



Impact of sugar factory effluent on mycoflora of seed and rhizosphere of Pearl millet (*Pennisetum typhoides*)

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Abstract: Microorganisms play an important role in the growth and ecological fitness of their host. Seed and soil health are important in growing crop. Seeds of Pearl millet in different concentration of effluent (0, 10, 50 and 100 %) have been treated to study germination and mycoflora grown during germination. Increased seed mycoflora were found in 50% treatment (*Aspergillus niger*, *A. flavus*, *A. terreus*, *Penicillium verruculosum*, *Fusarium oxysporum*, and *Curvularia lunata*). *Aspergillus flavus* were common in 100 %, 10 %, and control (0%). Percent germination was 0 % in 100 % effluent treatment. Highest percent germination was reported in control (86%) followed by 10 % (48 %), and 50 % effluent treatment (24 %)). The rhizosphere and non-rhizosphere mycoflora of Pearl millet at different growth period (15, 30, 45, and 90 day) were studied with different concentration of sugar factory effluent, (0, 10,50 and 100 %) following serial dilution plate method, (Timonin, 1940). Species of *Aspergillus*, *Fusarium* and *Penicillium* were very commonly isolated from the rhizosphere. The rhizosphere mycoflora was very high at flowering stage of plant growth i.e. the microbial population was increased with age of plant up to flowering stage then it was decreased. Qualitatively 16 fungal species were recorded from the rhizosphere and soil. It is seen that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. terreus*, *Alternaria* sp., *Penicillium funiculosum*, *Rhizoctonia bataticola*, *Rhizopus stolonifera* were dominant both in the rhizosphere and soil. The species like *Cladosporium oxysporum*, *A. ustus*, *curvularia lunata*, *Helminthosporium* sp., *Mucor* sp., *Aspergillus acculeaus* and *Trichothecium* sp. were occurred randomly. Results on an average basis indicated that *Aspergillus niger*, *A. fumigatus*, *A. ustus*, *Curvularia lunata*, *Fusarium oxysporum*, *Mucor* sp. *Penicillium funiculosum*, and *Trichothecium roseum* appeared to be stimulated due to effluents whereas species like *Aspergillus nidulans*, *Aspergillus terreus*, *Alternaria* sp., *Cladosporium oxysprum*, *Rhizopus stolonifera*, *Torula* sp., and *Aspergillus acculeatus* were found to be inhibited due to effluent treatment. The R/S ratio also corresponded to this.

Keywords: Sugar factory effluent; *Pennisetum typhoides*; seed mycoflora; rhizosphere mycoflora; soil mycoflora; R/S ratio.

Introduction

In India, sugar industry is the second largest agro-based industry and it contributes significantly to the socio-economic development of the nation. Indian sugar industry is also a major sector to create employment probably 7.5 per cent in Indian economy. The sugar industry plays a leading role in global market being the world's second largest producer after Brazil, producing nearly 15 and 25% of global sugar and sugarcane respectively. The sugar industry produces around 300-350million tonnes (Mt)

cane, 20-22 Mt white sugar and 6-8 Mt jiggery and khandasri to fulfill the domestic consumption of sweeteners.

The industry is able to export around 1300 MW of power to the grid. Sugar industry is also involved to make avail of sugar complexes by manufacturing sugar, bio-electricity, bio-ethanol, bio-manure and chemical. (Venkatesh and Venkatewarlu, 2017). Sugarcane farming is the source of livelihood for nearly 2.5 crore people in rural Maharashtra.

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The sugarcane industry provides direct employment to about 1,65,000 workers, besides eight lakh workers engaged in harvesting and transport operations every year for six months. Rising trends of using the waste water (industrial effluents) for irrigation has the advantage of pollution removal where the pollutants are partly taken up by the plants and partly transformed in the soil without causing any damage. The findings of Damodharan and Reddy, (2012) suggested that the irrigation with the treated sugar industry effluent characterized by high nutritive value can improve the overall growth of the sugarcane compared to the bore well water. Dilute sugar mill effluent could be used for irrigation in nutrient deprived environment, (Hussain, 2013). The growth parameters like height of the plant, length and breadth of leaves, number of leaves per plant enhanced with increase in concentration of distillery effluent up to 75% but decline in growth parameters was observed in 100% treatment in sugarcane (Rath *et al.*, 2010). Higher concentration of sugar mill effluent could inhibit seed germination and seedling growth and eventually yield in some crops such as green gram (Baskaran *et al.*, 2009), peanut (Siva Sathi and Suja Pandian, 2012). Furthermore, irrigation with treated effluent minimizes the use of mixed compound chemical fertilizers, increases the soil organic matter, improves soil physical and chemical properties, upgrade soil fertility, and it is helpful for building good soil ecosystem and sustainable sugarcane production. It was interesting that the seedling growth was lower in control than the effluent, (Khan, 2019). Sugar mill effluent is acidic in nature and dull white in colour with decaying molasses smell. It affects photosynthesis and reduced the composition of substances by microbes (Bhuvaneshwari *et al.*, 2013, Saurabh and Shailaja, 2014). Large amount of organic and inorganic substances present in higher concentration in sugar factory effluent which

adversely affected the seed germination because of the high salt content which caused change of osmotic pressure outside of the seed. It decreased water absorption of the seed and then inhibited the seed germination (Adriano *et al.*, 1973). Highly acidic nature of effluent might be responsible for the damage of seeds and inhibition of percent germination. A total number of 15 species belonging to 9 genera of fungi were isolated during investigation in various sugarcane industries of Madhya Pradesh by Awasthi *et al.*, (2011). Sangeeta *et al.*, (2017) isolated *Aspergillus*, *Rhizopus*, *Alternaria* fungal flora and *Bacillus* and *Staphylococcus* bacteria from the sugar factory effluent.

In 1904 the German agronomist and plant physiologist Lorenz Hiltner first coined the term "**rhizosphere**" to describe the plant-root interface, a word originating in part from the Greek word "rhiza", meaning root (Hiltner, 1904). Hiltner described the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced, by the chemicals released from plant roots. The rhizosphere inhabiting microorganisms compete for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant (Hartmann *et al.*, 2009). These microorganisms play important roles in the growth and ecological fitness of their host. Furthermore, irrigation with treated effluent minimizes the use of mixed compound chemical fertilizers. The plant growth was highly affected due to the excess amount of chloride, alkalinity, hardness, calcium, magnesium, sulphate and phosphate in the sugar factory effluent. The root length was severely affected when compared to control (Ayyasamy, 2008). Sakhala *et al.*, (2012) observed increase in dry matter in 50% sugar factory effluent treated Pearl millet. During the experimental work there were two offseason

showers of rains and might be responsible for increased values in growth parameters in 100% treatment. Effluent treatment favours the growth except germination.

Pearl millet is usually grown as a dryland dual-purpose grain and fodder crop although it is sometimes irrigated in India, particularly the summer crop grown mainly as a forage crop. Pearl millet grain is the staple diet for farm households in the world's poorest countries and among the poorest people (Basavraj *et al.*, 2010). Pearl millet is common crop cultivated in North Maharashtra part of India for food and fodder and farmers nearby sugar factory used untreated effluent for irrigation in shortage of water. The flour made from the grain is used for making bread. The crop has an enormous yield for green fodder. The straw is used for thatching and as fuel.

Soil, a dynamic living matrix, is an essential part of the terrestrial ecosystem. In relation to plant growth, soil can be distinguished into two types, namely rhizosphere soil and non-rhizosphere soil. Rhizosphere is the narrow region of soil immediately surrounding the plant roots (Marschner *et al.*, 2004). It is the region where the soil and plant roots make contact, thus, characterized by increased microbial activities. The rhizosphere can also be described as a mixture of solid particles and active community of microorganisms, mostly bacteria (Haghighi *et al.*, 2011). The non-rhizosphere soil, also called the bulk soil is the soil free of plant roots and which is not part of any rhizosphere soil. Fungi belonging to the following genera are common in the rhizosphere soil: *Aspergillus*, *Cladosporium*, *Cephalosporium*, *Botrytis*, *Chaetomium*, *Fusarium*, *Mucor*, *Penicillium*, *Verticillium*, *Trichoderma*, *Rhizopus*, *Gliocladium*, *Monilia*, *Alternaria* and *Pythium*, etc. (www.agriinfo.in).

Microorganisms in the rhizosphere play a very important role in the growth and productivity of crop plant. The rhizosphere and rhizoplane microflora increased with plant age but declined after about twelve weeks (Eze *et al.*, 2014). Sakhala and Gangawane (2012) studied effect of molasses on the growth and rhizosphere mycoflora of *Cajanus cajan* L. and found inhibition in growth with higher R/ S ratio in 50% treatment of molasses. First flowering was also observed in 10 % treatment of molasses. Literature regarding effect of sugar factory on rhizosphere and soil mycoflora is rare therefore this work has been taken. In the present investigation impact of sugar factory effluent on seed germination, mycoflora appearing during germination and mycoflora in soil and rhizosphere at different period with different concentration of effluent have been studied with Pearl millet crop.

Materials and Methods

Collection of samples

The effluent sample was collected in a pre-cleaned, plastic container from the point of disposal of Belganga sugar factory located at Bhoras, Jalgaon District, and (M. S.) India. The collected effluent was stored at 5°C to maintain original characteristics

Effect of sugar factory effluent on the seed mycoflora

200 seeds of *Pennisetum typhoides* were placed on the moist plates for studying seed mycoflora. The seeds were dipped in the effluent for nearly 2 minutes and the filler paper was also moistened with sugar factory effluent. The seeds kept in the plates without treatment of effluent used as control. Observation for the presence of fungal species on the seed and germination was recorded after a week onwards. Percentage appearance was then calculated according to the protocol designed by Deshpande and Kulkarni, (1988)

Effect of sugar factory effluent on soil and rhizosphere mycoflora

The seeds of *Pennisetum typhoides* were sown in the soil, placed in earthenware pots (6 "X 9") and pots were irrigated with different concentration (10, 50 and 100 %) of effluent. The pots irrigated with tap water serves as control. All the treatments set including control were prepared in triplicates. Irrigation of all the set with water and factory effluent was done thrice a week during first and second month and twice a week in third month. The rhizosphere mycoflora was studied by serial dilution technique (Timonin, 1940). Five uprooting from each set was done after 15, 30, 45, and 90 days. The roots were carefully removed from the soil, shaken to remove excess soil and cut at the crown to separate the roots from the rest of plants, and were transported to the laboratory in sterile polythene bags. These roots were placed in 200 ml distilled sterile water in 250 ml conical flasks. The soil clinging to the roots was removed by shaking the roots thoroughly. Twenty ml Martins's Rose Bengal Agar Medium (for fungi) was poured in the sterilized Petri plate with 1ml of the soil suspension. Plates were moved clockwise and anticlockwise to mix the soil suspension. Plates were prepared in triplicate and incubated at room temperature (26+3°C). Similarly, non-rhizosphere soil was also collected from a depth of 1 cm. from the earthenware pots. The soil was diluted in sterile distilled water (10³). Enumeration of fungi in rhizosphere and non-rhizosphere soil was done by methods given by Dubey and Maheshwari (2002). The isolated fungi were identified on the basis of colony and morphology characters up to species level (Barnett and Hunter, 1973, Ellis, 1993)

Determination of rhizosphere effect (R: S ratio).

Rhizosphere effect is an indication of the degree of stimulation of microorganisms (fungi) in the

root region of a plant and is determined by dividing the population of fungi in cfu/g in the rhizosphere soil by that obtained in the non - rhizosphere soil (Dubey and Maheshwari, 2005).

$$\text{R/S ratio} = \frac{\text{Fungal population in the rhizosphere zone}}{\text{Fungal population in the non-rhizosphere zone}}$$

Results and Discussion

Effect on seed mycoflora and germination

Table 1 depicted percent seed germination and appearance of seed mycoflora with different concentration of effluents. Pure effluent which is acidic in nature inhibited percent germination. Results are in accordance with Adriano *et al.*, (1973). High osmotic pressure of the soil makes imbibition more difficult and retards germination. Similar observation was reported by Vijayaragavan *et al.*, (2011), Doke *et al.*, (2011), Khan *et al.*, (2019). *Aspergillus niger* and *Aspergillus flavus* shows occurrence in all treatments including control. Percent frequency of these fungi are quite higher in 100% (48%), and 50% (23%) as compared to 10% effluent treatment (3%) and control (3%) results are in accordance with the findings of Avasthi *et al.*, (2010) Sangeetha *et al.*, (2017) who studied dominance of these fungi in effluent. *Penicillium verruculosum*, *Fusarium oxysporum*, *Curvularia lunata* appears in 50 % effluent may be due to presence of nutrients present in the effluent. Results are not according to workers who studied treated effluent during their study, (Damodharan and Reddy, (2012), Rath *et al.*, (2010), Khan, (2019).

Table 1: Effect of sugar factory effluents on seed mycoflora of *Pennisteum typhoides*

Fungal species	% effluent			
	100	50	10	0
Percent germination	0.00	24.00	48.00	86.00
<i>Aspergillus niger</i>	48	23	3	3
<i>A. flavus</i>	12	12	1	1
<i>A. terreus</i>	0.0	2	--	--
<i>Penicillium verruculosum</i>	0.0	2	--	--
<i>Fusarium oxysporum</i>	0.0	1	1	--
<i>Curvularia lunata</i>	0.0	1	--	--

Effect of effluent on the rhizosphere and soil mycoflora

Altogether 16 fungal species were recorded from the rhizosphere and soil. The percentage frequency of fungal species occurrence at different growth periods and different concentrations of the effluent given in Table 2. It was seen that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. terreus*, *Alternaria* sp., *Penicillium funiculosum*, *Rhizoctonia bataticola*, *Rhizopus stolonifer* were dominant both in the rhizosphere and soil. Other species shown their occurrence randomly. Percentage frequency of occurrence of the species was highly variable when considered the rhizosphere and soil at different growth period of the plant. This was also proved when the treatment of effluent was considered. The species like *Cladosporium oxysporum*, *A. ustus*, *Curvularia lunata*, *Helminthosporium* sp., *Mucor* sp., *Torula* sp., *A. acculeatus* and *Trichothecium roseum* were occurred randomly.

When the results were compared on average basis *Aspergillus niger*, *A. fumigatus*, *A. ustus*, *Curvularia lunata*, *Fusarium oxysporum*, *Mucor* sp., *Penicillium funiculosum* and *Trichothecium roseum* appeared to be stimulated due to the effluents, whereas species like *Aspergillus nidulans*, *A. terreus*, *Alternaria* sp., *Cladosporium oxysporum*, *Rhizopus stolonifer*, *Torula* sp., and *A. acculeatus* were found to be inhibited due to the treatment of effluent. *Aspergillus niger* showed its occurrence in all growth period (15, 30, 45, and 90 days) in all treatments (0, 10, 50, and 100). Results indicated that rhizosphere mycoflora greatly inhibited (Table 2) due to treatment of effluent. When results compared at different periods of growth, it has been observed that rhizosphere and soil mycoflora inhibited in all concentration except 10 % treatment at 45 days (Fig-1). Results are in conformity with Andreote (2010), Sule and Oyeyiola (2012) Olahan *et al.*, (2015), Olahan *et al.*, (2016).

In the present study most of the common saprophytic fungi showed their occurrence more in number in rhizosphere than soil. Results are in conformity with earlier workers (Sakhala Shaila, 2012, Sakhala *et al.*, 2012) Increased mycofloral frequency in all treatment showed increased values due to the presence of mycoflora in the effluent, (Avasthi *et al.*, 2010, Sangeetha *et al.*, 2017).

R/ S ratio

The R/ S ratio of *Pennisetum typhoides* plant on an average showed inhibition in microbial interaction due to treatment of effluent. With pure effluent the R/ S ratio is 0.99 indicates inhibition in microbial population both in soil and rhizosphere as compared to control. Increased R/ S ratio values. Rhizosphere and R/ s ratio was observed in control at 30 days of growth following 10 % treatment. At 100 % and 50 % treatment R/ S ratio inhibited (Fig -1 and Table -2). At 90 days decreased R/ S value was observed. Results are in agreement with Eze *et al.*, (2014).

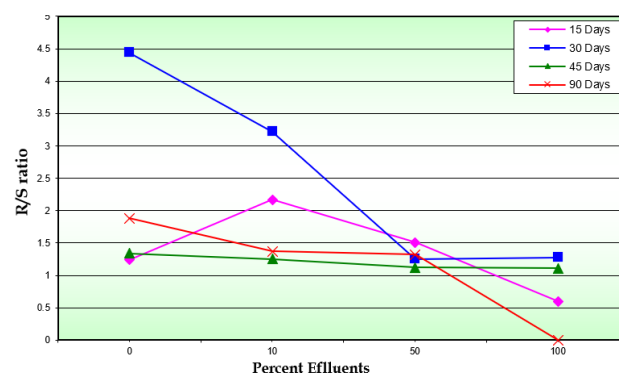


Figure 1. Effect of sugar factory effluents on R/S ratio in *Pennisetum typhoides* at different growth period.

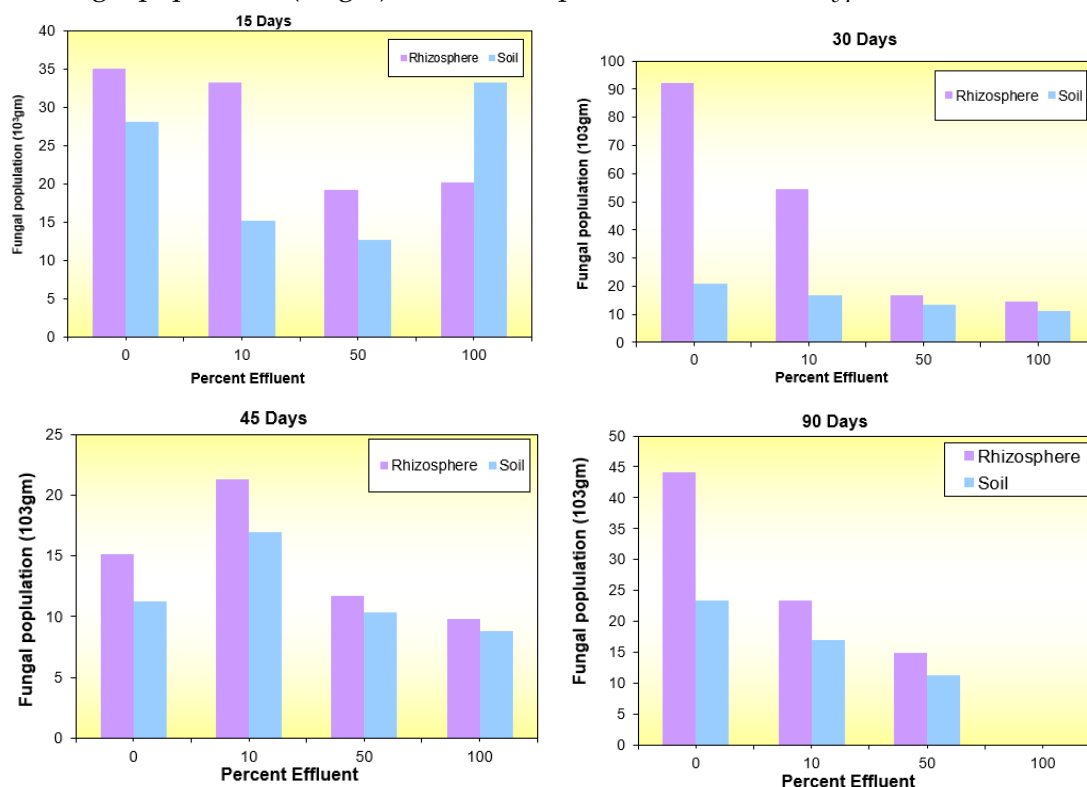
Conclusion

Untreated effluent discharged through sugar factory may cause detrimental effect to crop plant by changing soil composition, mycofloral diversity and frequency. Dilute concentration may be useful if properly treated. Species of *Aspergillus* occurred frequently, may be used for Bioremediation. Further research should be done in this field.

Table 3: Effect of sugar factory effluent on rhizosphere mycoflora of *Pennisetum typhoides*

		Growth of plants (days)				
		15	30	45	90	Average
Rhizosphere	0**	35.07*	92.40	15.14	44.06	46.66
	10	33.19	54.49	21.33	23.37	33.09
	50	19.22	16.78	11.71	14.95	15.66
	100	20.21	14.42	9.83	--	14.82
Soil	0	28.17	20.80	11.27	23.33	20.89
	10	15.23	16.90	16.97	17.00	16.52
	50	12.72	13.32	10.39	11.29	11.90
	100	33.26	11.26	8.81	--	17.89
R/S	0	1.24	4.44	1.34	1.88	2.2
	10	2.17	3.22	1.25	1.37	2.00
	50	1.51	1.25	1.12	1.32	1.3
	100	0.60	1.28	1.11	--	0.99

*Fungal population (10^3 / gm); **Concentration of effluent

Figure 2. Fungal population (10^3 gm) in the rhizosphere of *Pennisetum typhoides*

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Table 2: Percentage Frequency of mycofloral species in the rhizosphere of *P.typhoidus* at different growth periods treated with effluent.

Fungal Species		Days/ Effluent																Average			
		15				30				45				90							
		100	50	10	0	100	50	10	0	100	50	10	0	100	50	10	0	100	50	10	0
<i>A.niger</i>	R	29.13	36.76	12.25	18.36	29.12	2.10	32.63	11.26	14.10	18.26	8.42	5.44	--	10.41	--	3.33	18.08	25.19	13.32	9.59
	S	69.4	18.51	56.66	18.84	21.16	7.60	17.24	12.51	13.75	7.71	18.51	6.66	--	12.12	--	--	26.07	11.48	23.10	9.50
<i>A.flavus</i>	R	--	2.45	--	--	--	7.91	--	2.25	1.26	--	2.10	--	--	6.87	4.42	--	0.31	6.89	1.63	0.25
	S	--	3.70	--	--	5.23	6.33	2.84	--	4.21	--	--	--	--	--	7.75	--	2.36	2.50	2.64	--
<i>A.fumigatus</i>	R	12.94	9.80	36.11	11.29	29.12	60.12	16.31	15.76	1.26	4.56	29.62	11.29	--	59.02	106.19	--	10.83	33.37	47.05	9.58
	S	33.33	29.62	13.33	2.89	26.45	39.29	20.11	25.29	6.32	--	--	--	--	18.18	7.75	--	16.52	21.77	8.36	7.61
<i>A.nidulans</i>	R	12.94	14.70	25.00	25.42	6.47	16.87	90.90	18.01	1.26	2.28	1.05	8.23	--	12.12	--	--	5.16	11.49	29.23	12.91
	S	--	16.04	--	25.42	15.87	--	--	18.06	2.10	--	--	--	--	12.12	--	--	4.49	7.04	--	7.62
<i>A.terreus</i>	R	3.20	7.35	--	25.42	6.40	12.65	16.31	3.37	--	4.56	2.10	2.74	--	--	--	--	2.40	6.14	4.5	7.85
	S	--	2.46	5.00	1.43	5.23	12.67	25.86	--	3.16	4.69	--	2.22	--	--	--	--	2.09	4.95	7.71	0.91
<i>A.ustus</i>	R	--	--	--	--	--	1.05	--	--	--	--	--	--	--	--	--	--	--	9.25	--	--
	S	--	9.25	5.00	--	--	--	8.84	--	3.16	6.25	--	--	--	--	--	--	0.79	3.87	3.46	--
<i>C.oxysporum</i>	R	--	--	--	--	--	2.10	--	2.22	--	--	--	--	--	--	1.46	--	--	0.52	0.36	0.55
	S	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>F.oxysporum</i>	R	--	--	--	--	3.20	6.32	--	--	23.07	25.11	9.36	17.83	--	6.94	1.47	--	5.56	11.17	2.70	4.45
	S	--	--	--	--	--	--	--	--	43.38	14.08	22.22	26.66	--	12.12	15.50	--	10.84	6.55	9.43	6.56
<i>Helminthosporium Sp.</i>	R	--	--	--	2.82	--	--	--	1.12	--	--	--	--	--	--	--	--	--	--	--	0.98
	S	--	--	--	1.43	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.35
<i>Mucor</i>	R	--	--	--	--	--	--	--	--	2.54	--	--	--	--	--	--	--	0.63	--	--	--
	S	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>P.funiculosum</i>	R	--	--	--	4.23	16.18	6.32	3.49	2.25	17.94	6.84	29.96	12.34	--	3.47	--	3.33	8.53	4.15	9.23	5.53
	S	--	2.46	10.00	--	12.61	2.53	8.62	3.17	12.69	34.42	29.62	12.69	--	--	109.80	--	6.32	9.87	39.5	3.96
<i>R.bataticola</i>	R	38.83	284.31	25.00	14.83	12.94	5.27	6.99	25.90	--	--	--	--	--	1.46	36.26	12.94	72.39	8.36	19.22	
	S	8.33	19.75	13.33	82.60	--	32.31	48.85	9.43	--	--	--	--	--	1.09	3.87	--	2.08	5.21	16.51	23.00
<i>R.stolonifer</i>	R	24.27	--	363.60	211.86	--	--	266.99	126.68	7.69	22.83	18.72	49.38	--	17.36	2.94	13.33	7.99	10.04	164.06	100.39
	S	22.00	--	3.33	75.36	--	--	2.84	485.84	--	14.08	22.22	17.17	--	12.12	7.75	--	5.50	6.55	9.03	144.25
<i>Torula sp.</i>	R	--	--	--	1.42	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.35
	S	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>A.oculeatus</i>	R	--	--	--	--	3.20	--	--	--	--	--	--	--	--	7.75	--	0.80	--	1.93	--	
	S	--	--	--	17.39	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4.34
<i>T.roseum</i>	R	--	--	--	--	--	--	--	--	--	--	--	--	--	3.47	--	--	--	0.86	--	--
	S	--	--	--	--	--	--	--	--	--	--	--	--	--	--	11.62	--	--	--	2.90	--