Influence of Meteorological Parameters on Occurrence and Composition of Fungal Spores in Guava Orchard at Nasik

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Abstract
The present study deals with an aeromycological sampling, monitoring and analysis of fungal spore incidence over selected guava orchard in Nasik city. It involved monitoring on occurrence of some important air borne fungal spores, and their changing composition due to fluctuating weather conditions. The study was conducted by using volumetric Tilak air sampler during a period of January 2018 to December 2018; on Guava (Psidium guajava Linn.) fruit plant to trace pathogenic fungal mycoflora associated with the changing climatic conditions; responsible for different diseases. The meteorological parameters like temperature, relative humidity, wind speed and precipitation, in that order of importance, significantly influence the composition of airborne fungal spores. Variations and daily fluctuations in these weather conditions may provide a stimulus to the sporadic outbreaks of certain pathogenic airborne fungal spores that further cause a plant disease. An attempt was made to assess airborne fungal spore distribution patterns in relation to meteorological variables during the period of study. Also, it was observed that, the occurrence of fungal spores was in correlation with the weather changes, field operation, plant growth and disease incidence on the crop. The major diseases caused due to an outbreak of fungal spores on guava orchard were found to be stimulated by moderate precipitation and rapid fluctuations associated with high percentage of humidity.

Keywords Meteorological Parameters, Fungal Spores, Nasik

1. Introduction
Variation in the weather conditions may provide a stimulus to the sporadic outbreaks of certain pathogenic airborne fungal spores that further cause a plant disease. The diseases to which a plant may become susceptible is influenced by a local climatic condition of a particular region which is determining its susceptibility and quality that makes the crop commercially profitable. Whether or not a plant disease is significant; in crop production; often depends upon how the local or seasonal climatic conditions meet the requirements for the development and spread of the pathogen. The relation between the disease development and weather; is the basis on which, occurrence of disease can be predicted. The meteorological parameters like temperature, relative humidity, wind speed and precipitation, in that order of importance, significantly influence the composition of airborne fungal spores. Boddy et al., (2014); Grinn-Gofron et al. (2018).

It is well known that the presence of fungi, formation and dispersion of their propagating structures are responsible for disease incidence on variety of agricultural crops. This is significantly influenced and interacted by abiotic factors and meteorological parameters. Tilak et al. (2009). Many of the fungal spores are endowed with unique structures and capacity to survive under unfavourable environmental conditions and these probably account for their predominance in the air. Mishra and Deshmukh (2009). The major diseases caused due to an outbreak of fungal spores on guava orchard were found to be stimulated by moderate precipitation and rapid fluctuations associated with high percentage of humidity. The fungal spores which are always present in an air tend to fluctuate in terms of their concentration, composition and periodicity; due to the continuous complex type of interactions between biotic and abiotic factors of an environment such as topography, soil and climatic conditions, anthropogenic activities etc. The occurrence of the fungal spores in terms of their production, release, dispersal and pathogenicity is mainly influenced by meteorological factors. However; not a single but
combination of two or more factors are generally responsible for the complex and dynamic behaviour of the fungal spores. Azcón-Bíeito and Talón (2000). The colonization of any fungi infecting fruit products, as well as the production of mycotoxins, is dependent on an array of factors that are of intrinsic or extrinsic nature. Sardella et al. (2016).

The relationship between fungal spore concentration and the prevalent climatic conditions could be ascertained by the aerobiological studies. However, proper analysis of aerobiological data thus obtained, is required for getting more reliable view of fungal spore occurrence in the air. Grinn-Gofron and Bosiacka (2015).

As the agro climatic conditions in Nasik are quite pleasant; the cultivation of horticultural plants is gaining importance. Guava (*Psidium guajava* Linn. Family Myrtaceae) is an important fruit crop of after grapes and is extensively cultivated fruit plant in Nasik region. Also; it is hardy crop, can be cultivated successfully even in neglected soils & it is attacked by large number of pathogens mainly fungi. Gupta et al. (2010). Guava fruit contains phenolic compounds that are helpful for skin and diseases like cancer, it possesses anti-viral, anti-inflammatory activities Naseer and Hussain et al. (2018). Weather conditions may cause rapid production, germination, dispersal and spread of infection by the fungal spores. Surendranathan (2005); Zhu (2006). Though the effect of meteorological factors on fungal spores is an extensively studied phenomenon; little is known about the field epidemiology of guava diseases and importance of early disease detection in disease management. Fischer et al. (2017).

**2. Materials and Methods**

**2.1. Study Area**

The Nasik city is one of the megacities; also known as grape city or wine capital of Maharashtra, located in North part of Maharashtra. Though the Nasik is popularly known for its onion and tomato production; Guava is an emerging important horticultural crop of Nasik and has a tremendous scope for area expansion in guava cultivation, processing and marketing after grapes and pomegranates. The guava orchard situated at gangapur region which is about 10 km away from Nasik city; was selected for the trapping of fungal spores by operating continuously Tilak air sampler. Tilak and Kulkarni; (1970).

The Tilak air sampler was installed on iron made stand at a constant height of 4½ feet from the ground. It is an electrically operated device (AC-230V) consists of a cubical tin box of 10.4” x 10.4” x 8” dimension with the closing lid at the top. Air is sucked in through the orifice projecting tube at the rate of 5 litres/minutes. As the air rushes in, it impinges on the transparent cellophone of the rotating drum coated with the thin layer of petroleum jelly and thus entrap the bioparticles including fungal spores from the air. The air is sucked through the tube with the help of small fan having three prongs and fixed in the circular opening in the cover of air sampler, so as to force the air out the collection chamber causing a negative pressure. Air was sampled at the rate of 5 litres/minute and the transparent cellophone coated with white petroleum jelly which was changed every 8 days. The mounting of the cello tape having catches of spores; is done in glycerine jelly that was prepared separately. The preparation of permanent microscopic slides and their scanning by using compound microscope as well as binocular research microscope; was done regularly on weekly basis. The counting of fungal spores and other bioparticles was done by Hirst short transverse method (1952) and identification of fungal spores; was based on microscopic diagnostic features, reference slides & available literature. The meteorological data such as daily temperature, relative humidity, average rainfall, wind velocity etc. were obtained from the Hydrology unit, Maharashtra Engineering Research Institute, (M.E.R.I.) Nasik.

The data of trapped fungal spores was compiled and their final concentrations expressed in terms of spores m⁻³ of the air sampled. Also, the data was analysed statistically by applying two-way ANOVA test (significance at the 0.05) using windows Microsoft version 19, 2014 with respect to the changing climatic conditions during three seasons of study period.

**3. Results and Discussion**

Low temperature and a slight rainfall with high humidity favours the occurrence of most of the fungal spores. Mohime & Koprenwar; (2015). A change in temperature may influence the colonization and growth of fungi directly through the physiology of individual organisms, or indirectly through physiological effects on their host plants or substrates. Charlotte Sindt et al. (2016). Local meteorological conditions have strong effects on temporal and spatial variations in concentration of fungal spores. Kumar and Attri (2016). Also, it is well established that the air is always carrying many bioparticles, especially fungal spores however; the concentration or fungal spore load may change according to the local climate, vegetation and anthropogenic activities.

During the present studies; the spores belonging to Deuteromycotina dominated among all fungal spore groups with 12 spore types followed by Ascomycotina with 08 spore types, Phycycomycotina with 04 spore types and Basidiomycotina with 02 spore types. (Table 1). The Deuteromycotina contributed maximum to the total airsora during three seasons with the average contribution of 9.79% & 82% (monsoon) 4.85% & 67% (winter) and 3.66% & 63% (summer) whereas; Basidiomycotina contributed least i.e. 0.65% & 05% (monsoon), 11% (winter 0.83% and summer 0.61%)
similarly; Ascomycotina contributed maximum of 1.01% & 18% (summer), 0.98% & 13% (winter) and 1.02% & 09% (monsoon). (Table 2 & Table 3) (Fig. 2).
### Table 1. Average monthly contribution of spore types (spore m⁻³) during the period of investigation (1st Jan. 2018 to 31st Dec. 2018)

| Sr No | Spore Type | January | February | March | April | May | June | July | August | September | October | November | December | Average | Total no. of spores | Percent Contribution (%) |
|-------|------------|---------|----------|-------|-------|-----|------|------|--------|-----------|---------|----------|-----------|---------|----------------------|--------------------------|
| 1     | ASCOMYCOTINA | Botryosphaeria Ces de Not. | 1132 | 1061 | 872 | 741 | 535 | 421 | 695 | 526 | 799 | 565 | 785 | 911 | 753.58 | 9043 | 2.53 |
| 2     |            | Pringheimia Schults. | 921 | 833 | 621 | 513 | 314 | 594 | 826 | 655 | 742 | 530 | 622 | 741 | 659.33 | 7912 | 2.21 |
| 3     |            | Hypoxylon Bull ex. Fr. | 321 | 413 | 290 | 112 | 132 | 277 | 435 | 459 | 510 | 156 | 342 | 367 | 317.83 | 3814 | 1.07 |
| 4     |            | Didymosphaeria Fuck. | 1243 | 1197 | 791 | 812 | 572 | 832 | 1112 | 945 | 973 | 652 | 876 | 1154 | 929.92 | 11159 | 3.12 |
| 5     |            | Lacanidion Endl. | 324 | 361 | 223 | 216 | 189 | 310 | 342 | 342 | 495 | 289 | 381 | 368 | 320.00 | 3840 | 1.07 |
| 6     |            | Lophiotrema Ces de Not. | 122 | 109 | 77 | 71 | 67 | 95 | 117 | 127 | 102 | 87 | 108 | 134 | 101.33 | 1216 | 0.34 |
| 7     |            | Sporormia de Not. | 142 | 256 | 270 | 587 | 623 | 456 | 138 | 243 | 205 | 67 | 43 | 129 | 263.25 | 3139 | 0.88 |
| 8     |            | Pleospora Rabh. | 198 | 267 | 356 | 510 | 598 | 411 | 112 | 149 | 105 | 56 | 34 | 123 | 243.25 | 2919 | 0.82 |
| 9     | BASIDIOMYCOTINA | Basidiospores | 72 | 91 | 88 | 56 | 72 | 352 | 445 | 465 | 394 | 426 | 416 | 118 | 249.58 | 2995 | 0.84 |
| 10    |            | Smuts | 3280 | 2834 | 1721 | 1634 | 2321 | 1692 | 2587 | 2132 | 1320 | 2450 | 2720 | 2340 | 2252.58 | 27031 | 7.55 |
| 11    | DEUTEROMYCOTINA | Alternaria Nees. | 789 | 1160 | 840 | 1551 | 1670 | 6570 | 7420 | 7213 | 6138 | 1729 | 1268 | 989 | 3111.42 | 37337 | 10.43 |
| 12    |            | Aspergillus Micheli ex Link. | 156 | 176 | 160 | 232 | 136 | 1210 | 1456 | 1389 | 1612 | 687 | 834 | 193 | 686.75 | 8241 | 2.30 |
| 13    |            | Cercospora Fr. | 514 | 412 | 452 | 1253 | 1574 | 5872 | 6268 | 5983 | 6032 | 1343 | 889 | 657 | 2604.08 | 31249 | 8.73 |
| 14    |            | Cladosporium Link. | 3542 | 3271 | 2621 | 2232 | 1723 | 1811 | 1432 | 1643 | 1430 | 2123 | 2630 | 2944 | 2283.30 | 27402 | 7.65 |
| 15    |            | Colletotrichum Corda | 1124 | 1235 | 965 | 933 | 873 | 4897 | 5912 | 6578 | 7867 | 3245 | 2987 | 1897 | 3209.42 | 38513 | 10.76 |
| 16    |            | Corynephora Berk and Br. | 521 | 692 | 447 | 684 | 801 | 858 | 851 | 638 | 424 | 693 | 652 | 801 | 671.83 | 8062 | 2.25 |
| 17    |            | Curvularia Boed. | 2514 | 2636 | 1459 | 1291 | 1223 | 3268 | 3530 | 2611 | 2267 | 2129 | 2132 | 2532 | 2299.33 | 27592 | 7.71 |
| 18    |            | Epichloë Link ex.Wallr. | 512 | 544 | 142 | 158 | 225 | 138 | 172 | 96 | 118 | 141 | 286 | 484 | 251.33 | 3016 | 0.84 |
| 19    |            | Fusarium Link. | 3155 | 3243 | 2544 | 1123 | 1988 | 3869 | 3698 | 3723 | 2988 | 1961 | 2876 | 3110 | 2856.50 | 34278 | 9.57 |
| 20    |            | Helminthosporium Link.ex Fr. | 1223 | 989 | 979 | 1113 | 1067 | 2234 | 2476 | 2832 | 2489 | 1357 | 1117 | 992 | 1572.33 | 18868 | 5.27 |
| 21    |            | Nigrospora Zimm. | 1234 | 1533 | 1619 | 995 | 944 | 2967 | 2430 | 2820 | 3336 | 2710 | 2312 | 2630 | 2127.50 | 25530 | 7.13 |
| 22    |            | Stemphylium Wallr. | 205 | 304 | 102 | 78 | 93 | 91 | 121 | 134 | 172 | 187 | 225 | 194 | 158.83 | 1906 | 0.53 |
| 23    | PHYCOMYCOTINA | Cunninghamella Mattr. | 934 | 804 | 603 | 697 | 543 | 704 | 398 | 689 | 718 | 614 | 831 | 857 | 699.33 | 8392 | 2.34 |
| 24    |            | Phytophthora (Mont) De Bary | 1232 | 1120 | 932 | 844 | 754 | 987 | 567 | 876 | 987 | 776 | 923 | 1157 | 929.58 | 11155 | 3.12 |
| 25    |            | Plasmopara Berk and Curtis. | 12 | 26 | 93 | 34 | 78 | 105 | 167 | 224 | 276 | 364 | 238 | 163 | 148.33 | 1780 | 0.50 |
| 26    |            | Rhizopus Chrenb. | 92 | 107 | 176 | 68 | 45 | 357 | 185 | 123 | 78 | 123 | 113 | 137 | 133.67 | 1604 | 0.45 |
Table 2. Average month wise contribution of fungal spore groups and meteorological factors during the period of investigation (1st January 2018 to 31st December 2018)

| Months   | Fungal spore Groups Average contribution (%) | Meteorological factors (Average) |
|----------|---------------------------------------------|----------------------------------|
|          | Ascomycotina | Basidio/mycotina | Deutero/mycotina | Phycomycotina | Temp. (OC) | Rainfall (mm) | Humidity (%) | Wind speed (m s⁻¹) |
| January  | 1.23         | 0.93            | 4.32             | 0.64          | 23         | --            | 32             | 2.41           |
| February | 1.25         | 0.81            | 4.52             | 0.58          | 23         | --            | 28             | 2.19           |
| March    | 0.97         | 0.50            | 3.44             | 0.51          | 24         | --            | 30             | 3.11           |
| April    | 0.99         | 0.47            | 3.25             | 0.46          | 26         | --            | 32             | 3.47           |
| May      | 0.84         | 0.66            | 3.44             | 0.39          | 31         | --            | 28             | 5.11           |
| June     | 0.95         | 0.57            | 9.44             | 0.61          | 17         | 74.61         | 72             | 5.12           |
| July     | 1.06         | 0.84            | 9.99             | 0.37          | 20         | 138.68        | 82             | 6.83           |
| August   | 0.97         | 0.72            | 9.97             | 0.54          | 21         | 73.87         | 85             | 7.86           |
| September| 1.09         | 0.47            | 9.74             | 0.57          | 21         | 29.1          | 78             | 4.11           |
| October  | 0.68         | 0.80            | 5.12             | 0.52          | 24         | 2.1           | 46             | 3.44           |
| November | 0.90         | 0.87            | 5.08             | 0.59          | 19         | 5.8           | 42             | 3.69           |
| December | 1.09         | 0.72            | 4.86             | 0.65          | 18         | --            | 30             | 3.5            |
| Total    | 12.02        | 8.36            | 73.17            | 6.43          |            |               |                |                |

Table 3. Distribution of fungal spore groups and meteorological parameters for each season during the period of investigation (1st January 2018 to 31st December 2018)

| Season    | Average fungal spore group contribution (%) | Meteorological factors (Average) |
|-----------|---------------------------------------------|----------------------------------|
|           | Ascomycotina | Basidio/mycotina | Deutero/mycotina | Phycomycotina | Temp. (°C) | Rainfall (mm) | Humidity (%) | Wind speed (m s⁻¹) |
| Summer    | 1.01         | 0.61            | 3.66             | 0.49          | 26         | --            | 29.5          | 3.47           |
| Monsoon   | 1.02         | 0.65            | 9.79             | 0.52          | 19.75      | 79.07         | 79.25         | 5.98           |
| Winter    | 0.98         | 0.83            | 4.85             | 0.6           | 21         | 3.95          | 37.5          | 3.26           |

Figure 1. Season wise average meteorological parameters
Figure 2. Season wise average percentage contribution of fungal spore groups during the Period of investigation (1st January 2018 to 31st December 2018)

The spore load was found to be comparatively less in monsoon season (except in case of Deuteromycotina) than that of winter and summer season. In winter season; spore load increases slightly, especially in case of Deuteromycotina i.e. maximum of 5.12% with an average contribution of 4.85% to the total airspora. (Table 2 & Table 3). This may be due to moderate temperature (average 21°C) with adequate amount of relative humidity. (Weber; 2003). The sporulation in different fungal genera are influenced in varied manner due to various climatic factors like temperature, moisture content and precipitation as observed in case of spore belonging to Ascomycotina and Deuteromycotina. Though the spore discharge and dispersal is enhanced by dry air with high wind velocity; the spores like Curvula, Nigrospora showed increase in their concentration with the increase in moisture content associated with slight decrease in temperature. Such observations are supported by earlier reports of Chakrabarti et al. (2012). Discharge of fungal spores may be stimulated by precipitation that was observed in case of Ascomycotina, Basidiomycotina and Deuteromycotina; the spores show significant increase with an increase in relative humidity and rainfall in monsoon season; same findings have been recorded previously by Gregory (1973); Roy et al. (2017). The spores like Aspergillus is also found to be highest during the monsoon season followed by winter and summer seasons. Oberle, M. et al. (2015); Gunthe et al. (2017). It was observed that the months with high relative humidity and rainfall witnessed significant increase in fungal spore collection throughout the period of sampling. The occurrence of spores like Cladosporium, Alternaria was rarely found to be associated with high temperature values unlike it was observed in the study of Grinn-Gofron and Bosiacka (2015), Grinn-Gofron et al. (2018). A gradual increase has been observed in collection of some fungal spore types during the months of January, February, and June; especially in case of spores like Alternaria and Fusarium; these were found to be highest in the month of June as observed by Odebode A. et al. (2020). Also, fungal spores like Fusarium oxysporum in association with root knot nematode is found to be a cause for wilt disease on young guava trees. Singh (2020). Similarly, Colletotrichum gloeosporoides is also responsible for Anthracnose disease. Shinde and Ahire (2017); Shinde (2020). Similarly; Smuts spores are found to be showing positive correlation with the wind velocity and their blowing away (deflation) occurred easily as pointed by Gregory (1961). Among the Deuteromycotina; though the spore load was found highest with its maxima of 9.99% in monsoon and 5.12% in winter season and was recorded minimum during summer season (3.25%); the fungal spores like Fusarium, Cladosporium, and Phytophthora which are known pathogens and causal agents for most of fungal diseases in guava; these have been found to occur more in number at low temperatures during winter season as compared to summer and monsoon season. Fusarium was found to be one of the dominant fungal spore in monsoon seasons. Sri Harsha Kota et al. (2018). This observation is slightly divergent to the earlier findings of Pitt; (1979), Pitt and Hocking (2009).

In case of Ascomycotina; the spore load was recorded sufficiently high i.e. 1.01% with average maximum of 1.25% during the summer season when there is no precipitation, low humidity (29.5%) with moderate wind velocity (3.47 ms⁻¹). (Table 2 & Table 3). However; the
Ascospores like *Didymosphaeria*, *Lophiostruma* and *Pringshemia*, *Lacanidion* and *Hyphoxylon* were found to occur more during low temperatures and high moisture periods in the months of monsoon and winter season and that was partially agreed with previous findings of Kulkarni (1971). Likewise, the Ascospore types like *Pleospora*, and *Sporormia* were present in the dry period and mainly occurred during increase in relative humidity with decreased temperature in winter season. (Table 1). The present study revealed that, the rainfall is having its immediate impact on the spore release of Ascomycotina, also; the temperature and rainfall are the two external factors which greatly affects the development of reproductive structures. Bauer (2008); Ball and Ketterson (2008). Many of the fungal spores have been found to occur throughout the period of investigation and this could be due to agricultural run-off through irrigation of the neighbouring crop fields.

The spore load of Phycomycotina was found to be in the range of 0.37% to 0.65% during the months of monsoon and winter seasons as seen in case of *Rhizopus* temperature between 19°C to 21°C and high percentage of humidity (79.25%) favours discharge and dispersal of conidiophores and spores that is facilitated by rainfall and high percentage of relative humidity. (Table 2). Also, the average size of the spores is comparatively larger, which helps in quick settlement of the spores. As the germination of spores is stimulated by first showers of rain; the concentration of spores was found to be higher during the months of June. However; during the month of July and early August the spore concentration was found to be less; that might be due to the heavy rainfall and rapid deposition of spores on the growing vegetation and the soil surface. Shinde and Ahire (2017).

It has been well established that the changes in the climate especially in temperature and annual rainfall have a significant influence on the timing, duration and intensity of sporadic outbreak. Boddy et al. (2014); Pakpour et al. (2015); Jana Scevkova et al. (2016). The fungal spores such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Helminthosporium*, *Nigrospora*, *Cercospora*, *Colletotrichum*, *Fusarium* etc. were found to occur with their high concentration when high precipitation (average 79.07 mm) and slight decrease in the average daily temperature (19.75°C) with high percentage of humidity (79.25%) as recorded during the months of monsoon season like of July, August, and September. (Table 2). Also, the fungal spore types like *Epicoccum* and *Stemphylium* are found to occur in high concentration during the late winter season when the climate is quite warm, dry and sunny. Timmer et al. (1998).

Increased temperatures (mean, maximum or minimum) may enhance the amount of spores in the atmosphere may induce longer spore seasons and advance sporulation time of fungi Reyes et al. (2009); De Linares et al. (2010). In the recent years; many megacities like Nasik have experiencing fluctuations in the daily average temperature; and a development of a specific microclimate and high proportion of build-up areas Alabi (2012). The changes in daily average temperature can affect the production, release and dispersion of fungal spores in the air. Hollins et al. (2004).

Analysis of the two-way ANOVA for average contribution of fungal spore groups and meteorological parameters in three seasons showed no significant difference in between average contribution fungal spore groups over the different seasons and changing climatic conditions during the study period. (Table 4). The differences were observed with respect to the changing meteorological parameters and fungal spore concentrations of individual spore types as well as spores groups with average monthly concentrations, but no statistically significant difference was observed when the overall total average percent contributions of spore groups were considered. The possible reason might be the insignificant changes of environmental factors as well as the fungal growth substrates at the sampling site during the one-year sampling. (Pawan and Manjunath; 2014).

The results of fungal spore occurrence and the associated changing meteorological parameters particularly for guava fruit crop of Nasik region may provide useful input for the study of airborne, plant pathogenic fungal airspora. However; the findings may vary according to the vegetation types, fungal growth patterns, sampler used, sampling techniques etc. hence these may not be generalized for the other such locations.

### Table 4. Two way ANOVA for fungal spore group and meteorological parameters

| Source of Variation | Sum of Squares (SS) | Degrees of freedom(df) | Mean Squares(MS) | F Value | P-value | F crit |
|---------------------|---------------------|------------------------|------------------|---------|---------|--------|
| Spore groups        | 1080.994314         | 1                      | 1080.99431       | 2.46732336 | 0.167291 | 5.987377607 |
| Seasons             | 6151.227271         | 6                      | 1025.20455       | 2.339985595 | 0.16229 | 4.283865714 |
| Error               | 2628.745786         | 6                      | 438.124298       |         |         |        |
| Total Variation     | 9860.967371         | 13                     |                  |         |         |        |
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