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Fungal Diversity and Composition in River Nile Water Polluted with Chlorpyrifos Insecticide

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ABSTRACT

In the current study, the occurrence of different microorganisms in a microcosm experiment of polluted River Nile water with different concentrations of chlorpyrifos insecticide was estimated. The obtained results showed the presence of twenty-three fungal species related to nine fungal genera. *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma* were the most prevalent fungal genera. The highest occurrence was recorded for *Trichoderma* sp. at 0.05 mL/L chlorpyrifos and the lowest occurrence was recorded for *Botrydiplodia* and *Curvularia* at control microcosm experiment and *Stachybotrys* at 0.2 mL/L chlorpyrifos. The highest fungal taxa (S) and individuals were estimated in the control experiment and the lowest fungal taxa and individuals were assessed at 0.1 mL/L chlorpyrifos. Whereas, the highest fungal Dominance (D) was monitored at 0.1 mL/L chlorpyrifos and the lowest fungal dominance (D) index was recorded at 0.05 mL/L chlorpyrifos insecticide. So, the highest fungal biodiversity indexes of Simpson (1-D) and Shannon (H) were estimated at 0.05 mL/L concentration of chlorpyrifos insecticide and the lowest fungal Simpson and Shannon indexes were recorded at 0.1 mL/L concentration. From the correlation results between the physicochemical characteristics and fungal occurrence, it was found that *Aspergillus fumigatus* was impacted by both the temperature and total dissolved sulphates (TDS), whereas *Penicillium duclauxii* was affected by pH, conductivity, and total salts (Ts), and *Aspergillus niger* was influenced by both total dissolved carbon (TDC) and total dissolved nitrogen (TDN). The cluster analysis of fungal composition estimated that fungal groups were detected with different concentrations of insecticide chlorpyrifos.

INTRODUCTION

The increase in world population in recent years, there is a pressure on the existing agricultural system, and nowadays, the prime objective of most of the countries is to increase the food production to meet the demands of a growing population which are expected to grow nearly to 10 billion by the year 2050 [١,٢]. The need of food requires constructive pesticide requests [٣]. Lose in global agricultural yield was over 45% due to wide range of using pests [٤]. Pesticides are widespread in agricultural practices. They are artificial compounds that can manage pests but non-target organisms can be poisoned [٥]. They have a negative effect on freshwater environment in surface and subsurface waterways [٦-١٠]. Organophosphorus pesticides, which make up more than 38% of world`s pesticide trade, have lately replaced organochlorine pesticides because of their high effectiveness and have short-term range in the environment [١١].

Chlorpyrifos (O, O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) is a frequently applied diethyl phosphorothioate organophosphate pesticide [١٢,١٣]. It`s widely spread OPs in the globe [١٤]. It was launched by Dow chemicals in the United States in 1965 to control leafage and soil-borne insect pests and insects in agricultural [15]. Usage of large-scale chlorpyrifos in soil result in aquatic adulteration via rainwater and exterior runoff [16]. Chlorpyrifos has a negative influence on non-target creatures after being released into the environment. Green algae growth was suppressed, and the rate of growth reduced progressively as concentration enlarged [17]. While the excessed presented in the environment [18]. These toxins provide an unwanted threat to human health and ecosystem [19,20]. Moreover, influence of chlorpyrifos on aquatic ecosystems was being concentrated on large-size species by early researches and neglected community-level reactions of aquatic microorganisms [21].

In the aquatic environment, microbes behave as growers, users, and heterotrophic and their involvement in nutrient series keep the balance of ecosystem [22-25]. Exogenic pollutants are destructive to freshwater microbes, as fungicides, herbicides and insecticides, they are also vital markers of contamination and aquatic environmental health [26-29]. Utilizing microorganisms to transfer hazardous or poisonous compound into non-toxic particles is a technique known as microbial bioremediation [30-32]. In this regard, numerous bacteria [33-37], fungi [38-43], actinomycetes [44], yeast [45] and microalgae [46] have been identified and distinguished for pesticide biodegradation. The use of specific microorganism to eliminate chlorpyrifos from the environment has gained a popularity as study issue due to it`s cost-effective and eco-friendly [47-49]. The study aimed to investigate the effect of chlorpyrifos on occurrence in aquatic ecosystems as well as their impact on fungal diversity and community using a microcosm experiment in the Nile River.

MATERIALS AND METHODS

Sampling and measurement of physicochemical parameters of water samples

Fifteen liters of water were collected from the Nile River in a sterilized container and transported immediately to the laboratory and kept in the dark at *in situ* temperatures for

further experiments. Water samples were collected from Paradise Park in Assiut, Kornish Al Nile (Al Thawra), El Fateh, Assiut Governorate.

Physico-chemical analysis of collected water.

The water temperature was measured *in situ* using a laboratory thermometer (liquid mercury in glass tube), pH value, conductivity and the total dissolved solids (Tds) were measured *ex-situ* using a combined pH meter-conductometer (Jenway Model 3540 conductivity/pH meter). Total dissolved organic carbon (TDOC), Total dissolved organic nitrogen (TDON), and total dissolved sulfate (TDS) were analyzed using (analysensysteme GmbH, Donaustr-7, D-63452 Hanau-Germany) in Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt. All parameters were measured at September 2021(zero time) and December 2021(after 90 days).

Microcosm Experiment

The microcosm experiment was managed to assay the impact of chlorpyrifos as an insecticide on fungal biodiversity and community. In the microcosm experiment, treatments were divided into four groups. Each group was performed in triplicate. The control group and three treatment groups (0.05, 0.1 and 0.2 mL/L) of different concentrations of chlorpyrifos were added to a sterilized glass bottle containing 1000 mL of Nile River water and 2 g of fresh Nile River mud were added to each bottle. The experiment was incubated at the ambient temperature with gentle shaking in the dark. The impacts of Chlorpyrifos insecticides on fungal biodiversity and composition were assayed at different time intervals (0, 30, 60 and 90 days) for three months from September 2021 to December 2021. As well as, a gas-tight glass bottle containing 1 L natural water from Nile River was set as control experiment.

Isolation and identification of fungi

The PDA medium was prepared by weighing 200 g of freshly peeled and washed potato in the laboratory. It was then boiled, mashed and the pulp squeezed through a fine sieve. Twenty grams of agar was added and boiled to dissolve and again 20 g dextrose was added. Chloramphenicol antibiotic 250 mg/L was added as bactericidal and with rose bengal (1/30000 g/mL) added as a bacteriostatic agent [50], then the total volume was made up to one liter with water. The content was then sterilized at 121 ^{O}C for 20 min in an autoclave. It was then removed and allowed to cool to an ambient temperature before use. After PDA medium was cooled, it was poured in the Petri dishes (9 cm in diameter) three replicates for each bottle. PDA media was left to solidify then 500 µL /L of water were taken of all water microcosm bottles and spread in the petri dishes with spreader. All the Petri dishes were incubated at 28±2°C for 7 days. The identification of fungal genera and species was performed based on morphological and cultural characteristics according to [51-58].

Assessment of fungal community and diversity:

Fungal biodiversity, Cluster analysis and Canonical correspondence analysis were estimated using Paleontological Statistics (PAST), Version 4.03, USA [59]. In order to understand the correlation of the determined physicochemical parameters with fungal

species, Canonical correspondence analysis (CCA) was used. Canonical correspondence analysis [60] is an analysis of species matrix where each site has given values for one or more environmental variables. The ordination axes are linear combinations of the environmental factors. Diversity indices (Taxa_S", Individuals, Dominance_D", Simpson "1-D", Shannon "H" and Evenness_e^H/S index) of each sample were calculated by using the Past version 4.03.

RESULTS

1-Physico chemical Parameters of water samples:

The result of physiochemical features of collected water samples of the river Nile were shown in Table (1). Water Temperature, pH, electrical conductivity, total dissolved solids (Tds), Total dissolved organic carbon (TDOC), Total dissolved organic nitrogen (TDON), and total dissolved sulfate (TDS) were observable and detectable relayed on time of isolation and concentration of insecticide chlorpyrifos. The highest values of these characteristics were regularly recorded in water samples which were collected from the Nile River either that collected at zero time or that collected after ninety days.

2- Fungi isolated in the present work

The obtained data in Table (2) showed the total count and the number of cases of isolation of fungi isolated from the microcosm experiment. The observed data showed that 23 fungal species related to 13 fungal genera microcosm experiment with different concentration of insecticides chlorpyrifos at different time intervals. The obtained results showed that *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. were the most widespread in all experimented concentrations but with the different occurrence and species diversity. *Aspergillus* spp. was represented by 7 species, of which *Aspergillus flavus* was presented only at 0.1 mL/L (0.3% of total samples) while *Aspergillus parasiticus* was showed at control and 0.05 mL/L (0.09% and 0.13% of total samples). Highest diversity of *Aspergillus fumigatus* was at control and 0.2 mL/L (0.6% and 0.5% of total samples). The highest fungal taxa of *Aspergillus* sp. were *Aspergillus niger* at control and 0.05 mL/L (1.69% and 1.56% of total sample). At 0.2 mL/L *Aspergillus terreus* was recorded in 0.89% of total samples. *Aspergillus ustus* presented only in control with 0.22% of total samples. *Aspergillus sydowii* was high estimated at control (0.21% of total samples). *Fusarium* sp. exhibited the broadest species spectra in control and all concentrations of insecticides chlorpyrifos. *Penicillium* species were recorded with 5 species, of which *Penicillium duclauxii* exhibited mostly in 0.1 mL/L (0.67% of total samples). *Penicillium funiculosum* recorded in control and 0.1 mL/L (0.14% and 0.11% of total samples, respectively). *Penicillium islandicum* record in control (0.11% of total samples). *Penicillium oxalicum* presented only in 0.05 mL/L with 0.36% of total samples. *Penicillium purpurogenum* represented in 0.2 mL/L with 0.48% of total samples. *Trichoderma* sp. was among the most widespread species of all fungi and recorded highly in 0.2 mL/L (2.05% of total samples). Similar variation patterns were recorded for many other genera and species (Table 2). Eighteen identified taxa *Alternaria alternata, Aspergillus parasiticus, A. fumigatus, A. niger, A. terreus, A. ustus, A. sydowii,*

Botryodiplodia sp., *Cladosporium* sp., *Cochlibolus sativus, Curvularia, Fusarium, Neosartorya, Penicillium duclauxii, P. funiculosum, P. islandicum, P. purpurogenum, Trichoderma sp.* were recorded in control. Sixteen fungal taxa *Acremonium* sp., *Alternaria* sp., *Aspergillus parasiticus, A. fumigatus, Aspergillus niger, A. terreus, A. sydowii, Chaetomium, Cladosporium, Fusarium, Neosartorya, Penicillium duclauxii, P. islandicum, P. oxalicum, P. purpurogenum, Trichoderma* were estimated in 0.05 mL/L of insecticides chlorpyrifos. Fourteen taxa *Acremonium, Alternaria, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, A. sydowii, Chaetomium, Fusarium, Neosartorya, Penicillium duclauxii, P. funiculosum, P. islandicum, Trichoderma* were represented at 0.1 mL/L of the insecticides chlorpyrifos. Ten identified species *Alternaria, Aspergillus fumigatus, A. niger, A. terreus, Chaetomium, Fusarium, Penicillium duclauxii, P. purpurogenum, Stachybotrys, Trichoderma* were exhibited in 0.2 mL/L of the insecticides chlorpyrifos.

Aspergillus ustus, Cochilobolus sativus, Curvularia sp. Were isolated only at control, whereas, *Stachybotrys* sp. was recovered only at 0.2ml/L. Also, some species were identified from one or two concentrations and not recorded at the other concentrations of Chlorpyrifos insecticide (Table 2).

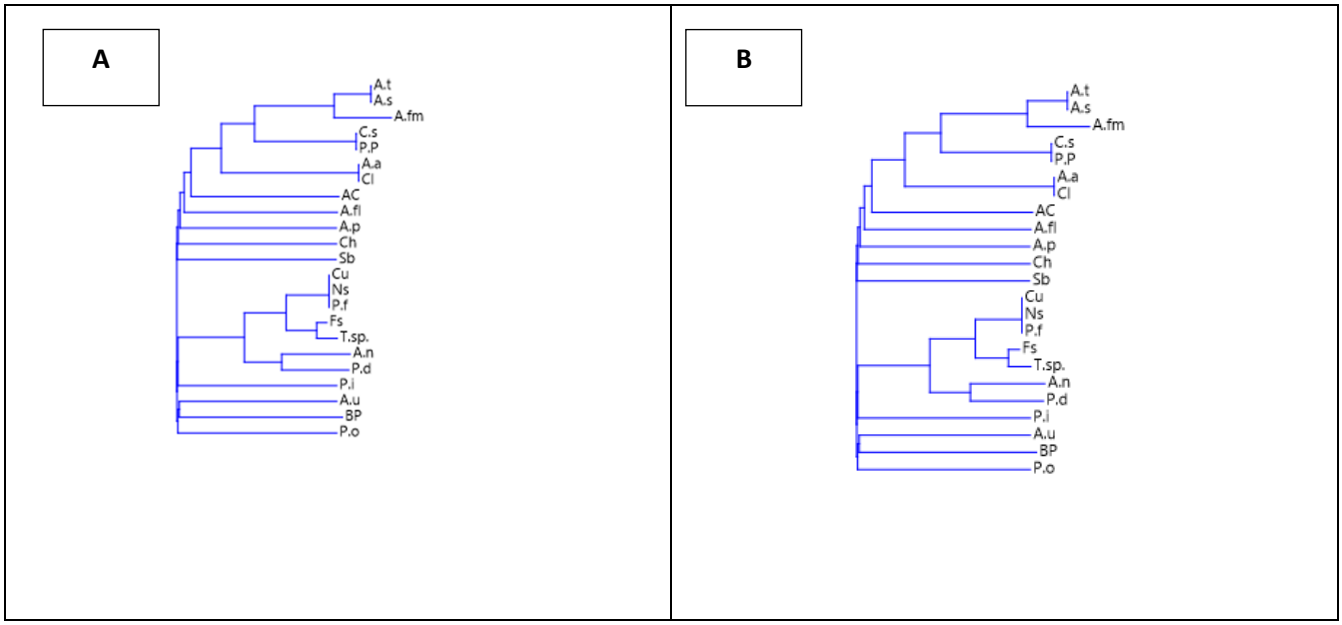
Biodiversity and fungal communities recovered from microcosm experiment

The diversity of aquatic fungal communities from the Nile River was calculated including fungal taxa (s) that specifies the number of isolated fungal strains from collected samples, dominance (d) indicating the superiority of fungal count in particular site; the Simpson index determines the equality of the aquatic fungal groups and Shannon index is the variety guide that stimulates the sum of fungal individuals and taxa. The presented data in table (3) exhibited that, the highest fungal taxa (9) was recorded at the control microcosm experiment after 30 days of incubation and the lowest taxa was estimated after 30 days of incubation at 0.1 insecticides chlorpyrifos. The highest fungal individuals (11) recovered from microcosm was recorded after 30 days of incubation at control experiment and the lowest fungal individuals were recorded after 30 days of microcosm incubation at 0.1 and 0.2 mL/L chlorpyrifos. The highest fungal dominance of isolated fungi from insecticide-polluted water was observed after 30 days of incubation at the concentration 0.1 mL/L chlorpyrifos and the lowest fungal dominance (0.124) was estimated at the control microcosm after 30 days of incubation. The highest Simpson and Shannon indexes of recovered fungi from the microcosm experiment were observed at control after 30 days of incubation recording 0.876 and 2.146, respectively and the lowest Simpson and Shannon diversity indexes were estimated at 0.1 mL/L chlorpyrifos after 30-day incubation.

The cluster analysis:

The cluster analysis of isolated fungi showed an obvious grouping of the fungal communities. Data in Fig.1A showed that, nine separated fungal groups existed in the control microcosm experiment (without insecticides chlorpyrifos). Acremonium, Aspergillus flavus, A. parasiticus, Chaetomium, Stachybotrys, Penicillium islandicum, A. ustus, Botrydiplodia, Penicillium oxalicum were recorded as individuals. Whereas, Curvularia, Neosartorya, Penicillium funiculosum, Fusarium sp., Trichoderma sp.,

Aspergillus niger, Penicillium duclauxii form one group together. Aspergillus terreus, A. sydowii, A. fumigatus, Cochlibolus sativus, Penicillium purpurogenum, Alternaria alternata, Cladosporium grouping with each other. Furthermore, results In Fig.1B revealed the presence of nine different fungal groups at 0.05 mL/L chlorpyrifos. Penicillium islandicum, Aspergillus ustus, Botrydiplodia, Penicillium oxalicum, A. parasiticus, Chaetomium, Stachybotrys were presented separately of each other. Curvularia, Neosartorya, Penicillium funiculosum, Fusarium sp., Trichoderma sp., Aspergillus niger, Penicillium duclauxii were grouped together. Aspergillus terreus, A. sydowii, Cochlibolus sativus, Penicillium purpurogenum, Alternaria alternata, Cladosporium, A. fumigatus, Acremonium, A. flavus cluster into a group. On the other hand, In Fig.1C twelve discrete fungal communities were presented at 0.1 mL/L of insecticides chlorpyrifos. Aspergillus terreus, Botrydiplodia, Cladosporium, Curvularia, Penicillium purpurogenum, A. ustus, Chaetomium, Cochlibolus sativus, Penicillium oxalicum, Stachybotrys were single and not grouping. Aspergillus flavus, A. niger, Penicillium duclauxii, P. funiculosum, Trichoderma sp., Alternaria alternata form one group. Aspergillus fumigatus, Acremonium, Penicillium islandicum, Aspergillus sydowii, Fusarium sp., Neosartorya, Aspergillus parasiticus occupying a group. In (fig.1.D) fifteen particular fungal classifications were presented at 0.2 mL/L of insecticides chlorpyrifos. Botrydiplodia, Cochlibolus sativus, Penicillium funiculosum, Curvularia, Chaetomium, Trichoderma sp., Penicillium islandicum, Aspergillus ustus, A. sydowii, Cladosporium, Neosartorya, Penicillium oxalicum were represented singly. Alternaria alternata, Aspergillus terreus, A. niger, Penicillium duclauxii, Aspergillus parasiticus form one cluster. Aspergillus fumigatus, Acremonium gathering into one group. Stachybotrys, Fusarium sp., Penicillium purpurogenum, Aspergillus flavus form another group with each other.



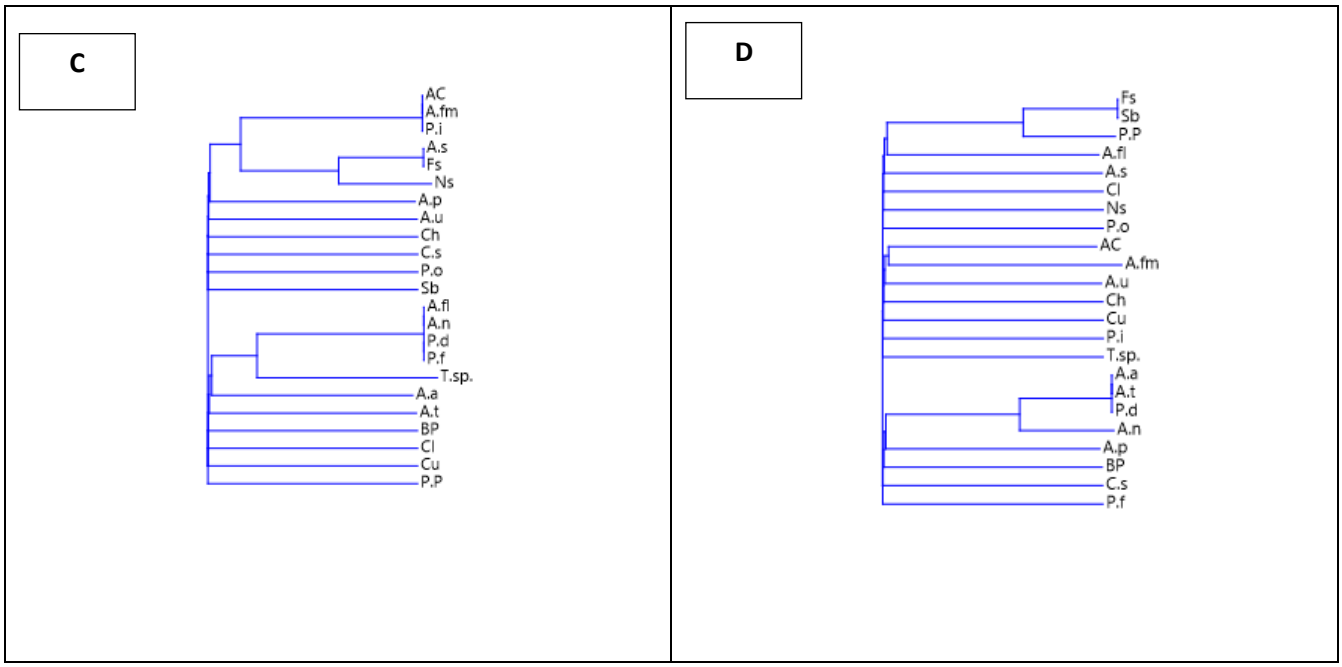


Figure (1): Cluster analysis showing the fungal communities at different concentrations control (A) ,0.05 ml/l (B), 0.1 ml/l (C) ,0.2 ml /l (D)

5- The interaction of physicochemical parameters and isolated

Canonical correspondence analysis demonstrated that the measured physicochemical properties remarkably affected the occurrence of aquatic fungal taxa in the water body (Figs 3 a, b, c, d). The physicochemical characteristics of water samples gathered from the microcosm experiment showed that temperature and total dissolved sulfate (TDS) had an influence on *Aspergillus fumigatus* at zero time in the control microcosm experiment. Whereas pH, conductivity and total dissolved solids affected on *Penicillium duclauxii* after 90 days incubation and the occurrence of *Aspergillus niger* after 30 days. Data in Fig. (2b) revealed that, 0.05 mL/L of insecticides, chlorpyrifos revealed that pH, Conductivity and temperature affected on *Chaetomium* and *Alternaria alternata* after 90 days and the presence of *Cladosporium, Aspergillus terreus* and *Trichoderma sp.* after 60 days of incubation. The total dissolved organic nitrogen (TDON), and total dissolved sulfate (TDS) affected other fungi at zero time. The interaction of physicochemical properties and fungal occurrence at 0.1 mL/L of insecticides chlorpyrifos (fig.2.c)

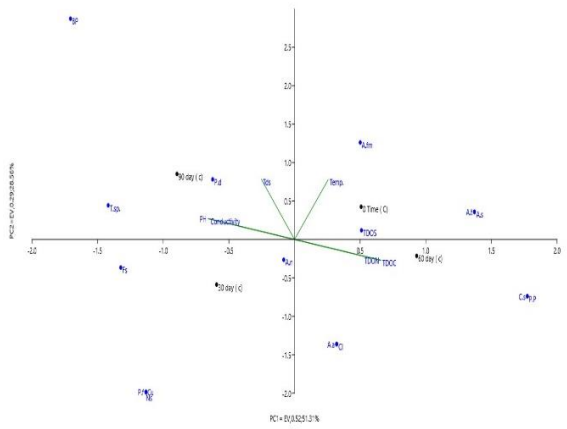


fig.2. a. Canonical correspondence analysis (CCA) of aquatic fungi and physicochemical variables at control

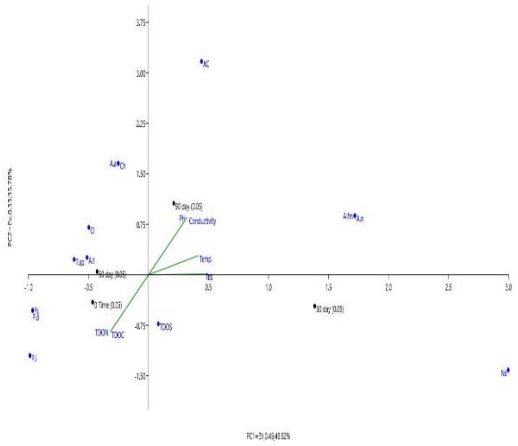


fig.2. b. Canonical correspondence analysis (CCA) of aquatic fungi and physicochemical characters at 0.05 ml/l of chlorpyrifos insecticide.

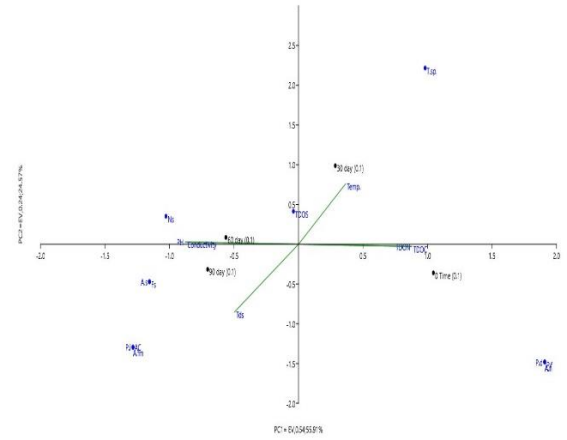


fig.2. c. Canonical correspondence analysis (CCA) of aquatic fungi and physicochemical parameters at 0.1 ml/l of chlorpyrifos insecticide.

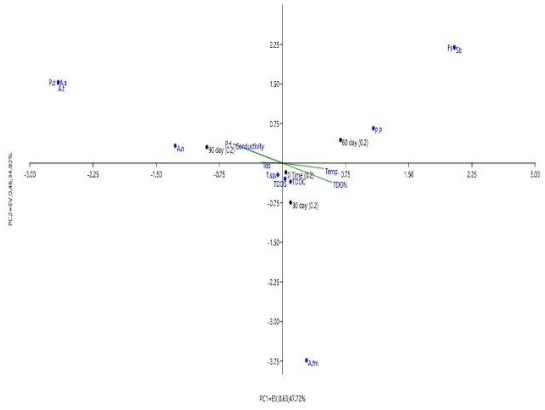


fig.2. d. Canonical correspondence analysis (CCA) of aquatic fungi and physicochemical features at 0.2 ml/l of chlorpyrifos insecticide**.**

showed that total dissolved solids, pH and Conductivity interacted with *Aspergillus sydowii* and *Fusarium sp.* after 90 days incubation of insecticides polluted water. Whereas, the total dissolved sulfate (TDS) interacted with *Neosartorya* after 60 days of microcosm experiment. Temperature, Total dissolved organic nitrogen (TDON) and Total dissolved organic carbon (TDOC) affected *Trichoderma sp.* after 30 days incubation of contaminated water with insecticide. results in Fig.2 d revealed that the persistence of *Penicillium purpurogenum* after 60 days of incubation of microcosm experiment 0.2

mL/L of insecticides chlorpyrifos. As well as, the pH value and Conductivity reacted with *Aspergillus niger* after 90 days of insecticide-treated water. Total dissolved solids interacted with *Trichoderma* sp.

DISCUSSION

There have been reports of freshwater fungi in variable environments involves the ocean [61,62], coastal regions [63,64], lakes [65-67], and rivers [68-69]. In the current study, a microcosm experiment was performed to examine the impact of insecticide on fungal diversity in fresh water and their association with fungal communities. Microcosms are artificial and simplified ecosystems that are used to mimic and predict the reaction of natural ecosystems under controlled conditions, which provides an experimental approach for ecologists to study natural ecological processes [70]. Thus, in this study, the effects of physicochemical parameters and pesticides on fungal diversity and on breakdown rate were studied by microcosms. One of the uninvestigated biodiversity barriers that constitute a challenge to modern microbial ecosystem is presented by fungi [71]. The diverse of fungi is enormous. *Penicillium* and *Aspergillus* were the most two relevant species and they were widely scattered around the world as in Mandovi estuary [72]., Cabo Rojo Solar Salterns [73], the Gulf of Aqaba [74]. and the Changjiang River [75]. *Aspergillus* spp. Were often observed in Suruga Bay in marine underground residual cores [76]. Some of the fungi isolated from the superficial water of Indian rivers were *A. niger, A. flavus,* and *P. crysogenum* [77].

In our study, twenty-three species were identified in the river Nile. *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp., *Trichoderma* sp., *Acremonium* sp. and *Curvularia* sp. were the most common fungi. In natural water environments, autochthonous species are most often represented by microscopic fungi from the following classes: *Chytridiomycetes*, *Oomycetes*, *Trichomycetes*, and *Mucoromycetes* (which were once named *Zygomycetes*). In agreement of our study of the millions of estimated fungal species, only 3,000–4,000 are classified as freshwater fungi [78] for which the water environment is a natural place of persistence. According to the published data, the filamentous fungi from different genera (*Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Trichoderma, Mucor,* and *Rhizopus*) have often been observed in surface-, ground-, and tap water [79-81]. An abundance of presented or novel chemicals with largely undiscovered bioactivities are found in marine fungi [82–84]. *Aspergillus, Cladosporium, Penicillium,* and *Trichoderma* are powerful pigment markers which have been isolated from marine ecosystem [85–89]. Temperature is an essential physicochemical characteristic as it controls the physiological activities and dispersion of aquatic species [90,91]. The medium temperature found in this study might be attributed to the time of sample collection and the vegetation cover of the study region [91, 92]. In our study the optimal temperature was 35-38°C in zero time and after 90 days of incubation. In the presented study the pH values varied from 6.66 to 7.85. This pH values could be as result from surface runoff or decay of organic matters presence in the water [90-93]. During the previous decade, fungi and other microorganisms were isolated to break down chlorpyrifos and its intermediate product TCP at the same time [94]. Purification natural pollutants is a critical ecological procedure which can be obtained by fungal groups [95]. When fungal species are unable to compensate for the

loss of functions relevant to chlorpyrifos-sensitive fungi through functional repetition, changes in fungal community structure may disrupt these processes [96]. Chlorpyrifos is essential for upgrading agricultural yield. However, overdose impact on increase pests` resistance and environmental threats [97]. In this study, we used different concentrations (0.05, 0.1, 0.2 mL/L) of chlorpyrifos to find out if fungal diversity had been affected due to the presence of chlorpyrifos. Additionally, fungi are crucial to the use of biodegradation technology. There have been seldom reports of fungal detoxifying of chlorpyrifos which relevant to tolerance of excessive pesticides residuals with high concentrations [98-100]. The highest values of fungal taxa (S) and individuals were evaluated after 30 days of incubation at control microcosm. Whereas, the highest fungal Dominance (D) (1) was analyzed after 30 days of incubation at 0.1 mL/L chlorpyrifos insecticide. Furthermore, the highest fungal biodiversity indexes of Simpson (1-D) and Shannon (H) were recorded at 0.05 mL/L concentration of chlorpyrifos insecticide recording, 0.876 and 2.146, respectively. However, contrary results had been reported by Singh *et al.* [101], where the overall metabolic diversity and evenness of soil microorganisms were little affected in the soil after chlorpyrifos application at a concentration of 10 mg/kg. *Ascomycota* were previously assumed to be the most prevalent group within aquatic fungal communities [102,103]. However, recent studies based on culture-independent methods are revealing high proportions of *Chytridiomycota* in various aquatic ecosystems [104], which is consistent with our findings. In this study cluster analysis of fungal communities were analyzed at different concentration of chlorpyrifos insecticide which reveals nine fungal groups were presented in control and 0.05 mL/L However, twelve fungal communities existed at 0.1 mL/L. of insecticides chlorpyrifos. Whereas, fifteen particular fungal groups were appeared at 0.2 mL/L of insecticides chlorpyrifos. This part of the present investigation was planned to detect the physico-chemical properties of water samples in river Nile Assiut and study the effects of the changes in fungal distribution at different time intervals. Also, Canonical Correspondence Analysis (CCA) analysis was conducted for twenty-three species of fungi genera and seven selected environmental variables [temperature pH, conductivity, total dissolved salts (Tds), Total dissolved organic carbon (TDOC), Total dissolved organic nitrogen (TDON) and total dissolved sulfate (TDS)] at different concentration of chlorpyrifos insecticide. On the other hand, it was reported that pH always contributed a major explanation to the shaping of the fungal communities in a full-scale membrane bioreactor (MBR) for urban wastewater treatment [105]. Lower pH is known to select for acid-tolerant fungi and to reduce overall fungal diversity increasing. Bai et al. [106]. indicated that of the environmental variables, organic matter, total nitrogen, water temperature, and pH were the dominant factors affecting fungal community composition. Abdel-Raheem et al. [107]. concluded that pH value particularly plays a crucial role in determining the fungal diversity and composition. Temperature is a significant parameter that affect water density which enhanced persistence of nutrients impacting aquatic fungal structure [108]. In different study, it was discovered that the structure and abundance of fungi exist along the Natori River in Japan [109]. Increasing in temperature in other field reports and microcosm tests enlarge microbial activity [110]. In microcosm experiments different temperature led to decrease in fungal accumulation [111]. According to earlier studies fungal diversity in the Songhua River of China, organic matter and total nitrogen are necessary influencing characters for fungal groups

[112,113]. Overall, the composition of fungi is considered to be affected by nitrogen consumption [114]. The conclusion from these results is chlorpyrifos insecticides had an effect on fungal biodiversity. In control and in 0.05 mL/L a diverse was present in fungal communities and many groups but in 0.1 mL/L some of fungi went out of groups and few formed patterns. On the other hand, in 0.2 mL/L most of fungi formed a group of it`s own in comparison with the other concentrations of chlorpyrifos insecticides.

CONCLUSION

In conclusion, the present study aimed to study the impact of chlorpyrifos on diversity and communities of fungi in aquatic ecosystems. The results indicated that chlorpyrifos could influence on the fungi isolated from the Nile River habitat at concentrations of (0.05, 0.1 and 0.2 mL/L). This study provided new viewpoints for evaluating the ecological threat of chlorpyrifos in aquatic ecosystems, the role of fungal biodegradation of chlorpyrifos as biocontrol elements and the limited use of chlorpyrifos in the environment.

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Table (1): The physico -chemical characteristics of the examined water

Treatment	**Temp. (^{0}C)**		**pH**		**Conductivity (µS/cm)**		**Tds (mg/I)**		**TDON (mg/L)**		**TDOC (mg/L)**		**TDOS (mg/L)**	
Sample	**0 time**	**90 day**	**0 time**	**90 day**	**0 time**	**90 day**	**0 time**	**90 day**	**0 time**	**90 day**	**0 time**	**90 day**	**0 time**	**90 day**

Control	**36**	**38**	**6.88**	**7.82**	**231.9**	**276.67**	**0.41**	**0.44**	**0.25**	**0.06**	**5.98**	**5.25**	**3.94**	**0.44**
0.05	**35**	**36**	**7.04**	**7.84**	**247.67**	**291.67**	**0.44**	**0.4**	**0.35**	**0.14**	**5.03**	**3.82**	**5.46**	**0.52**
0.1	**34**	**35**	**7.12**	**7.85**	**276**	**311**	**0.44**	**0.46**	**0.48**	**0.42**	**6.74**	**5.77**	**3.92**	**4.65**
0.2	**33**	**35**	**7.13**	**7.75**	**275**	**302.33**	**0.4**	**0.48**	**0.59**	**0.41**	**6.82**	**4.4**	**0.63**	**0.49**

Table (2): Periodic variations and frequency of occurrence of fungi recovered from the Nile River treated with chlorpyrifos insecticide at different concentrations from September 2021 to December 2021.

Fungi	**Control**			**O.05 mL/L**			**0.1 mL/L**			**0.2 mL/L**		
	TC	**TC%**	**NCI**	**TC**	**TC%**	**NCI**	**TC**	**TC%**	**NCI**	**TC**	**TC%**	**NCI**
Acremonium sp.	0	0	0	2	0.19	2	2	0.3	2	0	0	0
Alternaria sp.	4	0.33	2	2	0.21	2	1	0.2	1	3	0.64	2
Aspergillus flavus	0	0	0	0	0	0	2	0.31	2	0	0	0
Aspergillus parasiticus	1	0.091	1	1	0.13	1	0	0	0	0	0	0
Aspergillus fumigatus	6	0.62	5	2	0.21	2	2	0.3	2	1	0.5	1
Aspergillus niger	11	1.69	4	11	1.56	6	3	0.56	3	2	0.48	2
Aspergillus terreus	2	0.21	2	3	0.27	3	1	0.2	1	3	0.89	3
Aspergillus ustus	2	0.22	1	0	0	0	0	0	0	0	0	0

Aspergillus sydowii	2	0.21	2	1	0.09	1	2	0.35	2	0	0	0
Botryodiplodia sp.	1	0.13	1	0	0	0	0	0	0	0	0	0
Chaetomium sp.	0	0	0	3	0.33	3	2	0.4	2	1	0.25	1
Cladosporium sp.	6	0.53	4	3	0.29	3	0	0	0	0	0	0
Cochlibolus sativus	2	0.22	2	0	0	0	0	0	0	0	0	0
Curvularia sp.	1	0.05	1	0	0	0	0	0	0	0	0	0
Fusarium sp.	7	0.59	6	6	0.81	5	9	1.43	5	5	1.43	4
Neosartorya sp.	1	0.05	1	1	0.13	1	1	0.25	1	0	0	0
Penicillium duclauxii	4	0.37	4	3	0.33	3	4	0.67	2	1	0.14	1
Penicillium funiculosum	2	0.14	2	0	0	0	1	0.11	1	0	0	0
Penicillium islandicum	1	0.11	1	1	0.09	1	1	0.1	1	0	0	0
Penicillium oxalicum	0	0	0	2	0.36	2	0	0	0	0	0	0
Penicillium purpurogenum	1	0.11	1	3	0.34	3	0	0	0	2	0.48	2
Stachybotrys sp.	0	0	0	0	0	0	0	0	0	1	0.14	1
Trichoderma sp.	15	1.31	7	16	1.66	7	7	1.82	5	8	2.05	5

Table (3): Biodiversity of fungi isolated from the microcosm experiment treated with different concentrations of the insecticide chlorpyrifos

Diversity of Fungi																
Conc.	Control				0.05 ml/L				0.1ml/L				0.2 ml/L			
Time	0 time	30 day	60 day	90 day	0 time	30 day	60 day	90 day	0 time	30 day	60 day	90 day	0 time	30 day	60 day	90 day
Taxa_S	5	9	7	5	8	4	7	8	5	1	3	5	3	2	4	5
Individuals	6	11	7	6	10	4	7	8	6	2	4	6	3	2	5	5
Dominance_D	0.22	0.12	0.14	0.22	0.14	0.25	0.14	0.13	0.22	1	0.38	0.22	0.33	0.5	0.28	0.2
Simpson_1-D	0.78	0.87	0.86	0.78	0.86	0.75	0.86	0.88	0.78	0	0.63	0.78	0.67	0.5	0.72	0.8
Shannon_H	1.56	2.15	1.94	1.56	2.03	1.39	1.95	2.08	1.56	0	1.04	1.56	1.09	0.69	1.33	1.61
Evenness_e^H/S	0.95	0.95	1	0.95	0.95	1	1	1	0.95	1	0.94	0.95	1	1	0.95	1