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EFFECT OF SOME MACROPHYTES EXTRACTS ON GROWTH OF *ASPERGILLUS PARASITICUS*

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ABSTRACT

The effect of methanol extracts of some common and widely distributed macrophytes in Lake Manzalah (*Potamogeton pectinatus L., Ceratophllum demersuml L., Eichhornia crassipes (C. Mart.) Solms, Saccharum spontaneum L., Polygonum tomentosum* L.) on growth of toxigenic strain of *Aspergillus parasiticus* was studied experimental. The results revealed that, the extracts of all tested plants showed inhibitory effects on the growth of *Aspergillus parasiticus*. These effects increased with increasing concentrations of the extracts. The maximum inhibition for all tested plants was reported at extracts concentration of 40 mg/ml. Varying from 76.13±0.83 % for *Polygonum tomentosum* leaves to 88.05±1.89 % for *Potamogeton pectinatus*. The phytochemical screening for some active and important substances in all tested plants (Tannins, Flavonoids, Saponins, Terpenes, Alkaloids and Glycosids) was carried out in the methanol extracts. The results revealed the presence of alkaloids, flavonoids and glycosids in all studied plants. While saponins were absent from *Eichhornia crassipes*, terpenes were absent from *Potamogeton pectinatus* and tannins were absent from *Eichhornia crassipes, Saccharum spontaneum and , Polygonum tomentosum*.

1. INTRODUCTION

Aflatoxins are secondary metabolites of certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* which have been shown to be toxigenic, carcinogenic, mutagenic and teratogenic to several species of animals and fishes (Chu, 1977; Ellis *et. al*., 1991; Wood, 1992; Massey *et. al.,* 1995; Khalil, 1997; WU, 1999; Diab *et. al*., 2000; Hussein *et. al*., 2000; Abdelhamid *et. al*., 2002a & 2002b and El-Barbary, 2002).

The problem of food contamination with aflatoxins has been a constant concern and received special attention over the last few decades. The frequent incidence of these toxins in agricultural products is detrimental to the economy, especially in affected regions, in developing countries, where adequate harvesting and post-harvesting techniques for prevention of fungal growth are rarely implemented (Marth, 1967; Stoloff, 1980 and Bullerman, 1986) Therefore, the presence of aflatoxins or toxigenic fungi in food represents a potential hazard to human health (Fan and Chen, 1999). They cause diseases collectively known as aflatoxicosis (Purchase, 1971). An incident involving nearly 1000 people, of whom nearly 100 died. occurred in India in 1974 and was considered to be acute aflatoxin poisoning (Tandon & Tandon, 1988).

Fish wealth is considered as one of the most important sources which contribute animal protein to human particularly in developing countries. Most countries of the world cared of increasing the fish production whether naturally or via aquaculture, which depends on feeding complete artificial diets for fish growth. In tropical countries where climatic conditions are suitable for fungal growth and its toxins production, these artificial diets led to the appearance of various problems that affect fish production.

Mycotoxins are considered as one of the most important problems that appeared (El-Barbary, 2002).

Hence, many synthetic chemical compounds had been used in food preservation to prevent the fungal growth and aflatoxin formation (Wilson *et. al.*, 1979; Farag *et. al.*, 1989 and Barakat *et. al.*, 1992). However, many disadvantages are associated with the use of chemicals and there is a worldwide trend towards limiting their use in grain and foodstuff (Paster *et. a.l.*, 1995). Thereat, it seems to be essential to look for alternative preservatives, which could possess antifungal activity and cause no health problems to consumer. In this respect, natural plant extracts may provide an alternative to these preservatives.

A number of studies have been focused on the use of medicinal plants, spices essential oils and or their extracts in order to retard the growth of *A. parasiticus* and *A. flavus* and its aflatoxin production (Bullerman *et. al.*, 1977; Sharma *et. al*., 1979; Farag *et. al.*, 1989; Masood & Ranjan, 1991; Mahmoud, 1999, Alghalibi, 2004 and Ibrahim, 2004).

Nothing was found in literatures dealing with the used of macrophytes as antifungal agents, so the present study aimed at looking for a new resource of antifungal agents on the growth of *A. parasiticus* in five different macrophytes, which naturally growing and widely distributed in lake Manzalah (Egypt). The plants were *Potamogeton pectinatus L., Ceratophllum demersuml L., Eichhornia crassipes (C. Mart.) Solms, Saccharum spontaneum L.,Polygonum tomentosum* L. Also the extracts of theses plants had been subjected to testes for the presence or absence of some active and important substances (Tannins, Flavonoids, Saponins, Alkaloids, Terpenes and Glycosides).

2. MATERIALS AND METHODS

Lake Manzalah is the largest and most productive among the Egyptian northern Delta lakes (Manzalah, Edku and Burullus). It lies on the southeastern Mediterranean Coast, between the Damietta branch of the Nile River and the Suez Canal (Fig.1).

2. 1. Plant Extracts Preparation

The plants used in the present investigation (Table. 1) were collected from Lake Manzalah (Fig.1) during May 2004, washed with water, then air dried, ground to fine powder and stored in clean bottles, The methanol extracts were prepared according to (Saber, 1976 and Alghalibi, 2004). Forty grams of each plant materials were soaked in 400 ml methanol (95%), shaken for about 90 minutes and left in the laboratory in a sealed container for 24 hr. This procedure was repeated daily throughout five successive days; thereafter the extract was filtered through Whatman No.1 filter paper. The obtained methanol extracts was concentrated under vacuum at 4^{o}C in a Rota-vapour apparatus to dryness, the residues were dried to constant weight in a vacuum- desiccators and the physical properties of all residues were studied. One gram of the residue of each extract was then diluted to 10 ml using sterilized tween 80%, where each ml contains 100 mg of the plant extract.

2. 2. Test Organism

Aspergillus parasiticus used in this investigation was found to be the most potent toxigenic isolate from the rhizosphere of *Faba bean* grown in the Faculty of Agriculture Farm at Fayoum Governorate. The isolate was identified according to Gilman (1950) and Raper and Fennel (1965). Stock culture was maintained on slants of potato dextrose agar (PDA) Difco (1977), then stored at 4°C and subculture was made every two weeks.

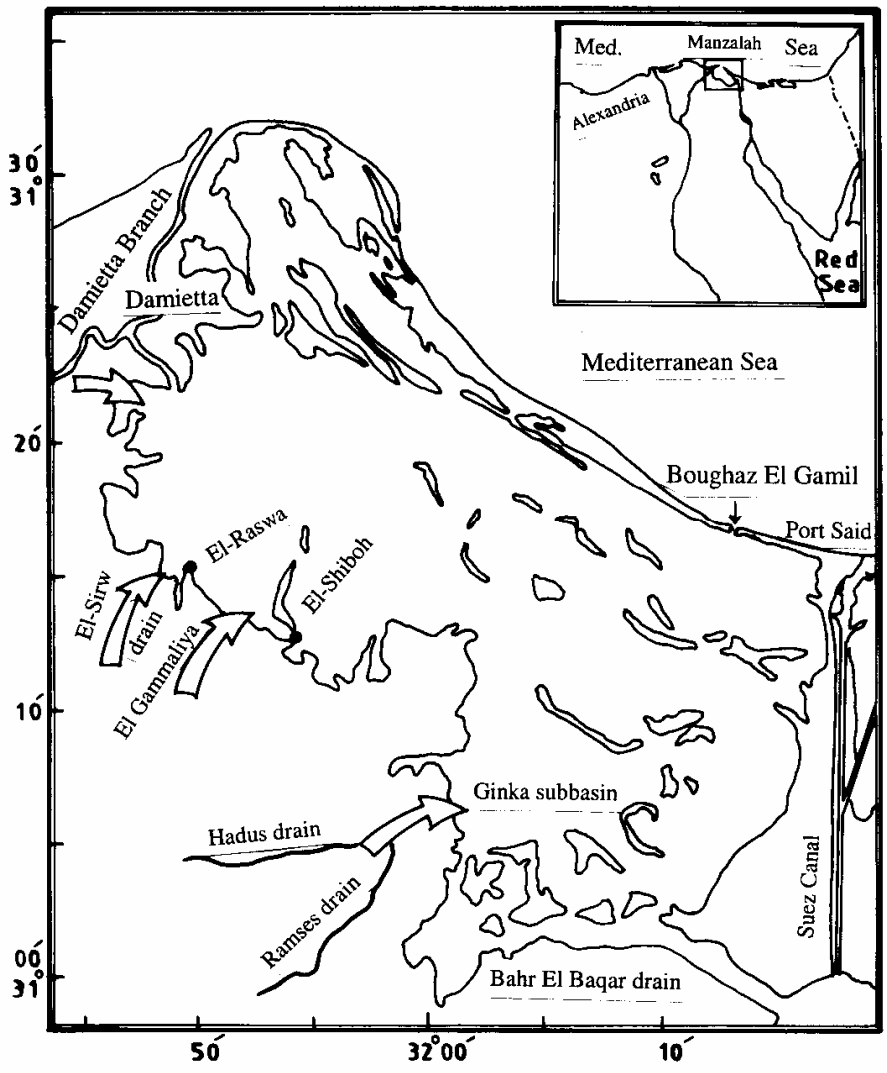

Fig. (1): Map of Manzalah Lake with points of samples collection.

Table (1): The plants used in the present investigation.

Plant scientific names	Family name	Part of plant used	Place of collection	Economic importance	References
Saccharum spontanum L.	Poaceae	The whole plant	El-Raswa	Planted to check soil-erosion, leaves are used for thatching. Used as material for sugarcane breeding, in medicine it is useful in treatment of dyspepsia, piles, burning sensation, respiratory troubles .	Bhandari(1990) and Sastri and Kavathekar(1990)
Ceratophyllum demersum L.	Ceratophyllaceae	The whole plant	El-Raswa &El-Shiboh	An important habitat plant for young fish, small aquatic animals, and aquatic insects.	Bhandari(1990),
Potamogeton pectinatus L.	Potamogetonaceae	The whole plant	El-Raswa &El-Shiboh	Edible uses, leaves roots and stems. Medicinal uses, a decoction of the plant is used in treatment of a feverish liver	Harrington (1967) and Duke and Ayensu (1985)
Polygonum tomentosum L.	Polygonacea	Leaves alone- roots & stems tougher	El-Shiboh	-	-
Eichhornia crassipes (C. Mart.) Solms	Pontederiaceae	The whole plant	El-Raswa &El-Shiboh	One of the most productive plants It have a small medicinal folklore, the flowers are used for medicating the skin of horses. It is also recorded as a source of biogas formation.	Duck and Wain (1981) and Smiyh and Dowd (1981)

2. 3. Fungistatic activity of the tested plant extracts

The antifungal activity of the tested plant extracts was assayed according to the method described by Chkhikvishvili and Gogiya (1995). Ten ml portions of melted potato dextrose agar (PDA) medium were allowed to cool, and the spores of the fungus were placed in the center of solid agar surface (control). Various amounts of reconstituted dry matter of plant extracts under investigation were mixed thoroughly with 10ml of melted PDA medium to give final concentrations of 20 mg/ml, 30 mg/ml and 40 mg/ml, then poured into Petri dishes. The spores were placed as described before representing the different treatments. All plates were incubated at 28±2°C for 7 days after which the fungal growth grown diameters were estimated compared to the control.

2. 4. Preliminary Phytochemical Screening

A preliminary phytochemical screening was carried out on the alcohol extracts of the powdered air dried samples of the five selected plants according to the following methods.

2.4.1. *Test for glycosides*

About 50 ml of alcoholic extract were concentrated under reduced pressure until

free alcohol and tested for carbohydrates and reducing sugars using Molish and Fehlings reagents (Harper, 1975).

2.4.2. *Test for sterols and terpenes*

Libermann-Burchard test (Lewkowitch, 1921; Wall *et al*., 1954 and Fieser and Fieser, 1959) was carried out to detect the presence of sterols and terpenes as follows:

Few ml of each alcoholic extract were evaporated to dryness, the obtained residue was dissolved in two ml chloroform and filtered. Each filtrate was subjected to Libermann-Burchard test by adding one ml acetic anhydride solution followed by addition of a few drops of conc. H_2SO_4 on the side of a previously dried test tube. A red tinge ring was formed, at the junction between the two liquids indicating the presence of sterols.

2.4.3. *Test for flavonoids*

Flavonoids were detected by adding conc. HCL dropwise to one ml of the alcoholic solution containing a fragment of magnesium ribbon (Shinoda, 1928 and Wall *et al*., 1954). A characteristic pink color was obtained indicating the presence of flavonoids.

2.4.4. *Test for alkaloids*

Test for alkaloids was carried out according to the method described by (Jenkins *et al*., 1957).

2.4.5. *Test for tannins*

Few ml of each alcoholic extracts were evaporated to dryness and each respective residue obtained was dissolved in few ml of water and filtered. Each filtrate was treated with $Fecl_3$ solution. A greenish color appeared in all extracts indicating the presence of tannins.

2.4.6. *Test for saponins*

Both foam and haemolysis tests were carried out. The foam test appeared positive when an alcohol-free extract was shaken vigorously in a test tube and the developed voluminous forth persisted for almost an hour.

Haemolysis was carried out, according to Ruyssen *et al*., (1947). Few ml of the alcoholic extracts were evaporated to dryness and the residue was dissolved in two ml of water. The aliquot was then added to one ml 1:40 suspension of erythrocytes in a physiological saline solution (Wall *et al*., 1954).

3. RESULTS AND DISCUSSION

Results in (Table. 2) show the effect of extracts of *P. pectinatus, C. demersum, E. crassipes, S. spontaneum, P. tomentosum* on growth of the tested fungus. The percent of growth inhibition showed gradual increase with increasing concentration of the tested extract.

The extracts of *P. pectinatus,C. demersum* and *E. crassipes* appeared to be the most effective, where their concentrations from 20-40 mg/ml reduced fungal growth by 35.79±1.72 to 88.05+1.89%, 44.09±0.671 to 84.69±1.18% and 60.8±2.14 to 83.27±1.79% of the control value respectively.

However, extracts of the other plants displayed relatively lower effect. The fungal growth reduction fluctuated from 42.17±1.98 to 76.13±0.83% with the extract of *P. tomentosum* leaves, 35.44±2.64 to 80.88±1.047% for *P. tomentosum* (roots and stems) and from 45.05±2.143 to 78.99±2.20% for *S. spontaneum.*

The physical properties of alcoholic residues were compiled in (Table. 3), the residues in all cases were semi-solid, varied in their physical properties and the weights. The highest weight was recorded for *P. pectinatus* (9.08% of dry wt.) followed by *P. tomentosum* (6.61%) and *S. spontaneum* (5.20%). For the other tested plants species the weights of residues formed 4.06-4.83% of dry weight.

Phytochemical screening for the tested plants revealed the presence of some important and active substances (tannins,

flavonoids, saponins, alkaloids, terpenes and glycosides) with a few exceptions (Table. 4). *C. demersum* was characterized by the presence of all these substances while tannins were absent from *E. crassipes*., *P. tomentosum* and *S. spontanum*, saponins were not detected in *E. crassipes* and terpenes were not found in *P. pectinatus*.

Table (2): The effects of plants methanol extracts on growth of *Aspergillus parasitics.*% of growth inhibition = (value of control- value of treatment)/ value of control ×100.

Plant Species	Extracts concentrations	% of growth inhibitions	% of growth
Potamogeton pectinatus .	20 mg/ml	35.78±1.72	64.215
	30 mg/ml	67.03±1.26	32.97
	40 mg/ml	88.05±1.89	11.955
Ceratophyllum demersum .	20 mg/ml	44.09±0.67	55.905
	30 mg/ml	67.01±1.20	32.995
	40 mg/ml	84.69±1.18	15.305
Eichhornia crassipes	20 mg/ml	60.8±2.14	39.2
	30 mg/ml	76.59±2.79	23.405
	40 mg/ml	83.27±1.79	16.73
Polygonum tomentosum . *leaves*	20 mg/ml	42.17±1.97	57.83
	30 mg/ml	67.03±1.26	32.97
	40 mg/ml	76.13±0.827	23.875
Polygonum tomentosum . *Roots& stems*	20 mg/ml	35.44±2.64	64.56
	30 mg/ml	73.24±2.16	26.76
	40 mg/ml	80.88±1.07	19.12
Saccharum spontaneum .	20 mg/ml	45.05±2.14	54.945
	30 mg/ml	58.41±1.38	41.595
	40 mg/ml	78.99±2.20	21.015

Table (3): The weight of the extracts residues as well as its physical properties.

Plant Species	Weight of residues gm/100gm of dry wt.	Physical properties of the extracts residue
Potamogeton pectinatus .	9.08	Yellowish- brown- resinous mass, having white crystals
Ceratophyllum demersum .	4.06	Yellowish- green resinous mass
Eichhornia crassipes	4.83	Reddish resinous mass
Polygonum tomentosum . leaves	6.61	Brown aromatic resinous mass
Polygonum tomentosum . Roots& stems	6.6	Brown aromatic resinous mass
Saccharum spontaneum .	5.20	Faint yellow resinous mass having favorable odor and containing crystals

Table (4): Preliminary phytochemical screening of the studied plants.

+ Small amount ++Moderate amount
+++High amount -Not detected

	Plant Species				
Tested substances	***Eichhornia crassipes***	***Ceratophyllum demersum***	***Potamogeton pectinatus***	***Polygonum tomentosum***	***Saccharum spontaneum***
Glycosides	++	++	+++	+++	++
Tannins	-	+	+++	-	-
Flavonoids	+	++	+++	+++	+
Saponins	-	+++	++	+	+
Terpenes	++	+	-	+	+++
Alkaloids	++	++	+++	++	++

As we concluded in these results the alkaloids, glycosides and flavonoids were recorded in all tested species, these substances were secreted in special cells. Alkaloids are supposed to be decomposition products of proteins. They have a marked physiological effect on animals and therefore, they are of high value in medicine and drugs. They include some of the most powerful plant poisons and narcotics. Caffeine and theobromine, are usually classed as alkaloids (Pandey, 1984). Glycosides are similar to alkaloids, but they are derived from carbohydrates and not from proteins. They are commonly used in the manufacture of medicine and drugs for man (Pandey, 1984).

Flavonoids are all structurally derived from the parent substance flavone, they are mainly water- soluble compounds. They are phenolic compounds and hence change in color when treated with base, they contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible region of the spectrum. They are generally present in plants bound to sugar as glycosides and any one flavonoid aglycone may occur in a single plant in several glycosidic combinations (Harborne, 1984). Flavonols, appear to be important in growth controlling (Galston, 1969) and adverse effects on insect feeding as a natural resistance factor (Isman and Duffey, 1981).

Tannins were also recorded, which are organic compounds chiefly glucosidal in nature, they have acid reaction and are very astringent tastes (Pandey, 1984). In the plant cell, the tannins are located separately from the proteins and enzymes of the cytoplasm but when tissue is damaged, e.g. when animals feed, the tanning reaction may occur, making the protein less accessible to the digestive juices of the animal. Plant tissues high in tannin are, in fact, largely avoided by most feeders, because of the astringent taste they impart. One of the major functions of tannins in plants is thought to be as a barrier to herbivory (Harborne, 1984). Also, they are of commercial importance because of their property of forming an insoluble colloidal compound with the hides of animals. They also react with the salts of iron to form dark-blue or greenish-black compounds, the basis of common inks (Pandey, 1984).

The presence of the active substances mentioned above in the extracts of the studied plants caused the inhibiting effect on growth of *Aspergillus parasiticus*. Such effect was related to the concentration of these substances in the plant, whereas, *P. pectinatus* extract contained the hightest amount among all tested plants and this lead to the greatest percentage of growth reduction of *A. parasiticus* (88.05±1.89%) as compared to the effect of other plants..

The present results are in agreement with those of (Ghazi, 1976) who mentioned that, the negative results recorded by (Azzouz and Bullerman, 1982) for the effect of powdered *Pomegranate peel* on growth of *A. flavus* or *A. parasiticus* may be due to that, the powdered crude material contains ferrous ion, which bind with the active substances (tannins and flavonoids) and inhibit its effect on the cytochrome, while our extracts are characterized by the presence of these substances in free form.

The present study shows that the phytochemical properties of the examined plants in Lake Manzalah appeared to be similar to those recorded by Williams, *et. al.*,1976; Ostrofsky and Zettler, 1986; Haroon,1989; Abo- Zeed, 1990 ; Edress; 1990 (Table, 5) but with a few differences, which may be related to the differences in environmental conditions and the age of plant used.

4. CONCLUSION

This study indicates that; *P. pectinatus* ,*C. demersum, E. crassipes, S. spontaneum* and *P. tomentosum* contain some medically important substances like, glycosides, flavonoids and alkaloids. However, in some of these plants other substances were lacked.

These substances were proved to have antifungal effect of *A. parasiticus* and consequently may offer some assistance for preventing aflatoxins production. There for, the extracts of these plants could be used as natural preservative for food materials in stead of chemicals. In such context, further and intensive experiments are needed on the safety and toxicity of these substances as well as on its ability to control pathogenic and storage fungi under storage conditions.

Table (5): The comparable results of phytochemical screening of the studied plants.

References	Comparable results
Williams, *et. al.* (1976)	Recorded the presence of flavone sulphate in saccharum and related genera.
Ostrofsky and Zettler (1986)	Separated nine types of alkaloids from *Potamogeton pectinatus*
Haroon (1989)	Recorded the presence of all tested substances except terpenes& saponins in extract of *potamogeton pectinatus* collected from Lake Burrllus.
Abo-zeed (1990)	Recorded the presence of all tested substances except saponins in extract of *Ceratophyllum demersum* L. from Idku Lake.
Edress (1990)	Recorded the presence of all tested substances in extract of *Polygonum Tomentosum L.* and the absence of saponins from extract of *Saccharum spontaneum* L. collected fromRosetta branch of the Nile river.

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