

ISOLATION AND IDENTIFICATION OF VEGETABLE MARKET AEROMYCOFLORA FROM THE ENVIRONMENT OF WARDHA CITY

Botany

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ABSTRACT

Airborne fungi behaves as an indicators of the levels of atmospheric biopollutants. The study was carried out during the period of March 2017-February 2018. The fungi were isolated from the area of vegetable market of Wardha city. The exposed petriplate method were used for the isolation of fungi from morning and evening hours and humidity and temperatures were recorded regularly. Total 835 and 800 fungal colonies were recorded during one-year study period and they were belonging to 95 fungal species. The fungus *Cladosporium cladosporioides*, *C. herbarum*, *A.niger*, *A. flavus*, *A. nidulans*, *A.fumigatus* was the prevalent isolated types from vegetable market area of Wardha city from morning and evening hours. Maximum percentage contribution of fungal colonies was observed at morning and evening hours during rainy (41.43%), (40.05%) and winter season (39.64%), (41.16%) followed by summer season (18.92%), (18.03) respectively.

KEYWORDS

Aeromycoflora, Fungi, Petriplate exposure method, Vegetable market.

INTRODUCTION:

The fungi are present in the outdoor and indoor environment of the city. The number of fungal spores present in the environment depends upon the environmental factors like rainfall, temperature and humidity present in the air of the city. Airborne fungi behave as an indicator of the level of atmospheric bio-pollution. The presence of fungal spores, volatile and mycotoxin in the air can impact health in all segments of the population (Kakde *et al.*, 2001). Different types of fruits and vegetables are part of our daily diet and they are an important source of carbohydrates, vitamins and mineral content. Soil borne fungi is the reason for producing less yield of vegetables and fruits. The rotten vegetables and fruits are the source of infection and a variety of fungal spores have been produced. The vegetable vendors were dumping a variety of garbage in the nearby place and day by day it gives a bad smell in that area. The residential people are suffering from different infections as well as suffering from breathing and itching problems. In the present research attempts was made to determine the airborne fungal spores present in vegetable markets of the Wardha city.

MATERIAL AND METHODS

Following material and methods were used for the completion of my work.

Area selection: The vegetable market area was selected and it was always rushed by the people and more than 100 vendors were sold daily their vegetables to people.

Study period: The study was carried out during the year of March 2017-February 2018 at morning and evening hours

List of Equipment: Hot Air oven, Autoclave, Zeiss Trinacular Microscope (40X,100X) Bunsen burner, Laminar air flow, Microwave oven for heating, PH meter, Needles and spatulas.

Glassware: Conical flask, Beaker, Glass rod, Petridishes, Test tubes, Slides and Coverslip and other glasswares.

Chemicals: Agar powder, distilled water, lacto phenol cotton blue stain, alcohol or Spirit, Dextrose or sucrose, Ambistrin.

Other required materials: Potato, Cotton swab

MEDIA PREPARATION METHOD:

The following material and methods were used for the media preparation.

Table 1. List of chemical contents used for the preparation of PDA Media.

S. N.	Chemicals constituents	Net quantity in Mg /Lit
01	Potato (sliced)	200gm.
02	Dextrose	20 gm/lit.
03	Agar-Agar Powder	20gm/lit.
04	Doubled sterilized Distilled water	1000 ml.

05	Ambistrin	250 mg/lit.
06	pH	6.7

One litre media were prepared by using 200 gms. of potato sliced were added in 500 ml of distilled water and boiled up to 30 min. After boiling, potato solution was used for media preparation. 20 gms. of dextrose and 20 gms. of agar powder were dissolved in the solution and the volume was made up to 1000 ml by adding doubled sterilized distilled water. Then added the pinch of ambistrin as a source of streptomycin antibiotics, it used to avoid the bacterial contamination in the media. pH of media was adjusted by using pH meter, acids and bases.

Isolation and Identification of airborne fungi /outdoor aeromycoflora fungi.:

Sampling was done by employing petriplate exposure methods (Jadhav, and Tiwari., 1994; Devi *et al.*, 2010; Kalbende *et al.*, 2012). Six sterilized petriplates containing PDA media were exposed once in a month, three at evening and three at morning hours for 10-15 minutes. Exposed Petri plates were brought into the laboratory and incubated at $27 \pm 1^\circ\text{C}$ for 4 to 5 days, (Singh, 2006). Lactophenol and cotton blue stain were used for preparation of culture slide and identification was made by using literature related with fungi. The colony identification was done by using colony characteristics such as colour, shape of the colony and other morphological features of the mycelia and spores which were visible under the microscope by employing different objectives. Micro-photography was performed by Trinacular Zeiss Microscope from the laboratory of Department of Botany, Bajaj College of Science, Wardha

Ecological studies:

Percentage contribution of individual species of survey period was calculated by using the standard formula (Jadhav. and Tiwari., 1994; Tiwari, 1999).

$$\text{Percentage contribution} = \frac{\text{Total number of colonies in individual species}}{\text{Total number of colonies in all species}} \times 100$$

Metrological data:

During the present investigation period relative temperature, relative humidity and rainfall data were obtained from RBCA, Pipri, Wardha.



Figure 1: Photographs of vegetable market.

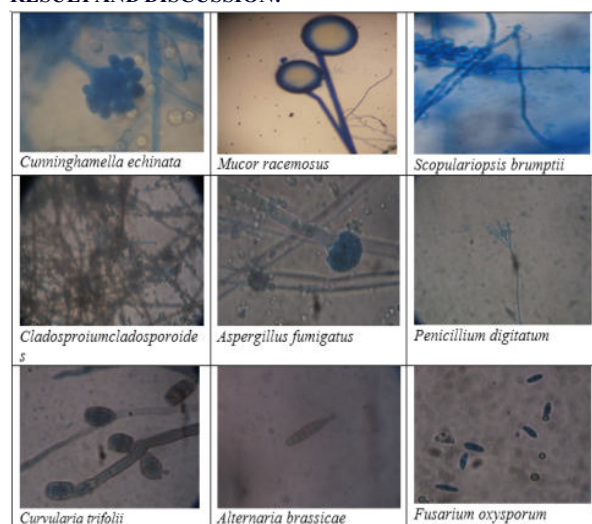
RESULT AND DISCUSSION:

Figure 2: Microscopic images of different fungal forms isolated from the area of vegetable market, Wardha.

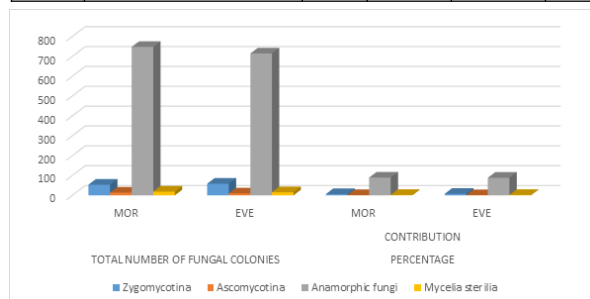
Table 2: Exploration and identification of Aeromycoflora from Vegetable market area during the year Mar 2017- Feb 2018 at Morning and Evening hours.

	NAME OF FUNGI	GRAND TOTAL		PERCENTAGE CONTRIBUTI ON (%)	
	ZYGOMYCOTINA	MOR NING	EVENI NG	MOR NING	EVENIN G
1	<i>Absidea corymbifera</i>	4	4	0.47	0.49
2	<i>Actinomucor elegans</i>	2	2	0.24	0.25
3	<i>Choenephra cucurbitarum</i>	6	9	0.71	1.11
4	<i>Cunninghamella echinata</i>	7	6	0.83	0.74
5	<i>Mucor circinneloides</i>	2	2	0.24	0.25
6	<i>Mucor hiemalis</i>	5	6	0.59	0.74
7	<i>Mucor racemosus</i>	5	5	0.59	0.62
8	<i>Rhizopus arrhizus</i>	7	11	0.83	1.35
9	<i>Rhizopus oryzae</i>	4	3	0.47	0.37
10	<i>Rhizopus stolonifer</i>	9	8	1.07	0.99
11	<i>Syncephalestrum racemosus</i>	3	3	0.36	0.37
	Total no of colonies	54	59	6.46	7.37
	ASCOMYCOTINA				
12	<i>Chaetomium globosum</i>	9	5	1.07	0.62
13	<i>Eupenicillium javanicum</i>	2	3	0.24	0.37
14	<i>Myrothecium roridum</i>	1	1	0.12	0.12
15	<i>Thielavia basicola</i>	2	1	0.24	0.12
	Total no of colonies	14	10	1.67	1.25
	ANAMORPHIC FUNGI				
16	<i>Alternaria alternata</i>	13	27	1.54	3.33
17	<i>Alternaria brassicae</i>	10	15	1.18	1.85
18	<i>Alternaria porri</i>	17	16	2.01	1.97
19	<i>Alternaria solani</i>	6	8	0.71	0.99
20	<i>Alternaria tenuissima</i>	12	12	1.42	1.48
21	<i>Aspergillus awamori</i>	6	5	0.71	0.62
22	<i>Aspergillus brassilensis</i>	7	9	0.83	1.11
23	<i>Aspergillus caesipitosus</i>	16	15	1.89	1.85
24	<i>Aspergillus clavatus</i>	4	2	0.47	0.25
25	<i>Aspergillus flavus</i>	33	61	3.91	7.51
26	<i>Aspergillus fumigatus</i>	25	30	2.96	3.69
27	<i>Aspergillus glaucus</i>	4	4	0.47	0.49
28	<i>Aspergillus luchensis</i>	3	3	0.36	0.37
29	<i>Aspergillus nidulans</i>	23	29	2.72	3.57
30	<i>Aspergillus niger</i>	51	66	6.04	8.13
31	<i>Aspergillus ochraceus</i>	7	7	0.83	0.86

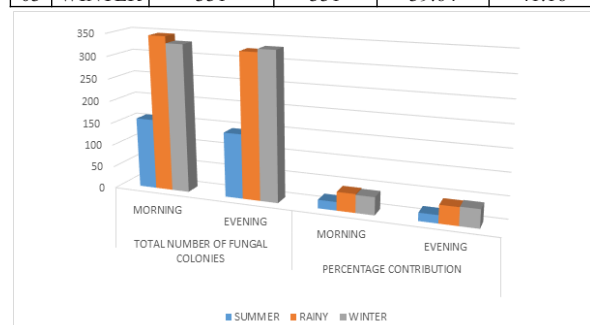
32	<i>Aspergillus oryzae</i>	4	5	0.47	0.62
33	<i>Aspergillus parasiticus</i>	8	8	0.95	0.99
34	<i>Aspergillus sulphureus</i>	0	4	-	0.49
35	<i>Aspergillus sydowii</i>	6	0	0.71	0.00
36	<i>Aspergillus tamarii</i>	7	2	0.83	0.25
37	<i>Aspergillus terreus</i>	7	7	0.83	0.86
38	<i>Aspergillus versicolor</i>	23	11	2.72	1.35
39	<i>Aureobasidium pullans</i>	2	4	0.24	0.49
40	<i>Beltrania rhombica</i>	1	0	0.12	0.00
41	<i>Bispora antennata</i>	4	3	0.47	0.37
42	<i>Candida albicans</i>	1	0	0.12	0.00
43	<i>Cladosporium cladosporioides</i>	103	87	12.19	10.71
44	<i>Cladosporium herbarum</i>	86	29	10.18	3.57
45	<i>Cladosporium oxysporum</i>	16	30	1.89	3.69
46	<i>Cladophialophora carrionii</i>	3	4	0.36	0.49
47	<i>Curvularia brachyspora</i>	6	5	0.71	0.62
48	<i>Curvularia clavata</i>	2	3	0.24	0.37
49	<i>Curvularia lunata</i>	5	7	0.59	0.86
50	<i>Curvularia pallescens</i>	9	5	1.07	0.62
51	<i>Curvularia trifolii</i>	17	4	2.01	0.49
52	<i>Drechslera tuberculata</i>	4	4	0.47	0.49
53	<i>Drechslera specifera</i>	4	3	0.47	0.37
54	<i>Drechslera tetramera</i>	7	5	0.83	0.62
55	<i>Diploidea olivarium</i>	7	4	0.83	0.49
56	<i>Epicoccum nigrum</i>	1	1	0.12	0.12
57	<i>Exerohilum rostratum</i>	6	7	0.71	0.86
58	<i>Fusarium chlamydosporum</i>	9	9	1.07	1.11
59	<i>Fusarium moniliforme</i>	7	4	0.83	0.49
60	<i>Fusarium oxysporum</i>	8	14	0.95	1.72
61	<i>Fusarium pallidoseum</i>	25	12	2.96	1.48
62	<i>Fusariella verrucosa</i>	2	5	0.24	0.62
63	<i>Geotrichum candidum</i>	7	7	0.83	0.86
64	<i>Gilmaniella humicola</i>	3	0	0.36	0.00
65	<i>Glucocladium roseum</i>	1	1	0.12	0.12
66	<i>Helminthosporium oryzae</i>	11	2	1.30	0.25
67	<i>Memnoniella echinata</i>	4	7	0.47	0.86
68	<i>Monillia sitophila</i>	8	2	0.95	0.25
69	<i>Nigrospora oryzae</i>	1	6	0.12	0.74
70	<i>Paecilomyces variotii</i>	9	1	1.07	0.12
71	<i>Penicillium citrinum</i>	6	9	0.71	1.11
72	<i>Penicillium chrysogenum</i>	5	13	0.59	1.60
73	<i>Penicillium digitatum</i>	7	5	0.83	0.62
74	<i>Penicillium expansum</i>	9	7	1.07	0.86
75	<i>Penicillium italicum</i>	5	9	0.59	1.11
76	<i>Penicillium oxalicum</i>	0	3	-	0.37
77	<i>Phoma glomerata</i>	1	0	0.12	0.00
78	<i>Periconia hispida</i>	4	1	0.47	0.12
79	<i>Pestalotiopsis glandicola</i>	2	3	0.24	0.37
80	<i>Pyricularia oryzae</i>	1	2	0.12	0.25
81	<i>Rhizoctonia solani</i>	0	1	-	0.12
82	<i>Scopulariopsis brumptii</i>	4	2	0.47	0.25
83	<i>Stachybotrys alba</i>	3	4	0.36	0.49
84	<i>Stachybotrys chartarum</i>	0	3	-	0.37
85	<i>Stemphylium solani</i>	9	0	1.07	0.00
86	<i>Torula caligans</i>	3	11	0.36	1.35
87	<i>Tricothecium roseum</i>	8	4	0.95	0.49
88	<i>Trichoderma harzianum</i>	8	10	0.95	1.23
89	<i>Trichoderma viridi</i>	0	6	-	0.74
90	<i>Ulocladium chartarum</i>	2	0	0.24	0.00
91	<i>Verticillium lecanii</i>	0	1	-	0.12
	Total no of colonies	748	715	89.58	89.375
	MYCELIA STERILIA				
92	<i>Sterile mycelium (ash)</i>	1	1	0.12	0.12
93	<i>Sterile mycellia (black)</i>	6	6	0.71	0.74
94	<i>Sterile mycelium (brown)</i>	4	2	0.47	0.25
95	<i>Sterile mycelium (white)</i>	8	7	0.95	0.86
	Total no of colonies	19	16		
	Monthwise total colonies	835	800	2.27	2.0

Table 3: Groupwise total seasonal percentage contribution of Aeromycoflora of Vegetable market area, Wardha at Morning and Evening hours during the study period of Mar 2017- Feb 2018.

SR NO	NAME OF FUNGAL GROUP	SUMMER		RAINY		WINTER		TOTAL NUMBER OF FUNGAL COLONIES		PERCENTAGE CONTRIBUTION	
		MOR	EVE	MOR	EVE	MOR	EVE	MOR	EVE	MOR	EVE
01	ZYGOMYCOTINA	14	12	23	23	24	17	54	59	6.46	7.83
02	ASCOMYCOTINA	03	01	09	07	02	02	14	10	1.67	1.24
03	ANAMORPHIC FUNGI	134	126	309	289	300	305	748	715	89.58	88.93
04	MYCELIA STERILIA	07	06	05	05	05	07	19	16	2.27	1.99
	Total	158	145	346	324	331	331	835	800		

**Fig 3: Group wise total seasonal percentage contribution of Aeromycoflora of Vegetable market area from Morning and Evening hours****Table 4: Seasonal percentage contribution of Aeromycoflora from Vegetable Market area, Wardha at Morning and evening hours during the study period Mar 2017- Feb 2018.**

SR NO	SEASON	TOTAL NUMBER OF FUNGAL COLONIES		PERCENTAGE CONTRIBUTION	
		MORNING	EVENING	MORNING	EVENING
01	SUMMER	158	145	18.92	18.03
02	RAINY	346	324	41.43	40.05
03	WINTER	331	331	39.64	41.16

**Fig 4: Seasonal percentage contribution of Aeromycoflora from Vegetable Market area from morning and evening hours**

Altogether 95 fungal species were recorded from vegetable market area of Wardha city at morning and evening hours. Out of these, total 89 fungal species were recorded in the morning and 88 fungal species were recorded in the evening hours. Total 835 and 800 fungal colonies were noted during one-year study period of March 2017-Feb 2018 (Table 2). The fungus *Cladosporium cladosporioides*, *C. herbarum*, *A. niger*, *A. flavus*, *A. nidulans*, *A. fumigatus* were the prevalent isolated types in the vegetable market area of Wardha city observed from morning and evening hours. Similar findings were recorded by Tilak and Chakra; 1977, Santra and Chandra; 1989.

According to their percentage, the highest percentage contribution was recorded by *Cladosporium cladosporioides* followed by *Cladosporium herbarum* and *Aspergillus niger* in the morning hours and similarly *Cladosporium cladosporioides* was recorded highest percentage contribution followed by *Aspergillus niger* and *A. flavus* in the evening hours respectively (Table 2). The genus *Aspergillus* and *Cladosporium* was the highest recorded fungal form in the vegetable market area of Wardha city. This type of similar findings were recorded by Nagadesi and Reddy, (2020) at Bangalore and Amit (2020) at Ashti.

Groupwise, the highest percentage contribution was recorded by Class Anamorphic fungi followed by class Zygomycotina, Ascomycotina

and Sterile mycelium in the morning and evening hours. (Table 3 & Fig 3).

Total 11 species of class Zygomycotina, 04 similar species of class Ascomycotina and Mycelia sterilia were recorded at both the hours and the fungal species of class Anamorphic fungi like *Aspergillus sydowii*, *Beltrania rhombica*, *Candida albicans*, *Gilmaniella humicola*, *Phoma glomerata*, *Stemphylium solani* and *Ulocladium charatum* were recorded only in the morning hours but their presence was not recorded in the evening hours. Similarly *Aspergillus sulphurii*, *Penicillium oxalicum*, *Rhizoctonia solani*, *Stachybotrys charatum*, *Trichoderma viridi* and *Verticillium lecanii* were recorded in the evening hours only due to extra moisture or humidity was present in the environment at the time of petriplate exposure.

Maximum percentage contribution of fungal colonies were recorded during rainy (41.43%), (40.05%) and winter season (39.64%), (41.16%) followed by summer season (18.92%), (18.03) in the morning and evening hours respectively (Table 4 & Fig4). These results were in accordance with the observations by Shanthamma and Jadhav (2014), Tiwari and Jadhav (2004), Jadhav and Kunjam (2009) in Raipur; Chauhan and Kulshrestha (2006) in Agra; Manoharachary *et al.* (1998) in Hyderabad; Kakde and Kakde (2012) in Nagpur.

CONCLUSION:

From the findings of our studies the Aeromycoflora of vegetable market area of Wardha city consisting different variety of fungal genera were present in that particular area and some fungi were restricted only in that area due to suitable conditions present for the growth of fungi and it was observed that the vegetable market area was never free from the fungal spores. Hence this study opens the door for exploring new fungal genera of this region. there was only the slight difference noted in the findings of Morning and Evening hours. Sometimes extra moisture was recorded in the evening hours.

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