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Studies on environmental bio-pollution by airborne fungi over a sugarcane field

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ABSTRACT

An aeromycological study at different sites viz., inner, outdoor and 100 meter away of sugarcane field in an agricultural village of Puducherry was carried out during the month from January to April by implementation of petriplate sedimentation method. During the study period, a total number of 420 fungal colony forming units (CFUs) were isolated from the entire site, of which centre of the field contributed the maximum (38%) followed by the border of the field (32%) and 100 meter (30%). Among the recorded taxa, members of Deuteromycotina were predominant in their occurrence followed by the members of Zygomycotina and Ascomycotina. In qualitative analysis, altogether 18 fungal species belonging to 10 genera and 3 sterile mycelia forms were isolated from the sites. Aspergilli are represented by 9 species. Plant pathogenic fungi like *Curvularia* and *Helminthosporium* were recorded from the field sites. It was revealed that the *Aspergillus awamori*, *Aspergillus niger*, *Aspergillus fumigatus* and *A. nidulans* were the highest with in the centre, border and 100 meters away of the sugarcane field. Besides the dominant ones, contributing substantially to the total fungal CFUs a large number of fungal types recorded incidentally one or three times during the study period contributing thus very negligibly. The month of February contributed the maximum spores in the outdoors but centre site shared the maximum spores in all the months studied. Isolates of *A. japonicus*, *A. nidulans*, *A. tamari*, *A. terreus*, *A. awamori*, *Cladosporium herbarum*, *Curvularia lunata*, *Penicillium fellutanum* and *Penicillium citrinum* accounted for less than or equal to 1 % of total CFUs isolated in both centre, border and 100 meter away of the sugarcane field.

Keywords: Environmental biopollution, Sugarcane field, airborne fungi

INTRODUCTION

Occurrence of fungal spores in the atmosphere has been a matter of concern for both aero-allergists and biologists with an interest in health and bio-pollution problems [1], since mould fungi are the causal agents of allergic rhinitis, bronchial asthma, hay fever and aspergillosis in the lungs of atopic human beings [2]. Such interest has fostered the studies aimed at the qualitative and quantitative characterization of the indoor and outdoor aero-mycoflora of crop fields throughout the world, as well as its relationship to meteorological factors and pathological dysfunctions among plant communities. Fungi, based on their biological features and ease of dispersion of their spores, make the chief agents of contamination of any type of substrate, so their spores prevail mainly in the air, both indoors and outdoors [2]. Because of their ubiquity, fungi generally cause major diseases in plants, animals and also in human beings. The importance of airborne microfungi has long been recognized, especially in plant pathology but their significance in human allergy has been recognized recently [2, 3]. The disease incidence was greatly influenced by quantitative assessment of pathogens i.e., fungi and bacteria in the bioaerosols, their seasonal concentrations and the

prevailing meteorological parameters for disease development between the growth stages of the host plant and presence of pathogen [4]. The Indian economy is basically agriculture. Most of the people of our country, a land of villages, are farm workers, with about 80 percent of its population living in the rural areas notwithstanding the rapid industrialization and technological progress made by India since independence. People in the rural areas are predominantly agricultural dependents. Due to unhygienic condition of the rural environment and prevailing aero-biopollutants, the people suffer from a large number of allergenic diseases mainly asthma, hay fever, dermatitis, rhinitis and conjunctivitis because the environment that plays an important role in precipitation of allergenic symptoms [1]. Further, the rapid industrialization and urbanization also enhance the risk of aero-biopollution in present days. Due to illiteracy, poverty, lack of awareness and distinct category of people, rich and poor, the risk due to aeroallergens in India is inevitable to industrial as well as to farm workers. For instance, *Didymella exitialis* can grow abundantly on ripening straw, a normal component of the air spora, but farm workers are likely to be more heavily exposed than people living at a distance from cereal fields [4]. Many works have been carried out in different states of India, but studies on airborne fungal spores in and around a sugarcane field is lacking in the state of Puducherry. The present study is an attempt to analyze the composition and concentration of airborne allergenic/pathogenic fungi in and around of a sugarcane field in an agricultural village.

MATERIALS AND METHODS

The present study was carried out in a sugarcane field of an agricultural village, Thondamanatham, Pondicherry during the cultivation period (January to April).

Study sites

The study site, Thondamanatham is a rural agricultural village, located 20 km away from Pondicherry city and 12 km away from coastal belt of Bay of Bengal. The village locality is 5 km away from two small towns like Thattanchavady and Villianur towards east and north respectively. The village occupies 1-1.5 sq.km area with a population of 4000-4500 people. It is not a socially and economically well-developed area, indeed development has been only in recent years. The chief occupation is agriculture and livestock. The village economy is mostly (75%) dependent on agriculture and cattle farming. The village with its natural fertile land and irrigation facility represents interesting types of vegetation and crop. The region comprises, cultivated land, rich vegetation around ponds and lake. Many food crops viz, paddy, sugarcane, ground nut, tapioca, black gram and crops like cotton and casuarinas, along with seasonal crops are cultivated. However during recent years, the cropland has been decreasing due to urbanization.

Air samplings

Air samplings were made at three distinct sites one of the sugarcane field viz., inside of the field (centre), outside (border) of the field and 100 meter away from the field at fortnight intervals during 10 AM to 11 AM for four months continuously. The media plates were exposed at 5 ft height from the ground in the three sites of the field. Three replicates of media plates (9cm) containing Potato Dextrose Agar (PDA) medium with streptomycin/penicillin (50mg) were carried to the study sites inside a sterilized container and exposed to the air for five minutes to receive the air borne fungal spores on the media plates by sedimentation method. Altogether 72 petriplates were exposed in the three study sites of the sugarcane field. Each site was air sampled at 15 day intervals and continued for total four months to complete the samplings. The exposure time was standardized to get countable number of fungal colonies/colony forming units (CFUs) per plate. Each set of plates exposed in different sites were brought separately to the Microbiology laboratory, Department of Biomedical Engineering, Sathyabama University, Chennai with utmost care and incubated in culture room at 25±3°C upside down for 15 days with constant observation after 3-4 days of incubation. Fungal colonies developed in plates were counted for individual species and to get the total number CFUs. Microscopic slides stained with lactophenol cotton blue were prepared from each CFUs and observed microscopically to identify them up to species level. The colony forming units CFUs that could not be identified directly from plates were sub cultured in PDA again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa [6, 7, 8].

RESULTS AND DISCUSSION

During the study period, a total number of 420 fungal colony forming units (CFUs) were isolated from all the sites, centre of the field, border and 100 meter away of the sugarcane field, of which centre of the field contributed the maximum (38%) followed by the border of the field (32%) and 100 meter away site (30%) (Fig 1). Incidence of

airborne fungal spores, their CFUs contribution and monthly occurrence recorded in each site of the sugarcane field are given in Table 1, 2 & 3. Among the recorded taxa, members of Deuteromycotina were predominant in their occurrence, followed by the members of Zygomycotina. In qualitative analysis, altogether 18 fungal species belonging to 10 genera and 3 sterile mycelia forms were isolated from all the sites. Centre of the field harbored the maximum number, 16 fungal species belonging to 8 genera and 2 sterile mycelia forms. Border site of the field contributed 13 species under 7 genera and 11 fungal species belonging to 7 genera and one sterile mycelia form were isolated from 100 meter away site of the sugarcane field. Among the total number of isolated fungal species, *Aspergillus* is represented by 9 species followed by *Penicillium* (2 species) and other genera had only species each. *Aspergillus* sp. contributed maximum in centre, border and 100 mt away followed by *Penicillium*, *Cladosporium*. Plant pathogenic fungi like *Curvularia* and *Helminthosporium* were recorded from the field sites. Besides the dominant ones, contributing substantially to the total fungal CFUs a large number of fungal types recorded incidentally one or three times during the study period contributing thus very negligibly. Isolates of *A. japonicus*, *A. nidulans*, *A. tamari*, *A. terreus*, *A. awamori*, *Cladosporium herbarum*, *Curvularia lunata*, *Penicillium fellutanum* and *P. citrinum* accounted for less than or equal to 1% of total CFUs isolated in both centre, border and 100 mt away of the sugarcane field. Monthly incidence of airborne fungal spores recorded in three experimental sites is given in the Fig 3. it shows the month of February contributed the maximum spores in the outdoors but beach site shared the maximum spores in all the months studied. *Curvularia* and *Penicillium* occurred regularly throughout the crop season.

Table 1: Relative incidence of air borne fungi spores recorded in the Centre site of the Sugarcane field

SI. No	Name of the Fungi	January	February	March	April	Occurrence
1.	<i>Aspergillus awamori</i>	3	8	-	10	13.29
2.	<i>Aspergillus niger</i>	2	4	3	-	5.69
3.	<i>Aspergillus nidulans</i>	1	-	-	12	8.22
4.	<i>Aspergillus japonicus</i>	2	-	10	-	7.59
5.	<i>Aspergillus tamarii</i>	4	3	7	-	8.86
6.	<i>Aspergillus fumigatus</i>	7	-	-	4	6.96
7.	<i>Aspergillus ochraceus</i>	3	1	-	1	3.16
8.	<i>Aspergillus ustus</i>	1	-	-	2	1.89
9.	<i>Cladosporium herbarum</i>	7	6	3	-	10.12
10.	<i>Curvularia lunata</i>	-	3	5	8	10.12
11.	<i>Drechslera indica</i>	1	-	2	-	1.89
12.	Green sterile mycelia	3	5	2	-	6.32
13.	<i>Penicillium fellutanum</i>	4	-	3	1	5.06
14.	<i>Penicillium citrinum</i>	-	4	1	-	3.16
15.	Pink sterile mycelia	1	-	-	1	1.26
16.	White sterile mycelia	5	3	1	1	6.32

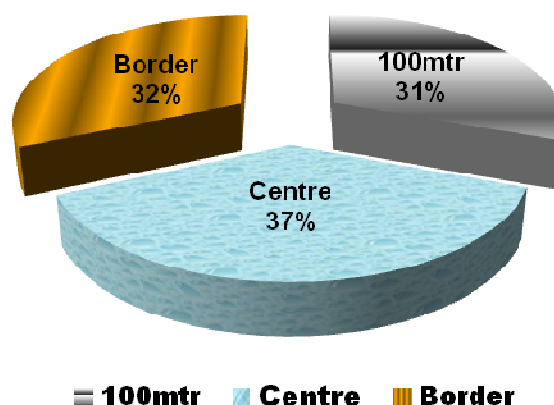
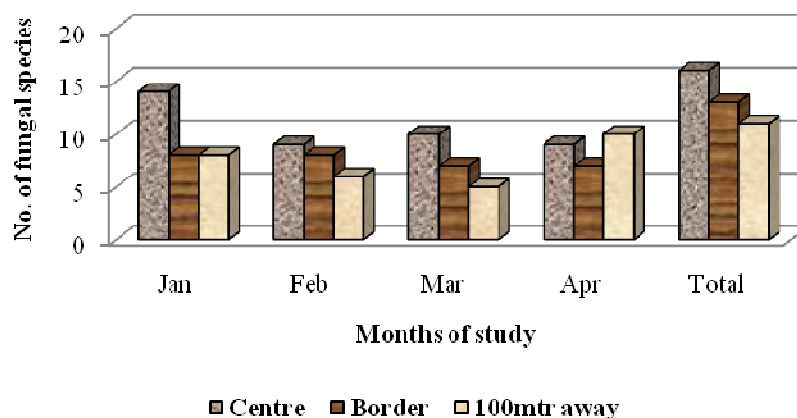
Table 2: Relative incidence of air borne fungal spores recorded in Border site of the Sugarcane field

SI. No	Name of the Fungi	January	February	March	April	Percentage %
1.	<i>Aspergillus awamori</i>	3	-	1	4	6.01
2.	<i>Aspergillus nidulans</i>	5	6	11	-	16.54
3.	<i>Aspergillus niger</i>	7	-	-	8	11.27
4.	<i>Aspergillus fumigatus</i>	-	3	-	4	5.26
5.	<i>Aspergillus japonicus</i>	2	-	-	3	3.75
6.	<i>Aspergillus terreus</i>	5	6	1	-	9.02
7.	<i>Cladosporium herbarum</i>	-	3	-	4	5.26
8.	<i>Curvularia lunata</i>	-	3	5	8	13.53
9.	Green sterile mycelia	3	-	2	-	3.75
10.	<i>Penicillium citrinum</i>	5	6	-	-	8.27
11.	<i>Penicillium fellutanum</i>	-	4	7	-	8.27
12.	Pink sterile mycelia	-	-	3	2	3.75
13.	White sterile mycelia	4	3	-	-	5.26

Table 3: Relative incidence of air borne fungal spores recorded at 100 meter away site of the sugarcane field

Sl. No	Name of the Fungi	January	February	March	April	Occurrence
1.	<i>Aspergillus awamori</i>	-	-	8	9	13.17
2.	<i>Aspergillus flavipes</i>	6	3	-	1	7.75
3.	<i>Aspergillus fumigatus</i>	1	-	6	3	7.75
4.	<i>Aspergillus niger</i>	11	3	-	4	13.95
5.	<i>Aspergillus tamaraii</i>	3	1	1	-	3.87
6.	<i>Cladosporium herbarum</i>	-	-	8	9	13.17
7.	<i>Curvularialunata</i>	4	3	-	3	7.75
8.	<i>Helminthosporium sp.</i>	-	-	4	9	10.07
9.	<i>Penicillium citrinum</i>	5	-	-	6	8.52
10.	<i>Penicillium fellutanum</i>	6	3	-	1	7.75
11.	White sterile mycelia	5	1	-	2	6.20

Total number of fungal species isolated from three study sites of sugarcane field is given in Fig 2, which described that the centre of the field harbored the maximum number of species and the months of January and April were found to be recorded with more number of species in comparison to other months. Fungal composition was found to be more prominent in the sugarcane field.

Fig 1: Percentile occurrence of fungal spores in the study sites of sugar field**Fig 2:** Fungal composition at different sites of the sugarcane field

Aeromycoflora study above a sugarcane field in an agricultural village was carried out for four consecutive months by means of the culture plate exposure technique. A more or less uniform spore concentration was observed during the early part of the vegetative stages, but later on the spore concentrations were increased and peak during the maximum vegetative growth period during March. A uniform spore count was found up until the flowering stages. A gradual increase in spore count after flowering was recorded in all the study sites. The dominant genera isolated were *Aspergillus*, *Curvularia*, *Cladosporium* and *Penicillium*. *Aspergillus* appeared in high concentrations from the crop field. The present report is agreed with the previous workers elsewhere [9]. Extensive studies on this field work were carried out by various workers such as Gregory [10] in UK, Kramer et al. [11] in USA. Singh and Singh [12] and Singh [13] reviewed aeromycology and crop disease in Manipur with special reference to cereals (rice), pulses (broad bean, *Vicia faba* L.; common bean, *Phaseolus vulgaris* L.; Pea- *Pisum sativum* L.) vegetables (tomato, potato, cabbage), oil seed (mustard) and cash crops (sugarcane) etc. Singh [9] also reported epidemiology of *Cercospora* leaf spot disease of broad bean. He concluded that there is a close correlation between spore cloud and danger of infection. *Aspergillus flavus*, a well known fungus for production of mycotoxin (aflatoxin), cause asthma in workers of food processing plants [12]. The high concentration of *A. flavus*, the causal agent of "aflatoxicosis" in birds, needs a detailed investigation in view of its role in Farmer's lung disease [14]. In tropical environments these fungi are among the dominant ones and are known for allergenicity [5]. *Rhizopus* and *Curvularia* are saprophytic fungi and weak pathogen of vegetables and crop plants were isolated in the present study. In addition to these prominent human allergens, a number of other fungi were recorded; many of them were prominent plant pathogens and saprophytic field and storage fungi.

CONCLUSION

During the present study it was found that the prevalence of airborne fungal spores is more prominent over the sugarcane field, which may attribute to the health hazard to the people working in the field since the mould fungi are attributed with in causing different allergic dysfunctions among the atopic human beings as well as to other cultivated crops nearby for infection of the pathogenic fungi. Moreover extensive work should be undertaken to find out the allergenic fungi over such crop field which are implicated in causing different diseases.

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