World Journal of Pharmaceutical research

Volume 3, Issue 2, 2347-2356. Research Article ISSN 2277 – 7105

INVESTIGATION ON FUNGAL DIVERSITY IN COASTAL MANGROVE SOIL AT THONDI RAMANATHAPURAM (DT), EAST COAST OF TAMIL NADU, INDIA

***S.Madavasamy[1] and A.Panneerselvam[2]**

[1]Department of Botany, PRIST University, Thanjavur, Tamil nadu. India.

[2]Associate Professor and Head, Department of Botany & Microbiology, A.V.V.M Sri Pushpam College, Poondi. Thanjavur, Tamil nadu. India.

Article Received on
05 December 2013
Revised on 06 January 2013,
Accepted on 05 February 2014

***Correspondence for Author:**
S.Madavasamy,
Department of Botany, PRIST University, Thanjavur, Tamil nadu. India.

ABSTRACT

The present study was confined to the Thondi mangrove ecosystem in Ramanathapuram District, Tamil Nadu. Mangrove Sediment (Soil) samples were collected for one year to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. Colony growth rate of the fungi was studied on solid media. The physico-chemical characteristics of soil samples were found to influence the distribution and population of fungi. Therefore it could be concluded that there is no uniformity in the diversity of marine fungi and their distribution pattern in different geographical regions. The factors were discussed in this manuscript which affecting the distribution of fungi.

Key words: Thondi, mangrove ecosystem, fungi, Sediment, physico-chemical parameters.

INTRODUCTION

Mangrove forests are located at the interface between land and sea, a unique and extreme environment. The soils in mangrove communities are muddy or sandy with loose sediment. They contain submerged mangrove roots, trunks and branches. These conditions attract rich communities of fungi and bacteria. Biodiversity of fungi is an important aspect to be dealt with utmost scientific accuracy and accountability.

Mangroves are coastal wetland forests established at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical

latitudes. Mangrove forests are also referred to as mangrove swamps, tidal forests, tidal swamp forests or mangals and considered a dynamic ecotone (or transition zone) between terrestrial and marine habitats. Approximately 25 % of the world's coastline is dominated by mangroves distributed in 112 countries and territories encompassing an area of 181,000 sq km. worldwide.

Mangrove forests are biodiversity "hotspots" for marine fungi. These fungi play an important role in the nutritive cycle and support the mangrove ecosystem. They commonly occur as saprophytes on decomposing organic matter such as wood, stem, leaf etc and as symbionts of plants and animals and as parasites of plants in mangrove ecosystem. Fungi being ubiquitous organisms occur in all types of habitats and are the most adaptable organisms. The soil is one of the most important habitats for microorganisms like bacteria, fungi, yeasts, nematodes, etc (Wahegaonkar *et al.*, 2011).

Mangrove forests generate considerable amount of detritus such as leaf litter, woody debris and infloresence (Wafar *et al.*, 1997) and hence constitute an ideal habitat for many detritus dependant fauna and microbes. Chandralata (1999) and Raghukumar and Raghukumar (1998) reported adaptation and activity of terrestrial fungi under marine/ mangrove ecosystem as facultatives or indwellers or residents.

Based on the necessary basic information obtained on marine fungi and mangrove ecosystem, the present study has been undertaken in Thondi mangroves, a coastal deltaic habitat Ramanathapuram District, along the East coast of Tamil Nadu.

MATERIALS AND METHODS

Study Area

This study was carried out in the permanent site of the Mangrove region at Thondi Ramanathapuram (DT), Tamil nadu. Between the months of March 2011 to February 2012. Thondi is a Panchayat town in Ramanathapuram district in the Indian state **of** Tamil Nadu**.** It is an ancient port site referred in the name of Tyndis**.** This village is situated in the Palk Bay, (Lat 9° 43' and 10° 2' N; Long 77° 47' and 78° 49' E). Thondi had a population of 15,298 (Fig-1).

Sampling schedule

Soil samples were collected monthly for a period of one year from March 2011 to February 2012.

Collection of soil sample

Soil samples were collected from mangrove region of Thondi, Ramanathapuram (DT), Tamil Nadu (Fig-2). Soil samples were collected from the study site at random during the study period. The samples were made at a depth within 10-15 cm from the surface of the soil. The collected soil samples were brought to the laboratory in sterilized polythene bags handpicked air, dried and stored in containers for further analysis.

Physico-chemical analysis of soil

The physico-chemical properties of the soil samples were determined in accordance with standard analytical methods (Subramanyam and Sambamurthy, 2002). The characteristics in relation to Temperature Pressure, pH and Salinity of medium (Masuma *et al.*, 2001) were analyzed.

Isolation of fungi (Warcup, 1950)

Cooke Rose Bengal agar was chosen as growth medium. Soil dilution and soil sprinkle plates were used as isolation techniques. Soil dilutions were made by suspending 1 g of each soil sample in 50 ml of sterile distilled water. These suspensions were stirred for 20 min before making 7-fold falling dilutions and distributing 0.1-ml aliquots onto the medium in the plates. Sprinkle plates were prepared by uniformly distributing the soil directly on the surface of the medium. The plates were incubated at 25±3°C for up to 7 days. Fungi growing on the agar plates were transferred by sub culturing from hyphal tips, colonies or spores to fresh beer agar.

Identification of fungi

The isolated fungi were identified to the genus level and to the species when possible on the basis of macromorphological and micromorphological characteristics using suitable media, slide cultures (obtained by inoculating microfungi directly on a small square of agar medium) and the most updated keys for identifications.

Macroscopic study

Colonies of fungi were cultivated on potato dextrose agar at corresponding isolated

temperature for 5 days. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc.

Microscopic study

Fungal spores were cultivated on potato dextrose agar medium in glass microchamber (3) at isolated temperature for 6 days. The germination and growth of mycelium, hyphal structure, spore size, shapes and spore bearing structure was observed daily under a light microscope. Identification has been done by referring the standard manual Ainsworth *et al.*, (1973). Spore identification was done by Spore atlases of Gregory (1973) and Anna (1990).

Presentation of data

Number of species is referred as species diversity. Population Density is expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factor. In order to assess the dominance of individual species in each site percentage contribution was worked out as follows.

$$\% \text{ contribution} = \frac{\text{No. of colonies of fungus in a sample}}{\text{Total number of all colonies of all the species in a sample}} \times 100$$

RESULTS AND DISCUSSION

The physico-chemical characteristics of the soil samples organic matter contents, macronutrients, micronutrients and pH values is shown in Table 1. Physico-chemical characteristics of the soil samples were collected from different month's environmental factors, including climate, geomorphology, hydrodynamics and soil characteristics control the structure and function of any ecosystems. Among the biotic factors, in a particular soil nutrient status, are believed to be the most significant factors.

The pH values of all the soils samples analyzed from ranging from 7.16 to 8.15. pH is an important soil property,having great effects on solute concentration and absorption in soil. Lack of coastal vegetation, higher amount of human activities and also oil pillage from the motorized vessels used for fishing activities as well as washout from the catamaran might be the possible reason for the lower species richness and diversity of these above stations with the noted abiotic factors existing in the coastal environment. The presence of soil micro-organisms capable of killing nonindigenous fungi by lysing their cell walls is well documented (Mitchell and Alexander, 1963.).

Table-1 Physico- chemical analysis of Thondi soil sample

S.No.	Name of the Parameters	2011 to 2012											
		Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
1.	pH	7.89	7.29	7.16	7.63	7.36	8.15	7.69	7.97	8.06	7.98	7.98	8.09
2.	Electrical Conductivity (dsm^{-1})	0.45	0.42	0.36	0.36	0.36	0.69	1.36	0.36	0.32	0.36	0.35	0.39
3.	Organic Carbon (%)	0.47	0.51	0.62	0.48	0.28	0.52	0.58	0.41	0.42	0.35	0.34	0.29
4.	Organic matter (%)	0.56	0.31	0.42	0.33	0.41	0.40	0.23	0.30	0.32	0.35	0.47	0.25
5.	Available Nitrogen (mg/kg)	97.2	89.6	86.2	87.5	87.6	96.5	99.8	101.5	110.4	106.3	93.8	102.4
6.	Available Phosphorous (mg/kg)	4.50	3.25	2.48	3.12	3.48	4.36	4.26	4.78	5.36	3.75	3.50	3.75
7.	Available potassium (mg/kg)	76	82	79	68	73	81	84	79	83	87	89	91
8.	Available Zinc (ppm)	1.09	0.74	0.96	0.63	0.65	1.06	1.45	1.06	1.36	1.26	1.23	1.21
9.	Available copper (ppm)	1.08	0.78	0.68	0.52	0.57	1.05	1.18	1.25	1.06	1.03	1.20	1.25
10.	Available Iron (ppm)	6.52	5.96	4.68	3.98	2.65	4.21	4.36	4.28	5.46	4.78	4.46	3.65
11.	Available Manganese (ppm)	3.45	3.48	1.64	1.29	1.49	2.54	3.45	3.18	3.25	3.54	3.25	3.15
12.	Cat ion exchange capacity (C.Mole	18.1	16.5	15.3	16.3	15.2	17.5	17.9	19.3	20.5	18.4	18.8	19.7
13.	Calcium (mg/kg)	6.5	5.3	6.2	6.5	6.9	5.4	7.2	6.8	5.6	6.7	5.3	5.6
14.	Magnesium (mg/kg)	4.9	5.4	3.7	2.9	2.7	3.9	4.6	3.9	3.5	5.3	4.6	4.2
15.	Sodium (mg/kg)	2.25	2.18	0.86	0.96	0.35	1.48	1.85	1.78	2.89	2.35	2.22	2.19
16.	Potassium (mg/kg)	0.18	0.16	0.25	0.19	0.25	0.25	0.34	0.16	0.28	0.22	0.19	0.26

Table-2 Total number of colonies, mean density (CFU/g) and percentage contribution of fungi from Thondi

S. No	Name of the organism	March 2011 to February 2012																						Total no. of colonies	% contribution
		March		April		May		June		July		August		Sep.		October		Nov.		Dec.		Jan.		Feb.			
		TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD	TNC	MD		
1	*Acremonium* sp.	2	0.67	-	-	-	-	2	0.67	-	-	-	-	-	-	2	0.67	-	-	-	-	2	0.67	-	-	8	0.98
2	*Acrocylindrium oryzae*	2	0.67	2	0.67	4	1.33	3	1.00	3	1.00	-	-	-	-	-	-	3	1.00	-	-	3	1.00	4	1.33	24	2.94
3	*Acrophialophora fusispora*	6	2.00	2	0.67	2	0.67	-	-	-	-	4	1.33	2	0.67	-	-	2	0.67	-	-	-	-	-	-	18	2.21
4	*Aspergillus awamori*	2	0.67	2	0.67	3	1.00	2	0.67	2	0.67	3	1.00	2	0.67	-	-	2	0.67	5	1.67	-	-	-	-	23	2.82
5	*A. alliaceus*	-	-	3	1.00	3	1.00	2	0.67	-	-	2	0.67	3	1.00	2	0.67	-	-	2	0.67	2	0.67	3	1.00	22	2.70
6	*A. carneus*		-	2	0.67	3	1.00	-	-	4	1.33	5	1.67	5	1.67	-	-	3	1.00	-	-	4	1.33	-	-	26	3.19
7	*A. clavatus*	2	0.67	-	-	-	-	-	-	3	1.00	-	-	4	1.33	2	0.67	2	0.67	2	0.67	-	-	4	1.33	19	2.33
8	*A. fumigatus*	-	-	4	1.33	4	1.33	-	-	-	-	2	0.67	2	0.67	-	-	5	1.67	-	-	-	-	-	-	17	2.08
9	*A. granulosis*	5	1.67	-	-	-	-	3	1.00	5	1.67	-	-	-	-	-	-	-	-	-	-	3	1.00	-	-	16	1.96
10	*A. koeningii*	-	-	-	-	-	-	-	-	-	-	3	1.00	4	1.33	2	0.67	3	1.00	2	0.67	-	-	3	1.00	17	2.08
11	*A. lentulus*	-	-	-	-	-	-	3	1.00	4	1.33	2	0.67	-	-	-	-	-	-	-	-	3	1.00	-	-	12	1.47
12	*A. luchensis*	-	-	-	-	-	-	-	-	2	0.67	4	1.33	4	1.33	6	2.00	4	1.33	6	2.00	-	-	-	-	26	3.19
13	*A. nidulans*	-	-	2	0.67	2	0.67	-	-	-	-	-	-	-	-	2	0.67	-	-	2	0.67	-	-	-	-	8	0.98

14	*A. niger*	**2**	**0.67**	**-**	**-**	**-**	**-**	**3**	**1.00**	**2**	**0.67**	**3**	**1.00**	**5**	**1.67**	**-**	**-**	**4**	**1.33**	**-**	**-**	**3**	**1.00**	**5**	**1.67**	**27**	**3.31**
15	*A. ochraceous*	**-**	**-**	**4**	**1.33**	**4**	**1.33**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**1.67**	**-**	**-**	**5**	**1.67**	**-**	**-**	**-**	**-**	**18**	**2.21**
16	*A. oryzae*	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.67**	**3**	**1.00**	**2**	**0.67**	**3**	**1.00**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.67**	**2**	**0.67**	**14**	**1.72**
17	*A. quercinus*	**2**	**0.67**	**-**	**-**	**-**	**-**	**3**	**1.00**	**-**	**-**	**-**	**-**	**4**	**1.33**	**-**	**-**	**3**	**1.00**	**-**	**-**	**3**	**1.00**	**-**	**-**	**15**	**1.84**
18	*A. restrictus*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**1.67**	**5**	**1.67**	**3**	**1.00**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**13**	**1.59**
19	*A. ruber*	**-**	**-**	**2**	**0.67**	**2**	**0.67**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**3**	**1.00**	**7**	**0.86**
20	*A. sojae*	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**3**	**1.00**	**-**	**-**	**4**	**1.33**	**-**	**-**	**3**	**1.00**	**2**	**0.67**	**-**	**-**	**-**	**-**	**12**	**1.47**
21	*A. sydowi*	**2**	**0.67**	**-**	**-**	**-**	**-**	**3**	**1.00**	**-**	**-**	**2**	**0.67**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**3**	**1.00**	**5**	**1.67**	**15**	**1.84**
22	*A. tamarii*	**-**	**-**	**2**	**0.67**	**-**	**-**	**-**	**-**	**4**	**1.33**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.67**	**-**	**-**	**-**	**-**	**2**	**0.67**	**10**	**1.23**
23	*A. terreus*	**-**	**-**	**-**	**-**	**4**	**1.33**	**-**	**-**	**-**	**-**	**3**	**1.00**	**-**	**-**	**2**	**0.67**	**3**	**1.00**	**2**	**0.67**	**-**	**-**	**-**	**-**	**14**	**1.72**
24	*A. versicolor*	**2**	**0.67**	**-**	**-**	**-**	**-**	**-**	**-**	**4**	**1.33**	**-**	**-**	**5**	**1.67**	**-**	**-**	**2**	**0.67**	**-**	**-**	**-**	**-**	**4**	**1.33**	**17**	**2.08**
25	*A. wentii*	**4**	**1.33**	**-**	**-**	**2**	**0.67**	**2**	**0.67**	**-**	**-**	**5**	**1.67**	**3**	**1.00**	**-**	**-**	**-**	**-**	**2**	**0.67**	**2**	**0.67**	**3**	**1.00**	**23**	**2.82**
26	*Botryotricum* sp.	**-**	**-**	**3**	**1.00**	**3**	**1.00**	**4**	**1.33**	**5**	**1.67**	**3**	**1.00**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4**	**1.33**	**5**	**1.67**	**27**	**3.31**
27	*Botrytis cinerea*	**2**	**0.67**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.67**	**3**	**1.00**	**2**	**0.67**	**-**	**-**	**2**	**0.67**	**-**	**-**	**7**	**2.33**	**18**	**2.21**
28	*Cephalosporium lignicolum*	**-**	**-**	**3**	**1.00**	**3**	**1.00**	**5**	**1.67**	**3**	**1.00**	**4**	**1.33**	**-**	**-**	**4**	**1.33**	**2**	**0.67**	**4**	**1.33**	**5**	**1.67**	**3**	**1.00**	**36**	**4.41**
29	*Cephalosporium* sp.	**5**	**1.67**	**-**	**-**	**-**	**-**	**6**	**2.00**	**-**	**-**	**-**	**-**	**3**	**1.00**	**2**	**0.67**	**2**	**0.67**	**2**	**0.67**	**6**	**2.00**	**-**	**-**	**26**	**3.19**
30	*Circinella minor*	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**0.67**	**3**	**1.00**	**2**	**0.67**	**4**	**1.33**	**2**	**0.67**	**-**	**-**	**2**	**0.67**	**2**	**0.67**	**-**	**-**	**17**	**2.08**

31	*Circinella* sp.	-	-	**3**	**1.00**	-	-	-	-	-	-	**7**	**2.33**	**2**	**0.67**	**6**	**2.00**	-	-	**6**	**2.00**	-	-	-	-	**24**	**2.94**
32	*Geotrichum candidum*	-	-	**2**	**0.67**	**2**	**0.67**	-	-	**3**	**1.00**	**3**	**1.00**	**6**	**2.00**	-	-	-	-	-	-	-	-	**3**	**1.00**	**19**	**2.33**
33	*Hypocrea virens*	**2**	**0.67**	**3**	**1.00**	-	-	-	-	-	-	**3**	**1.00**	-	-	**2**	**0.67**	**2**	**0.67**	**2**	**0.67**	**5**	**1.67**	-	-	**19**	**2.33**
34	*Masoniella grisea*	-	-	**2**	**0.67**	-	-	**2**	**0.67**	**5**	**1.67**	-	-	-	-	-	-	**3**	**1.00**	-	-	**2**	**0.67**	**3**	**1.00**	**17**	**2.08**
35	*Neurospora crassa*	**6**	**2.00**	-	-	-	-	-	-	-	-	-	-	**4**	**1.33**	**5**	**1.67**	-	-	**5**	**1.67**	-	-	-	-	**20**	**2.45**
36	*Penicillium* sp.	**2**	**0.67**	**3**	**1.00**	-	-	**2**	**0.67**	**5**	**1.67**	**3**	**1.00**	-	-	**2**	**0.67**	**2**	**0.67**	**2**	**0.67**	**2**	**0.67**	**4**	**1.33**	**27**	**3.31**
37	*Phoma hedericola*	-	-	**3**	**1.00**	-	-	**2**	**0.67**	**2**	**0.67**	**3**	**1.00**	**3**	**1.00**	-	-	**3**	**1.00**	**2**	**0.67**	-	-	-	-	**18**	**2.21**
38	*Rhizopus stolonifer*	**5**	**1.67**	**2**	**0.67**	-	-	-	-	-	-	**7**	**2.33**	-	-	-	-	-	-	-	-	**3**	**1.00**	-	-	**17**	**2.08**
39	*Sporotrichum* sp.	-	-	-	-	-	-	**3**	**1.00**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**	**1.00**	**6**	**0.74**
40	*Trichoderma polysporum*	-	-	**3**	**1.00**	**3**	**1.00**	-	-	**3**	**1.00**	**2**	**0.67**	-	-	**4**	**1.33**	-	-	-	-	**2**	**0.67**	-	-	**17**	**2.08**
41	*T. reesei*	-	-	-	-	**2**	**0.67**	**2**	**0.67**	-	-	**2**	**0.67**	-	-	-	-	**3**	**1.00**	**4**	**1.33**	**2**	**0.67**	-	-	**15**	**1.84**
42	*T. viride*	**5**	**1.67**	-	-	**2**	**0.67**	**2**	**0.67**	**3**	**1.00**	**3**	**1.00**	**6**	**2.00**	**2**	**0.67**	-	-	**2**	**0.67**	-	-	-	-	**25**	**3.06**
43	*Trichoderma* sp.	**6**	**2.00**	**4**	**1.33**	**4**	**1.33**	-	-	**5**	**1.67**	**3**	**1.00**	**2**	**0.67**	**2**	**0.67**	-	-	**2**	**0.67**	**3**	**1.00**	**5**	**1.67**	**36**	**4.41**
44	*Verticillium* sp.	-	-	-	-	-	-	**2**	**0.67**	-	-	**2**	**0.67**	-	-	-	-	**2**	**0.67**	**2**	**0.67**	**3**	**1.00**	-	-	**11**	**1.35**
	Total	**64**	**21.33**	**56**	**18.67**	**52**	**17.33**	**60**	**20.00**	**81**	**27.00**	**94**	**31.33**	**86**	**28.67**	**56**	**18.67**	**60**	**20.00**	**67**	**22.33**	**69**	**23.00**	**71**	**23.67**	**816**	**100.00**

A total of 44 isolates were obtained from the analyses of mangrove soil samples taken from the area of the Thondi, Ramanathapuram District, March 2011 to February 2012 through soil dilution and soil sprinkle plates techniques. The isolates from mangrove soil samples were identified as filamentous fungi belonging to the *Deuteromycetes.* The most frequently isolated species belong to the genera *Acremonium, Acrocylindrium, Acrophialophora, Aspergillus, Botryotricum, Botrytis, Cephalosporium, Circinella, Geotrichum, Hypocrea, Masoniella, Neurospora, Penicillium, Phoma, Rhizopus, Sporotrichum, Trichoderma* and *Verticillium*. The fungal isolates were presented in Table-1.

Percentage contribution of the individual species to the total fungal population. The maximum percentage contribution of 4.41% *Cephalosporium lignicolum* and *Trichoderma* sp., *Aspergillus niger, Penicillium* sp. (3.31%) was found with *Cephalosporium* sp., (3.19%), *Trichoderma viride* (3.06%) (Table 2).

CONCLUSION

In conclusion, in the present investigation a number of factors that can influence the diversity of fungi in the marine environment. No single factor can account for the diversity observe, the marine environment being a complex ecosystem with great variation in many parameters from ocean to ocean, from mangrove to mangrove and from shore to shore and sometimes over a narrow range. Therefore it could be concluded that there is no uniformity in the diversity of marine fungi and their distribution pattern in different geographical regions. Several factors of salinity, origin and nature of substrata, pH, macronutrients and micronutrients influence the occurrence and diversity of marine fungi. They are reliant on the nature of the substrate and temporal regions that favor the colonization, growth and substrate possession of the fungi.

ACKNOWLEDGEMENT

The authors thank Sri Gowri Biotech Research Academy, Thanjavur (Dt), Tamilnadu for the laboratory facility.

REFERENCES

[1] Chandralata, R., Asian Microbiological congress, Chennai, India, 1999. pp.12.

[2] Gregory, P. H., Microbiology of the atmosphere (2nd Ed.) Appendix I. Leonard Hillp. 1973, 31.

[3] Mitchell and M. Alexander, Lysis of soil fungi by bacteria, *Can. J. Microbiol.*, 1963, 9: 169-177.

[4] Raghukumar, C., and Raghukumar, S., *Aqua. Microbiol. Ecol*., 1998, 15: 153-163.

[5] Wafar, S., A.G. Untawale and Wafar, M., *Estuaries. Coastal. Shelf. Sci.*, 1997, 44: 111-124.

[6] Wahegaonkar, N., Salunkhe, S.M., Palsingankar, P.L., Shinde, S.Y., *Annals of Biol, Res*., 2011, 2(2): 198- 205.

[7] Warcup, J.H., *Nature*, Lond, 1950, 178:1477.