Fig. 4). However, for temperature effect, the results showed that *A. flavus* exhibited growth at 20 and 30 °C however, the highest growth of the identified microbial isolate was recorded under temperature degree of 30 °C and this temperature appears to be the optimum degree for growth of *A. flavus* (Fig. 5). Furthermore, the results showed that the growth of *A. flavus* increased with increasing malathion initial concentration and the highest growth of the identified microbial isolate was recorded at malathion initial concentration of 5 mg/L (Fig. 6).

Biodegradation kinetics of malathion by *A. flavus*

The ability of *A. flavus* for biodegradation of malathion under different environmental factors was illustrated in Table 1 and Figs. 7–9. The results showed that the optimum pH for malathion degradation by *A. flavus* was

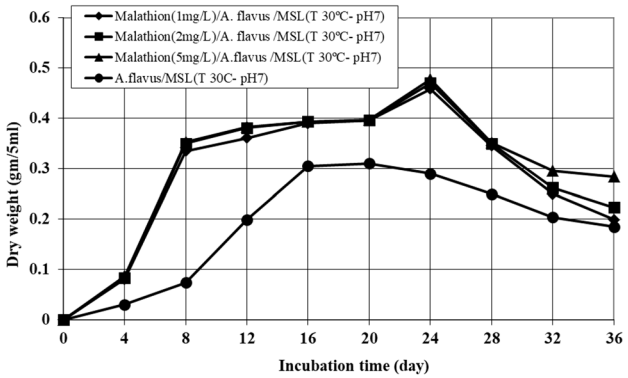

Fig. 6 Microbial growth of *A. flavus* in aqueous media under different initial concentrations of malathion

7 which showed the highest degradation rate of malathion by *A. flavus* compared to other pH values (Fig. 5). The highest degradation rate of malathion *A. flavus* was recorded at temperature degree of 30 °C and it appears to be the optimum degree for the degradation of malathion by *A. flavus* (Fig. 8). Moreover, the considerable degradation rate of malathion was achieved by by *A. flavus*

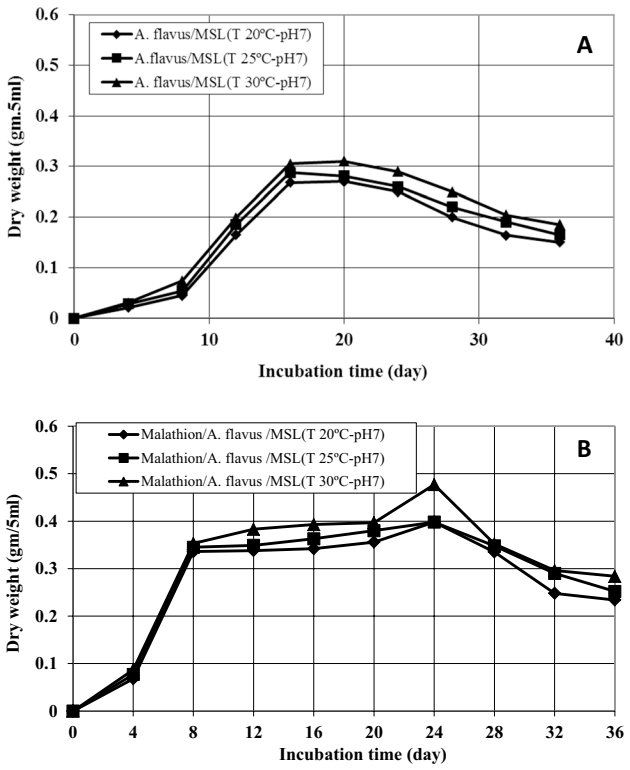

Fig. 5 Microbial growth of *A. flavus* under different temperatures in MSL medium with (**b**) and without (**a**) malathion in aqueous media

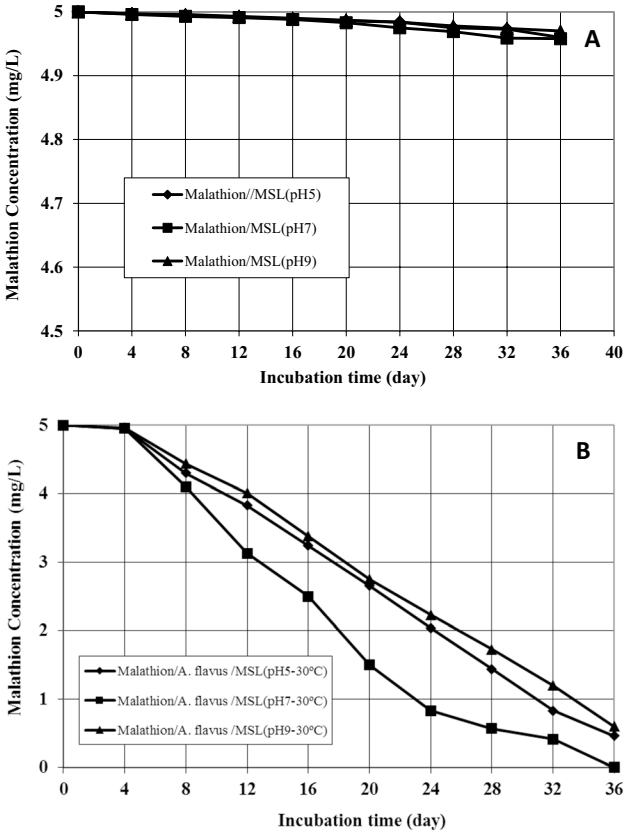

Fig. 7 Microbial degradation of malathion with (**b**) and without (**a**) *A. flavus* in aqueous media under different initial pH values

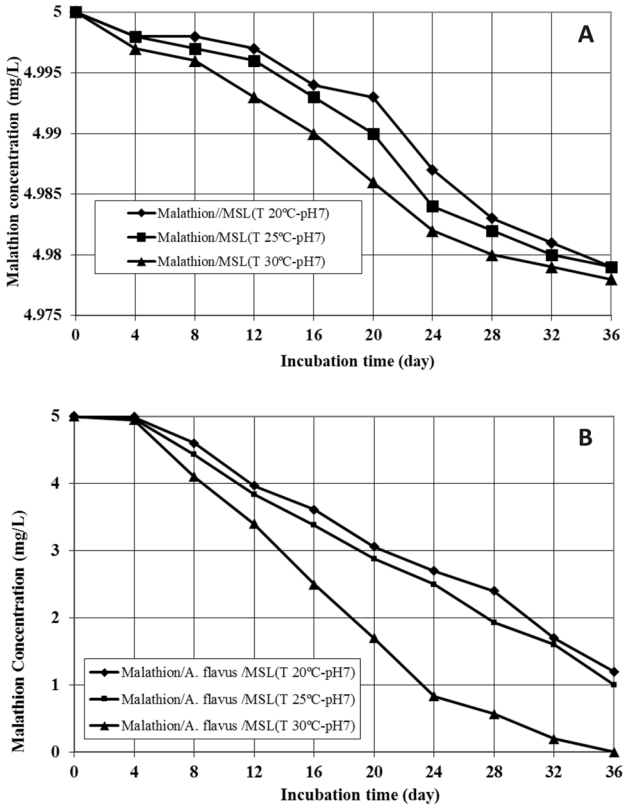

Fig. 8 Microbial degradation of malathion with (**b**) and without (**a**) *A. flavus* in aqueous media under different temperatures

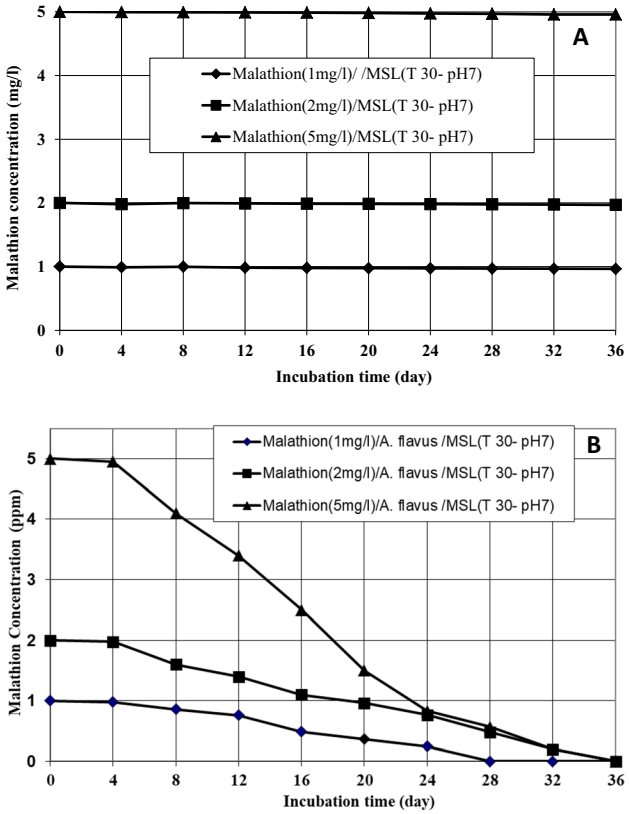

Fig. 9 Microbial degradation of malathion with (**b**) and without (**a**) *A. flavus* in aqueous media under different initial concentrations of malathion

exhibited at 20 and 40 °C. The effect of different malathion initial concentration on the degradation of malathion by *A. flavus* is shown in Fig. 9. The degradation rate of malathion by *A. flavus* was increased as a function of its initial concentration and the highest degradation rate of malathion was recorded at initial concentration of 5 mg/l. The results showed that the pH value of 7, temperature degree of 30 °C, and malathion initial concentration of 5 mg/l were the optimum conditions for achieving the highest degradation rate of malathion (100% degradation of malathion) by *A. flavus*.

The degradation rate constant and half-life values of the malathion by *A. flavus* under different environmental conditions are shown in Table 1. Half-life values of malathion were 24.4, 18.53 and 8.51 days in water treated *A. flavus* under 20, 25 and 30 °C, respectively. Half-life values of malathion were 15.78, 8.51 and 17.86 days in water treated *A. flavus* under pH values of 5, 7 and 9, respectively. Half-life values of malathion were 11.3, 15.47 and 8.51 days in water treated *A. flavus* under initial concentration of values of 1, 2 and 5 mg/L, respectively.

Table 1 Degradation rate constant and half-life values of malathion by *A. u* under different environmental conditions

Parameters	Degradation rate constant (day^{-1})	Half-life ($t_{1/2}$) (day)	R^2
pH			
5	0.044 ± 0.003a	15.78 ± 0.52a	0.94
7	0.081 ± 0.004c	8.51 ± 0.32c	0.93
9	0.039 ± 0.001ab	17.86 ± 1.10ab	0.95
Temperature (°C)			
20	0.028 ± 0.001a	24.40 ± 1.25a	0.97
25	0.037 ± 0.002b	18.53 ± 1.10b	0.9
30	0.081 ± 0.004c	8.51 ± 0.53a	0.93
Initial concentration of malathion (mg/l)			
1	0.056 ± 0.002a	11.33 ± 0.87a	0.94
2	0.041 ± 0.003b	15.47 ± 1.45b	0.98
5	0.081 ± 0.003c	8.51 ± 0.78c	0.93

Different letters indicate significant differences by Fisher's LSD at $P \leq 0.05$

Discussion

In this study, based on morphological and physiological characters, this microbe 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. 2007; Gontia-Mishra et al. 2013). Therefore, molecular characterization seems to be an authoritative tool in the identification of 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 *A. flavus* in this study was able to grow well in culture medium in the presence of malathion, it is able to survive and adapt to difficult environmental conditions (polluted areas). Also, the isolation of *A. flavus* from water contaminated pesticides contributes to 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 a wellspring of carbon for its development. Thus *A. flavus* having the ability to destroy the malathion biologically, confirms that it is used malathion as a source of carbon for its growth.

The results in this study indicated that *A. flavus* 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 (representing in mycelial dry weight) was increased gradually by increasing malathion degradation percentages. Moreover, the degradation of malathion by *Asperigillus* sp.(EM8) and *Penicillium* sp.(EMT) have 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 is indicating that the abiotic losses of the tested insecticide are negligible. This is 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 the fungal strain which is capable of degrading malathion (Karigar and Rao 2011).

Recently, it has been reported by many researchers that optimization of different environmental factors can play a vital role in accelerating the process of insecticides biodegradation (Derbalah et al. 2008; Vijayalakshmi and Usha 2012; Liu et al. 2012a, b). Therefore, in this study, the ability of *A. flavus* for growth and degradation of malathion was carried out as a function of different environmental temperatures, pH values, and malathion initial concentrations. The results showed that the optimum conditions for *A. flavus* to achieve the highest degradation rate of malathion were a pH value of 7, temperature degree of 30 °C and malathion initial concentration of 5 mg/l This result are in agreement with the findings of Derbalah et al. (2008). Moreover, *A. flavus* showed significant degradation of malathion under a wide range of pH and temperature. This variation is useful for using *A. flavus* in the degradation of malathion under different environmental conditions Derbalah et al. (2008). Moreover, it can be expected that this microbial isolate can tolerate the pH and temperature change during the degradation process thereby increase their degradation potential Derbalah et al. (2008).

The results also showed that the degradation of malathion by *A. flavus* increased with increasing the initial concentration of malathion. This is maybe due to the rate of the rates of uptake and mineralization of pesticides in the water were found to be proportional to the initial concentration of the substrate and the maximum specific growth rate of degrading microbes increased with the concentration. The high concentration of malathion leads to a high growth of *A. flavus* that use malathion as a source of carbon and energy and subsequently high degradation rate of malathion is expected.

In the study also the degradation rate of malathion by *A. flavus* was affected by temperature and this is maybe due to that the most microorganisms have the optimum growth temperature, and the degrading enzyme also has

the optimum reaction temperature. The change of temperature can affect the metabolism of microorganisms, the enzyme activity of degrading enzymes and even the physical state of pesticide pollution, thus affecting the degradation rate (Ye et al. 2018) Hu et al. (2013). tested the effects of different temperatures (20 °C, 25 °C, 30 °C, 35 °C and 40 °C) on the imidacloprid biodegradation rate and found that the optimum temperature for degradation was 30 °C. Derbalah et al. (2008) tested the effects of different temperatures (20 °C, 30 °C, and 40 °C) on the metalaxyl biodegradation rate and found that the optimum temperature for degradation was 30 °C. Also, Abd El-Ghany and Masmali (2016) reported that the increase in temperature enhances the degradation rate of diazinon, profenofos and malathion up to 30 °C.

Finally the isolation and identification of *A. flavus* from water naturally contaminated with pesticides which implied that this isolate has the ability to growth under stress conditions and in the presence of pesticides. Moreover, identification of *A. flavus* by sequencing of the 18 S rRNA genes seems to be an authoritative tool in the identification fungal isolates compared to morphological and physiological methods. The evaluation of the optimum growth conditions of *A. flavus* and degradation kinetics of malathion by it can play a vital role in accelerating the process of malathion biodegradation due to the optimization of different environmental factors. This study introduces *A. flavus* from natural water contaminated with pesticides and has the ability to grow under varied environmental conditions and can be used as an effective tool in bioremediation of malathion in natural water.

Conclusion

The tested microbe was isolated and molecularly identified as *A. flavus*. *A. flavus* showed much promise in the complete degradation and detoxification of malathion in contaminated water. The temperature of 30 °C, pH value of 7 and malathion initial concentration of 5 mg/l were the optimum conditions for the growth of *A. flavus* and degradation of malathion. However, further, research should be extended for isolation and characterizations of the gene(s) related to malathion biodegradation are needed. Moreover, the designed primer pairs from in this study could be used as a target of the most highly conserved regions of the 18S rRNA gene within *Aspergillus* sp.

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