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A preliminary study on distribution and screeningsome microfungi from saline soils of Pulicat Lake for the weedicidal property

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Abstract
A Preliminary study was conducted to identify the microfungi in saline soils of Pulicat lake, Tamil Nadu. A total of 8 genera belonging to 12 species were isolated during the study period. The fungal species recovered from the soil samples include *Aspergillus terreus*, *Aspergillus niger*, *Stachybotrys*sp., *Aspergillus* spp., *Penicillium*spp., Yeast form and Sterile forms. Hyphomycetes group were dominated among the microfungal assemblage of saline soils. Aspergilli represented more number of species and was found most abundant compared to other fungal species. Six fungal species were tested for the weedicidal activity in which *Aspergillus niger* showed promising activity against the *Lemna* sp.

Keywords: fungi**,** saline soil, Pulicat lake**,** *Aspergillus*, weedicidal activity

Introduction

Fungi are eukaryotic, ubiquitous diverse group of organisms, different from all other in their behaviour and cellular organization. Their unique characteristic features fits these entities in a separate kingdom representing one of the three major evolutionary branches of multicellular organisms by molecular taxonomists [1]. Apart from leading a parasitic, mutualistic, endophytic and saprophytic mode of life, they also proved to be fit to thrive in vagarious niche including nest of birds, guts of animals, rocks, oceanic depths, resins and dung of animals [2]. Further, current estimate on number of fungal species by predicts 2 to 3.8 million species, out of which only 7% is documented and many of them are yet to be explored [3]. In this context in recent years, diverse habitats have been continuously explored by mycologists in search of new fungal species, or metabolites derived from them for agriculture, industrial and pharmaceutical applications.

Fungi are one of the successful eukaryotes, able to adapt to a range of changes in external osmotic environment. Saline soils from different parts of the world have been surveyed for the presence of fungi especially rhizosphere soil [4] Solar salterns [5, 6] Saline soils [7]. Fungi growing in the stressful environments are known to elaborate novel metabolites that are antibacterial, anticancer, antiviral, antifungal [8]. There are reports on the production of enzymes like amylase, cellulase, protease and lipase from Halotolerant and Halophilic Fungi from the Great Sebkha of Oran in Northwestern of Algeria [9].

The present study is aimed to document and identify the microfungal population in saline soils of Pulicat lake, Tamil Nadu. In addition, some fungal species isolated from saline soils were tested for weedicidal activity.

Materials and methods

Description of the site

Pulicat Lake is the second largest brackish water coastal lagoon and is located between 13.33° to 13.66° N and 80.23° to 80.25°E, 45 km north of Chennai in the Nellore district of Andhra Pradesh and extending into the Tiruvallur district of Tamil Nadu, India (Fig.1)

Collection of soil sample

Soil samples were collected approximately 4 cm below the ground surface randomly from 10 regions spread approximately 1 km distance along the pulicatlake (light house) Tiruvallur District, Tamil Nadu. About 200 gm of soil samples were collected from each region, mixed thoroughly, brought to the laboratory in a sterile polythene bag and processed within 24 hrs of collection. The soil sample was tested in CVR Labs, Saidapet, Chennai for Physical and chemical parameters analysis (Table 1).

Sterilization of media and glasswares

Glasswares and Potato Dextrose Agar medium were sterilized in an autoclave at a pressure of 103kPa for 20 minutes. Simultaneously, Petri dishes were sterilized in hot air oven at 160^0 C for 3 hours. Potato Dextrose Agar (PDA) medium amended with antibiotic chloramphenicol (150 mg/l) was used for isolating fungi from soil.

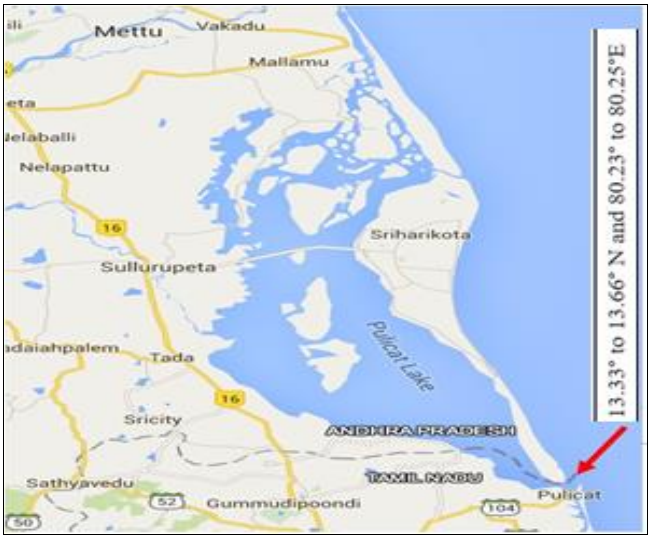


Fig 1: Map showing the Pulicat lake

Method for isolating soil fungi

Serial dilution plating method was used to isolate fungi from the soil samples [10]. In this method, one gram of the soil sample was dispersed thoroughly in 10ml of sterile distilled water. From this sample solution 1 ml was transferred to 9 ml of sterile water using micropipette. The resulting solution was mixed well and from this 1 ml was pipetted out into a conical flask containing 9 ml of sterile distilled water. From this dilution (10^3) one ml was transferred into a sterile petridish containing antibiotic amended PDA medium. Six replicates were maintained and the data obtained in this method was used for ecological analysis.

Incubation, identification and maintenance of cultures

The Petri dishes were incubated in light chamber maintained at 25 ± 3°C with 12 h light and 12 h dark cycle for 7 days. The isolated colonies were identified using standard identification manuals [11, 12, 13, 14, 15, 16] and pure cultures of soil fungi were stored, maintained in a refrigerator at 4^0C

Ecological analysis

Ecological parameters were used for determining the fungal population of the soil sample. Number of propagules, abundance and percentage frequency were calculated using the formulae:

1. Number of propagules/gm of soil = Average no. of colonies/plate / Weight of the soil x dilution factor
2. Abundance = Total no. of colonies of a species in all plates / Total no. of plates on which the species occurred
3. Percentage frequency = Total no. of plates in which the species occurs / Total no. of plates x 100

Weedicidal assay

The fungus was grown in PotatoDextrose broth for 20 days. About 25 ml of the culture filtrate was extracted with an equal volume of Ethyl acetate (EA). The organic phase was evaporated and the residue was dissolved in 25 ml of distilled water. Ethyl acetate extracts from uninoculated medium served as control. The inorganic E-medium – stock solution (KH_2 PO_4-680 mg; KNO_3-1515 mg; Ca $(NO_3)_2$-1180 mg; $MgSO_4.7H_20$-492 mg; H_3BO_3-2.86 mg; $MnCl_2$-3.62 mg; $FeCl_3.6H_2O$-5.40 mg; $ZnSO_4.7H_2O$-0.22 mg; $CuSO_4.5H_2O$-0.08 mg; EDTA-11.2 mg; Distilled water-1000 ml) was prepared and pH was adjusted by adding potassium hydroxide flakes up to 6–7. The Working E-medium was prepared by mixing 100 ml of stock solution and 900 ml of distilled water. Ethyl acetate extracts from uninoculated medium served as control. In eachpetridish, 20 mL working E-medium and 2ml extract along with the weed *Lemna*sp. having of 2 to 3 healthy fronds were added. *For each treatment 10 weeds were used.* It was placed in growth chamber by maintaining the temperature at 25 ± 3°C for 7 days. The status of the weed plant (browning and death) was recorded after the stipulated day of incubation. Results were analyzed as in percentage of weedicidal activity [17,18]

$$\text{Percentage of weedicidal activity} = \frac{\text{Number of weeds affected (turned brown)}}{\text{Number of weeds not affected}} \text{ X } 100$$

Result

Physical and chemical properties of the soil

The saline soil collected from Pulicat lake were analyzed for Physical and chemical parameters. (Table 1).

Table 1: Physical and Chemical parameters of the Saline soil of Pulicat lake

Parameter	Result (unit)
Texture	Sand 56% Silt 2% Clay 38%
Moisture	15.3%
Total soluble salts	5107.5 mg/kg
Nitrogen	3765.1 mg/kg
Phosphorus	366 mg/kg
Potassium	8968.9 mg/kg
Total organic carbon	0.38%

Isolation of micro fungi from saline soils of Pulicat lake.

The saline soil collected from the Pulicat lake was screened for the presence of fungi by using dilution plating method. A total of 12 fungal species could be isolated from the soil samples studied (Table 2). Non sporulating fungal colonies were classified under sterile forms and numbers were assigned based on the morphology of the colonies. On comparing different groups of fungi isolated from the soil samples 7 of them belong to hyphomycetes 1 yeast form and 4 sterile forms (Table 2 and Plate 1). Among these, hyphomycetes were considered as the dominated group in the micro fungal assemblage of saline soils (Fig.2).

Table 2: Number of propagules of fungi isolated from saline soils of Pulicat lake.

S.No.	Name of the fungus	No. of propagules per gram of soil
1	*Aspergillus terreus*	300
2	*Stachybotrys sp.*	130
3	*Aspergillus niger*	20
4	*Aspergillus* sp.1	20
5	*Aspergillus* sp.2	20
6	*Aspergillus* sp.3	10
7	*Penicillium*sp.1	30
8	Yeast form 1	40
9	Sterile form 1	40
10	Sterile form 2	20
11	Sterile form 3	10
12	Sterile form 4	20

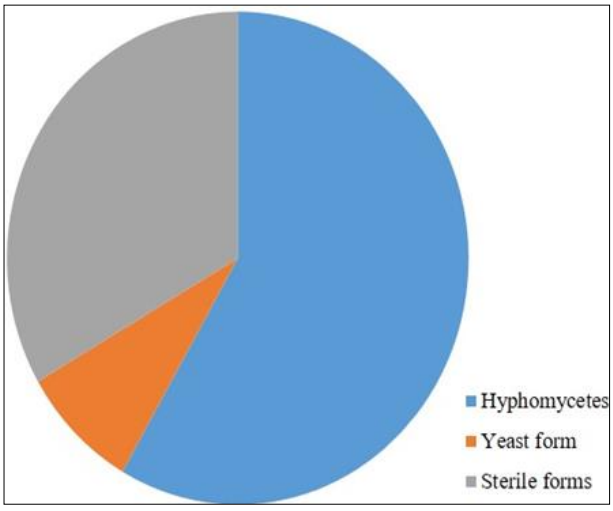


Fig 2: Percentage occurrence of different groups of fungi in saline soils of Pulicat lake.

In our study, *Aspergillus terreus*re presented more number of propagules and showed be maximum percentage frequency (Fig.3). Further it also was found to be the most abundant species (Fig. 4). Aspergilli represented more number of species in the soil sample screened indicates their adaptability and ubiquity to these stressful soils.

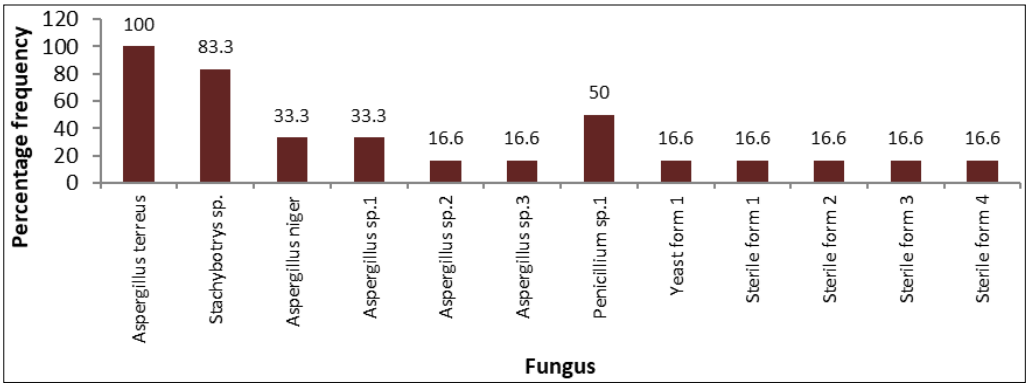


Fig 3: Percentage frequency of microfungiin saline soils of Pulicat lake

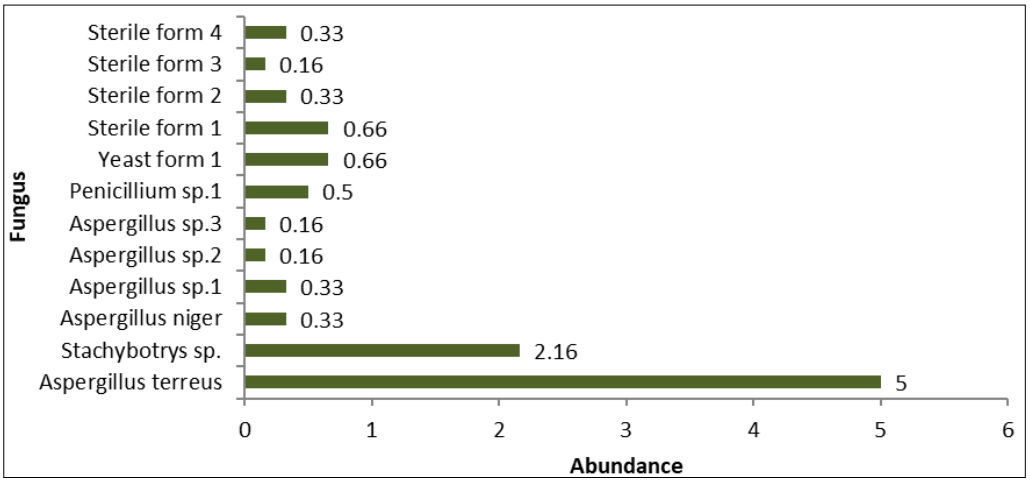
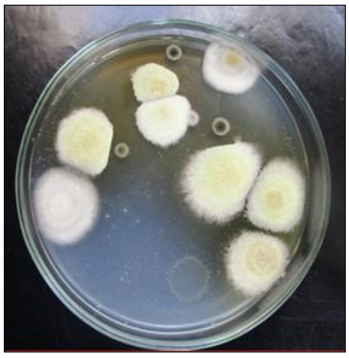
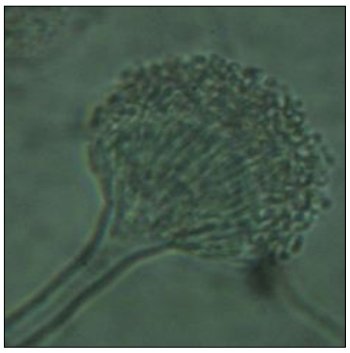


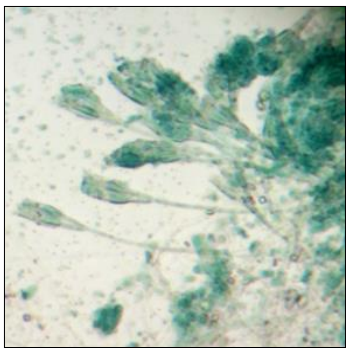
Fig 4: Abundance of microfungiin saline soils of Pulicat lake



A. Petri plate showing the growth



B. *Aspergillus terreus*



C. *Penicillium*sp.1

Plate 1

Effect of culture filtrate of fungi on *Lemna*sp.

Weedicidal activity was tested using culture filtrates of 6 fungal species (*Aspergillus terreus*, *Aspergillus niger*, *Stachybotrys*sp, *Aspergillus* sp.1, *Penicillium*sp.1, Sterile form 1) (Plate 2). Only three fungal species namely *Aspergillus niger*, *Stachybotrys*sp.and*Penicillium*sp.1 showed weedicidal effect as 100, 50 and 40%. Among these, *Aspergillus niger* showed the promising weedicidal activity (Table 3).

Table 3: Percentage of weedicidal activity of culture filtrate of fungi isolated from saline soil of Pulicat lake

S.No.	Name of Fungus	Activity (%)
1.	Control	-
2.	*Aspergillus terreus*	-
3.	*Aspergillus niger*	100
4.	*Stachybotrys*sp.	40
5.	*Aspergillus* sp.1	-
6.	*Penicillium*sp.1	50
7.	Sterile form 1	-

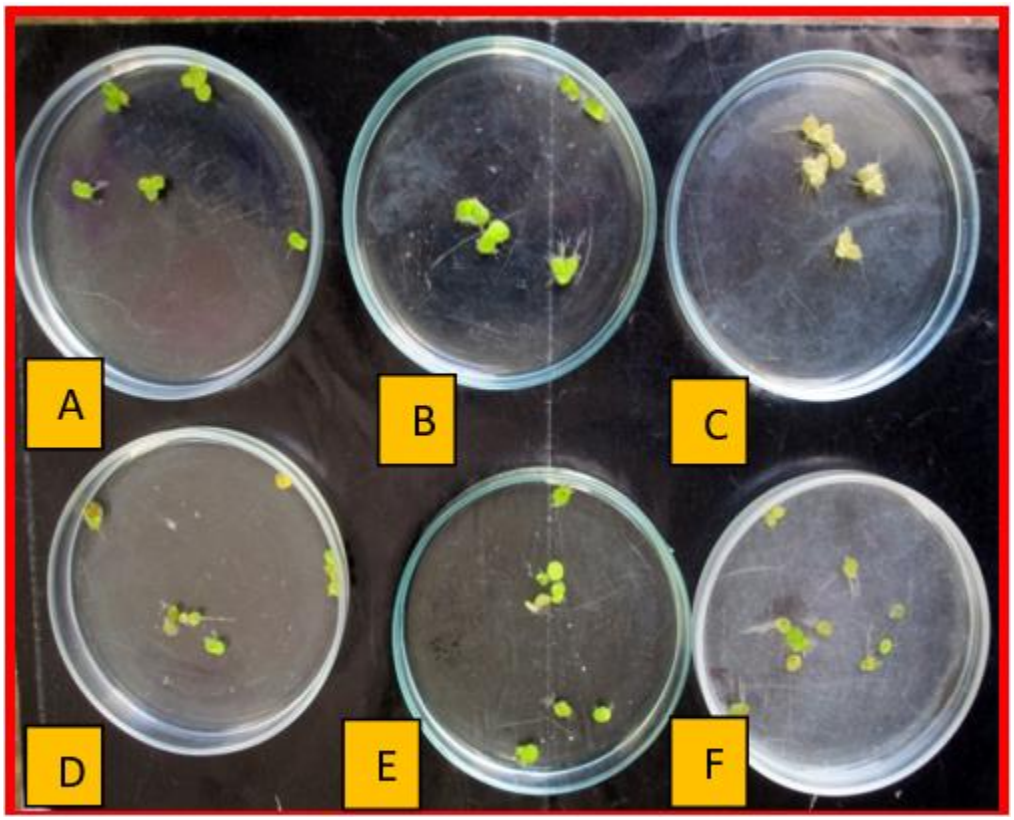


Plate 2: Weedicidal activity of culture filtrates of microfungi A=Control B=*Aspergillus terreus* C=*Apergillus niger* (Note: Browning of leaves)D=*Stachybotrys* sp.1 E=*Aspergillus* sp.1F=*Pencillium* sp.1

Conclusion

Pulicat lake soil sample was screened for the presence of fungi. The salinity was found high in the soil and low organic content was noticed. A total of 8 genera belonging to 12 species could be recovered from the soil by adopting dilution plating method. Among these, 7 belong to Hyphomycetes, 4 were sterile forms and one was a yeast form. Hyphomycete group dominated the fungal assemblage. Aspergilli represented more number of species. Six fungal species were tested for the weedicidal activity. Three fungal species showed promising activity against the weed *Lemna* sp. Thus, this study provided some presumptive evidence that fungal metabolites could be explored for the potential weedicidal activity in future.

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