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Colonization Abilities of Microflora to Attach Aquatic Plants By A.H. Aboellil & Amany H. Aboellil

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Keywords : Hydrophytes, epiphytic algae, fungal flora, enzymatic activities.

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I.

INTRODUCTION

he aquatic system is a complex and dynamic ecosystem composed of water, plants, soil bed and microflora, etc, in general this microflora colonizes the phylloplane, phyllosphere and rhizosphere of macrohydrophytes. It is may provides dynamic micro environment where bacteria and fungi in association to algal flora with plants detoxify hazardous organic compounds (Walton *et al.*, 1994). Microorganisms possess specific biochemical traits that account for their existence and prevalence in certain environment (Alexander, 1971).

Submerged and floating aguatic plants have different architecture offering different opportunities for epiphytes (Cattaneo, 1998 and Aboellil, 2006 and 2011). Fenchel and Jorgensen (1977) stated that. microorganisms have long been recognized important link between primary and secondary production in detritus-based food webs in aquatic environment. Manyinvestigators studies terrestrial fungal flora inhabitant macrohydrophytes like Gaueret al. (1992) and Galabraith (1986) which studied the relative contributions of fungi to water hyacinth decomposition while others like El-Morsyet al. (2000) study the occurrence of microfungi of some selected macrophytes from Nile delta of Egypt, Aboellil (2003) surveyed fungal

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and algal microflorainhabitant different types of hydrophytes in fresh water systems at middle Egypt belt. As far as, we know no one had been classified the fungal flora inhabitant macrohydrophytes according to their degree of attachment to the plant. On the other hand, Burkholder *et al.* (1990) and Cattaneo and Kalff (1978) classify the algal flora inhabitant hydrophytes to loosely attached and tightly attached ones, according to their degrees of attachment.

Kuehn and koehn (1988) stated terrestrial fungi in particular may be enzymatically better adapted than aquatic fungi in the initial colonization and utilization of submerged organic materials. Moreover, fungi may be enhancing or weakening the activity of allelopathic components (Gunnison and Barko, 1989). They possess a powerful system for penetrating the root as endophytes (El-Morsy, 1999b). Moreover cutin, pectin, cellulose is the short difficult structural obstacles to fungal invasion El-Morsy (1999).

Plant/microbial community feed back can have important consequences for species composition of both the plant and microbial communities (Gustafson and Casper, 2004).

The main objectives of the paper was to evaluate and classify the fungal and algal flora which inhabitants different categories of hydrophytes at middle Egypt belt according to their degree of attachment to plants and examine the enzymatic activities of some isolates, that's may help to colonize the hydrophytes surfaces.

II. MATERIALS OF METHODS

The studied area lies at Mid Egypt (Lat. 30-25 N) selected macrohydrophytes and water samples were collected from different water courses at great Cairo and Beni-Swif districts and representing available different types of eater course (Nile branch, channels, drains and pools). After preliminary survey 4 different hydrophytes were chosen to represent as far as the most abundant hydrophytes, and also monitoring the different categories hydrophytes, example of for Eichhonniacressipes (Floating), Ceratophyllumde -(Submersed), mersum Echirochloa Stagninum (emmersed) and Azolla (Ferns).

Isolation of fungus: the studied plants were collected aseptically and transported to lab. Three different technique were used to isolated the fungal flora according to their degree of attachment, 1st one washing

the sample and dilution technique using 1 g as disks of plants (Loosely attached flora), 2nd disks were punched from the washing sample using sterilized cork borer then planted on nutrient agar (Czapek's-Dox) (moderately attached flora) and finally maceration used to recognized the firmly attached flora (Dickinson, 1967; Salama*et al.*, 1986).

Isolation, fixation and identification of algal flora from different chosen hydrophytes per formal according to Prescott (1978).

Physicochemical characteristics of water at studied site were evaluated according to standard methods and recent instruments.

Enzyme activities of fungal flora isolated from different hydrophytes (pectinase, cellulose, lipase and amylase) detected by method described by Sall (1967), Hankin*et al.* (1971), Elwan*et al.* (1977) and whistler *et al.* (1984).

III. RESULTS AND DISCUSSION

Regarding to physical and chemical parameters average at studied sites reveals, the transparency of water was ranged between 35-68 cm by using secchi disk. DO_2 of Nile water was reworded the highest values with (5-6mg) comparing to drains which recorded the lowest values (2.1-3.8 mg) pH varied between 7.8-8.6 , while T.S.S. showed the lowest amount at River Nile (280-315 mg) and the highest amount at drain site (725-812 mg) (Table 1).

Fungal flora inhabiting floating hydrophytes *Eichhorniacrassipes*as shown from Table (1) were mainly belonging to 8 genera and about 13 species with total count (70colonies/g). Only *Asperigillus*spp. contributed about 47% of total count of isolated fungi, with high occurrence. Then *Alternariaalternate* and *Paecilomyces*sp. are represented about 14.29% of totalcounts for each with low occurrence. While *Cladosporiumherbarum* and *Fusarium*spp. showed about 10 and 7.14% of total count, respectively. *Trichoderma, Phomahemicola* and *Rhizopusnigricans* contributed 2.86, 2.86 and 1.43% of total counts, respectively.

Regarding to attachment degree of isolated flora, *A.alternata*, *C. herbarum*, *Fusarium*spp. and *P. humicola* were isolated after maceration process only so were com concluded that its belonging to firmly attached ones. While *Paecilomyces*sp. and *T. viride* restricted as moderately attached ones, isolated only at immersed stage. On the other hand, *Aspergillus* spp. generally may consider as biphase fungi because it was isolated as loosely and moderately attached fungi to *Eichhorniacrassipes*.

From table 2, fungi isolated from submersed hydrophyte *Ceratophyllumdemersum* were comprised about 16 species with total counts (60 colonies/g) varied between *Asperigillus* species contributed higher than 50% of total count with high occurrence, considered as loosely to moderately attached fungi. *Alternariaalternata*, with low occurrence, represented 18.42% of total count monitoring again as firmly attached fungi as well as in case of *Eichhorniacrassipes*. Also *Fusarium* spp. contributed about 11.67% of total count, showed the same trend. On the other hand, *Curvularia*, *Cladosporium* and *Ulocladim*are of the moderate rank showed 10, 5 and 5% of total count, respectively and partially appeared firmly attached isolated.

Helminthosporium and *P. humicola* were isolated once with low occurrence and considered moderately attached fungus.

Table (3) showed the range of occurance, total of fungi isolated count and types from Echinochloastagninum. Instead of the low occurrence ofAlternaria, it appeared to be most abundant species isolated from Echinochloa, it is represent 30% of total count and also recorded as firmly attached fungi while Fusariumspp. Contributed 16% of total count and considered as firmly attached fungi. Asperigullus here showed loosely attached ones and represented about 28% of total count with moderate occurrence. Sepedonumchrysosporum also recorded as moderate attached fungi isolated from the plant.

Helminthosorium and *P. humicola* were isolated once with low occurrence and considered moderately attached fungi.

Regarding to fungal flora isolated from *Azolla* fern, Table (4) confirmed that, again *Alternariaalternata* representing 4% of isolated fungal total count appears to be firmly attached fungi. *Trichodermaviride, Mucor*spp. contributed about 2.7% of total count and considered as moderately attached. The total count of fungi isolated from *Azolla* (84 colonies/g) comprise 12 species. *Aspergillus*spp. contributed 88.1% of total count and appears at 3 types (loosely, moderately and finely attached ones) by different ratio sloped to moderate one.

Difference of fungal flora may be due to the nature of leaching substances of different plants which considered as the media for growing the specific flora. Leaching substances are sugar, amino acids growth substances...etc have important consequences in microbial diversity (Gustafson and Casper, 2004).

From tables (2-5) we can concluded that A.alternata recorded only as firmly attached fungi for different investigated hydrophytes, while Aspergillus spp., instead of its recorded the most ascendant species its seems to be loosely to moderate attached fungi-Fusarium which isolated only from Ceratophylum and Echinochloa also showed to be finely attached fungi which isolated only by maceration. Newshan et al. (1995), were found Fusariumspp. implicated in the suppression of *Fusarium* wilt diseases and effecting the plant performance. Generally the above mentioned data came in harmony with El-Morsyet al. (2000) which stated that, fungi isolated from selected aquatic macrophysics at Nile Delta of Egypt were typical of terrestrial origin. The majority of isolated species were recorded from the rhizosphere of aquatic macrophytes isolated by Motta (1978) and Gunnison and Barko (1989).

On the other hand, Table (6) showed the enzymatic activities of isolated fungi, may explain to what extent the efficiency of these fungi to attached to hydrophytes depending upon their highly efficiency for decompose pectin. Cellulase, lipid and starch.

As shown from table (6) most isolates showed high to moderate efficiency to secrets pectinase, cellulose, lipase and amylase except some rare cases such *Mucor* which had lacking in secretion of cellulase and lipase, while, *F. moniliforme*, *A. carbanrous* and *A. alternata* showed no activities of lipase.

The best performance of enzymatic activities observed from *P.notatum*, *A. terreus*, *R. nigricans* and *Cladosporumherbarum*, on contrary the less performance showed in *Mucor*, *A. fumigatus* and *A. alternata*

While the moderate performance appeared in cases of *A. flavus*, *A. niger* and *F. moniliforme*.

In general the results obtained from detected the remarkable enzymatic activities of isolated fungi come in harmony with Kuehn and Koehn (1988) stated terrestrial fungi may be enzymatically better adapted than aquatic fungi in the initial colonization and utilization of submerged organic materials. Also with El-Morsy*et al.* (2000) which emphasized that the presence active fungal flora in the rhizosphere of aquatic macrophytes indicates their significant role in degradation of organic compounds they may be produced enzymes.

From the previous results, there is no clear relation between the degree of attachment of organisms to plants and their enzymatic potentiality. According to data, it is suggestion that the attachment degrees are due to the capability of the fungus to cause diseases to plants like *Alternaria, Fusarium, Curvularia Ulocladium.* Therefore, it may be more firmly attached to plant surfaces, but the saprophytic fungi like *Aspergillus, Mucor, Trichoderma, Rhizopus* are more or less loosely or moderately attached to plants.

The fungus perhaps has a high enzymatic system but can not caused diseases. Correlation of hydrophytes genera with microbial inhabitants influenced the enzymatic profiles of microbial communities from these plants.

On the other hand, the common constituents of aquatic systems, epiphyticalgae, which isolated from different chosen hydrophytes included cyanobacteria, chlorophyta and Bacillariophyta. Obtained data revealed that, the loosely attached algae included majority of cyanobactera such as *Oscellatoria* spp., *Lungbya*spp., *Microcystis*spp. and *Anabaena*spp.,in addition to chlorophyta membranes *Pediastrum*spp.,*Scenedesmus* spp., *Quadrigula* spp., *Botryococcus* and *Cladophora* spp. (Table 7).

While *Coleochaete* and *Melosira*, *Fragilaria*, *Nitzschia*, *Synedra* and *Rhizosolenia* from Bacillariophyta showed as moderately attached ones.

Firmly attached alga includes Navicula, Cymbella, Cocconies represented nonmotilepennates diatoms. Theabove mentioned data come in harmony with Zimba and Hopson (1997) stated, it is not surprising that significantly different removal efficiencies of epiphytes were obtained for the sampling types analyzed (mechanical agitation). Also with Cattaneo and Kalff (1978) and Burkholder et al. (1990) identified two distinct components of the epiphytic flora loosely and eighthly attached or adnate component. Goldsborough and Hickman (1991) revealed the importance of efficient removal of epiphyton from host tissues to assess the importance of macrophytes and epiphytes in ecological studies.

In general the data obtained from isolated epiphytic algae from different studied hydrophytes showed that, the main mean of loosely attached alga representing about 56% of algal taxa, while moderately attached ones harvested about 28% of total algal taxa most of them belonging to Bacillariorphyta, while firmly attached ones representing about 16% of total harvest and completely belongs to Bacillariophyta especially nonmotilepennatesdiatoams.

Algal communities collected from under four cooccurring hydrophytes depended on the medium (nutrient), temperature, transparency of water and pH. It can be hypothesizyed that some isolates were present as dormant structures while some others could be in growing forms.

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Table 1 ; Physical and chemical parameters average
at studied sites

Parameters	Average
Transparency of water	34-68 cm
Demand O_2 (DO ₂)	5-6 mgl ⁻¹
Low	2.1-3.8 mgl ⁻¹
Drain values	
High	725-812 mgl ⁻¹
рН	7.8-8.6
Total soluble salts (TSS)	280-315 mg l ⁻¹

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Organimm	Ч	iylloplane (coloni	es/g)	Total count	Total count (%)	No of cases	Rank of
	Washing	Immersion	Maceration	(colonies/g)		of isolation	Occurrence
Alternaria alternata	-	-	10	10	14.29	-	
Aspergillus sp.	16	12	5	33	47.15	3	Т
Aspergillus nodulans		Ļ	·	Ļ	1.43	Ļ	
A. flavus	4	2		9	8.57	2	M
A. carbonarius	10	-	-	10	14.29	1	Γ
A. sydowi	-	4	-	Ļ	1.43	1	Γ
A. terreus	-	-			1.43	-	Γ
A. niger	2	7	5	14	20	3	Т
Cladosdorum herbarum	-	-	7	7	10	-	Γ
<i>Furarium</i> sp.			5	2	7.14	Ļ	
F. moniliforme			ი	ი	4.29	.	
F. oxysporum			2	2	2.86		
Phoma humicola			2	2	2.86		
Rhizopus nigricans					1.43		L
Paecilomyces spp.		10		10	14.29		Γ
Trichoderma viride	2			2	2.86	-	Γ
Total count	18	23	29	70	100	17	

Table 2 : Fungal flora inhabitant Eichhornia crassipes and their occurrence range regarding to its attachment degree to hydrophytes

Rank of occurrence H, High = 3 cases, M, Moderate = 2 cases, L, Low = 1 cases

Table 3 : Fungal flora inhabitant Ceratophyllum demeritam and their occurrence range regarding to its attachement degree to hydrophytes

		:					
		Phylloplane	6	Total count	Total count	No of cases	Bank of
Organimm	Washing	Immersion	Maceration	(colonies/g)	(%)	of isolation	Occurrence
Alternaria alternata			7	7	11.67	, -	
<i>Aspergillus</i> spp.	20	6	2	3	51.67	1	Н
Aspergillus nodulans				1	1.67	-	
A. flavus	5	Э		8	13.33	2	Μ
A. carbonarius	2			2	33.3	1	Γ
A. sydowi		1		1	1.67	1	Γ
A. wentii		1		1	1.67	1	Γ
A. terreus		1		1	1.67	1	Γ
A. niger	12	3	2	17	38.3	2	Н
Cladosporum herbarum		1	2	3	5.0	2	Μ
Furarium			2	7	11.67	1	
F. moniliforme			ю	ю	5.0		
F. oxysporum			4	4	6.67	1	Γ
Phoma humicola			.	1	1.67	1	Γ
Rhizopus nigricans		1		1	1.67		Γ
Helminthosporium sp.				1	1.67		L
<i>Ulocoldium</i> sp.		1	2	3	5.0	2	Μ
<i>Curvularia</i> sp.		1	5	6	10.0	1	Μ
Total count	20	13	27	60	100	18	

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Table 4 : Fungal flora inhabitant Echinochloa stagninum and their occurrence range regarding to its	attachment degree to hydrophytes

Organimm	Phy	lloplane (color	nies/g)	Total count	Total count	No of cases	Rank of
	Washing	Immersion	Maceration	(colonies/g)	(%)	of isolation	Occurrence
Alternaria alternata			15	15	30	1	
Aspergillus sp.	12	2		14	28	2	Μ
Aspergillus nodulans	2			2	4	1	Γ
A. flavus	С	2		5	10	2	Δ
A. carbonarius	1			1	2	1	Γ
A. terreus	Ļ			1	2	1	Σ
A. niger	1			1	2	1	Γ
A. wentii	1			1	2	1	Γ
Cladosdorum herbarum	2		5	7	14	2	M
<i>Furarium</i> sp.			8	8	16	1	Ţ
F. moniliforme			ო	ო	9		
F. oxysporum			5	5	10	1	Γ
Rhizopus nigricans		2		2	4	1	Γ
Sepedonium chrysosporum		2		2	4	1	Γ
Total count	16	9	28	50	100	16	

Table 5 : Fungal flora inhabitant Azolla fern and their occurrence range regarding to its attachment degree to hydrophytes

Organimm	Phyl	loplane (colon	ies/g)	Total count	Total count	No of cases	Rank of
	Washing	Immersion	Maceration	(colonies/g)	(%)	of isolation	Occurrence
Alternaria alternata			Е	c	3.6	+	
Aspergillus sp.	28	31	15	74	88.1		Н
Aspergillus nodulans		1		, -	1.2	+	
A. flavus	7	20	8	30	35.7	3	Н
A. carbonarius	2			2	2.4	1	
A. sydowi			10	10	12.0	1	
A. terreus	12	2		14	16.7	2	Μ
A. niger	7	2	2		18.0	2	Н
A. ustus		5		5	6.0	1	
Cladosdorum herbarum		2		2	2.4	2	Μ
<i>Furarium</i> sp.							
F. moniliforme							
F. oxysporum							
Rhizopus nigricans	-			-	1.2	1	
Trichoderma viride		3		3	3.6	1	Γ
Total count	29	37	18	84	100		

Table 6 : Enzymatic activities of fungal isolates from different hydrophytes

Isolate	Pectinase	Cellulase	Lipase	Amylase
Alternria alternata	1+	3+	-	2+
Aspergillus carbonareus	3+	2+	-	3+
A. flavus	4+	2+	2+	4+
A. fumigatus	2+	2+	2+	4+
A. niger	2+	3+	-	2+
Cladospolum herbarum	4+	2+	2+	4+
Fusarium moniliforme	4+	2+	-	3+
Fusaruim oxysporium	-	3+	1+	2+
Helminthosporium sp.	4+	3+	2+	4+
<i>Rhizopus</i> sp.	2+	-	-	3+
Rhizopus nigricans	3+	4+	2+	3+
Sepedonum chrysosporum	1+	1+	4+	2+
Trichoderma viride	3+	1+	<u>±</u>	3+
<i>Ulocladium</i> sp.	4+	2+	3+	4+

Key of table used:

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= No activity, \pm = Slightly activity, 1+ = 1.1 – 1.4 cm in diameter, 2+ = 1.5-2.0 cm, 3+ = 2.1 – 2.5 cm and 4+ = 2.6 – 3 (\geq 2.6 cm)

Note: Cups were made (4 cups optimal) in each solidified plate using sterile cork-borer (1 cm in diameter).

Table 7	÷	Isolated	epiph	/tic algae	from	different	studied h	nvdro	ρh	vtes a	as t	heir	attachme	ent dec	iree
rubic r		loolatoa	opipity	riio aiguo	110111	amoroni	otaaloa i	iy ai O	PU	,100 ,	uυι	11011	anaonin	nin aog	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,

Firmly attached algae	Moderately attached algae	Loosely attached algae
<i>Nivicula</i> spp.	Coleochaete spp.	<i>Oscellatoria</i> spp.
<i>Cymbella</i> spp.	<i>Melosira</i> spp.	<i>Lungbya</i> spp.
<i>Cocconies</i> spp.	<i>Fragilaria</i> spp.	<i>Microcystis</i> spp.
	<i>Nitzchia</i> spp.	Anabaena spp.
	<i>Synedra</i> spp.	Pediastrum spp.
	<i>Rhizosolenia</i> spp.	<i>Scenedesmus</i> spp.
		<i>Guadrigula</i> spp.
		Botryococcus spp.
		Cladophora spp.

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