



Original Research Article

Biodiversity of statistical correlation between fungal population and physico-chemical parameters of soil fungi from sugarcane field of Dharmapuri District, Tamilnadu, India

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A B S T R A C T

Keywords

Sugarcane field, Biodiversity, Fungal population, Physico-chemical parameters, Phycomycetes.

Soil is a complex ecosystem delimited by physico- chemical parameters that hold enormous number of living organisms. This study deals with the monthly variation in soil fungal population of traditional sugarcane field in Dharmapuri District, Tamilnadu viz., Palakkodu and Harur. The fungi in sugarcane field soil samples were recorded by both direct examination and plating method. In the direct examination method totally 76 different species belonged to 32 genera were isolated. Among them 5 species were Ascomycetes, 68 species were deuteromycetes and 3 species were phycomycetes. They were isolated by using PDA medium and identified by using standard manual. The dominant species were *Aspergillus conicus*, *A. flavus*, *A. rugulosus* followed by *Fusarium semitectum*, *F. solani*, *Ceratocystis paradoxa*, *Trichoderma* sp, *Penicillium* sp and *Curvularia* sp from the sugarcane field soil of palakkodu in various months where as in Harur soil the dominant species were *Aspergillus awamori*, *A. funiculosus*, *A. sydowi*, *Cladosporium* sp, *Hypocrea virens* and *Setosphaeria rostrata* respectively. The six morphologically different isolates represented by sterile mycelia were isolated by plating method.

Introduction

Soil is one of the most diverse habitats on earth and contains the most diverse assemblages of living organisms. Biological activity in soils is largely concentrated in the top soil. The biological components occupy a tiny fraction (<0.5%) of the total soil volume and make less than 10% of the total

soil organic matter. This living component consists of plant root and soil organisms (Breure, 2004). It has been estimated that only between 1 and 5% of all microbes on earth have been named and classified. Fungi are a diverse component of soil microbial communities, in which they function as

decomposers, mycorrhizal mutualists and pathogens.

Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, Food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways. They are a diverse group of organisms comprising both single – celled and multicellular filamentous forms. Fungal bio technology has become an integral part of human welfare (Manoharacary *et al.*, 2005).

Microfungi play a focal role in nutrient cycling regulating soil biological activity (Arunachalam *et al.*, 1997). However, the rate at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions.

There have been a number of studies on the distribution of soil micro fungi in agricultural field. Some studies dealt with the influence of plant community (Chung *et al.*, 1997) and other attempted to examine monthly trends (Kennedy *et al.*, 2005). Large quantities of readily decomposable organic matter are added to agricultural soils every year as crop residues or animal wastes and have a significant outcome on soil microbial commotion. The plant species growing on the soil also equally influence the population and species composition of the soil fungi (Hackel *et al.*, 2000).

This study deals with the monthly variation in soil fungal population of traditional agricultural field in south India.

Materials and Methods

About 24 soil samples were collected from the two station viz, Palakkodu, Hurur in

Dharmapuri District, Tamil Nadu. The soil samples were collected for a period of 12 months in sugarcane field.

Sampling Schedule

Soil sample were collected in each sampling on monthly intervals for a period of one year from April 2009 to March 2010. The climate is monotonic and the calendar year has been divided in the 12 month viz., April–March.

Analysis

The mechanical and chemical analysis of the soils was made with the help of Lamotte's soil testing outfit, nitrogen and organic etc.

Isolation of Soil Mycoflora Dilution Plating Method

Dilution techniques described by Warcup (1950) was used to isolated the fungi from soil sample weighing 1g was diluted in 10ml of distilled water. One ml of the diluted sample was poured and spread on Petri plates containing sterilized PDA medium (Extract from 250g of potato [boiled and filtered], dextrose 15g, agar 18g and distilled water 1000ml, pH 7) in replicates. The inoculated plates were incubated in a dust free cupboard at the room temperature for 3 days. One percent streptomycin solution was added to the medium before pouring into Petri plate for preventing bacterial growth.

Observation

The colonies growing on PDA plates with different morphology were counted separately. A portion of the growing edge of the colony was picked up with the help of a paw of needles and mounted on a clean slide with lacto phenol cotton blue stain. The slide was gently heated in a sprit lamp so as to facilitate the staining and remove air

bubbles, if any. The excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope. Microphotography of the individual fungal species was also taken using Nikon phase contrast Microscope, Japan.

Identification

Colony colour and morphology were observed besides hyphal structure, spore size, shape and spore bearing structures. They were compared with the standard works of Raper and Thom (1949), Van Arx (1974), Anisworth *et al.*, (1973), Raper and Fennel (1965) and Ellis (1976) for identification of species.

Presentation of data

Number of species is referred as species diversity, population density expressed in terms of colony forming unit (CFU) per gram of soil with dilution factors.

In order to assess the dominance of individual species the percentage contribution worked out as follows.

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

Results and Discussion

Fungal Diversity in Sugarcane Soils

Altogether 24 soil samples from 2 different stations representing the entire Dharmapuri District were examined for fungal diversity. The study resulted the presence of 76 species of fungi in all of them 3 species belonging to two genera were Phycomycetes and the remaining 76 species belonging to 32 genera were assignable to Deuteromycetes.

Station Wise Occurrence

Altogether 41 species belong to 16 genera (3 phycomycetes, 2 Ascomycetes, 36 Deuteromycetes) were identified from Palakkodu and 45 species belong to 18 genera (3 Ascomycetes, 42 Deuteromycetes) were identified from Harur.

Species Composition

Among the 16 genera recorded, the genus *Aspergillus* was considered by more number of 12 species followed by *penicillium* (5 species) *Fusarium* and *Trichoderma* (4 and 10 species, respectively). All other genera were represented one species each (Table 2, 2a) and 18 genera recorded, the genus *Aspergillus* was considered by more number of 14 species followed by *Penicillium* (6 species) *Fusarium* and *Trichodrema* (2 and 4 species, respectively). All other genera were represented one species each (Table 3 and 3a, respectively)

Species Diversity

Altogether 76 species and 32 genera (3 Phycomycetes, 5 Ascomycetes, and 68 Deuteromucetes) were identified from Palakkodu and Hurur station (Table 1)

In the present investigation the survey was conducted to find out the fungal diversity in two different stations such as Palakkodu and Hurur. Totally 76 species isolated belonging to 32 genera from the soil of sugarcane field. Number of Deuteromycetes was representing by 68 species and the remaining 3 species belongs to phycomycetes and 5 species belong to Ascomycetes.

Table. I

Ascomycetes :

1. *Chaetomium* sp. kunze and schmit
2. *Cladosporium* sp. Link
3. *Laptosphaeria salvinii* cattanes
4. *Neurospora crassa* Shear and Dodge
5. *Nigrospora sphaerica* (Saccard) mason

Deuteromycetes :

1. *Acrocylindrium* sp. Bonorden
2. *Alternaria alternate* keissl
3. *Aspergillus awamori* Kawachi
4. *A.chevalieri* Thom and Church
5. *A.clavatus* Desmazieres
6. *A.conicus* Blochwitz
7. *A.flavipes* Bainier and Sartory
8. *A.flavus* Link
9. *A.fumigatus* Fresenius
10. *A.funiculosus* G.Smith
11. *A.granulosis* Raper and Thom
12. *A.humicola* Chaudhuri
13. *A.nidulans* Winter
14. *A.niger* Van Tieghem
15. *A.ochraceous* Wilhelm
16. *A.oryzae* (Ahlburgin Korschelt) Cohn
17. *A.repens* (Corda) de Bary
18. *A.ruber* Thomand Church
19. *A.rugulosus* Thom and Raper
20. *A.sulphureus* (Fresenius) Thom and Church
21. *A.sydowi* (Bainier and Sartory) Thom and Church
22. *A.tamarii* Kita
23. *A.terreus* Thom
24. *A.ustus* Thom and Church
25. *A.versicolor* Thom and Raper
26. *A.wentii* wehmer
27. *Bipolaris oryzae*
28. *Botrytis cinerea* Persoon
29. *Ceratocystis paradoxa* (Dade) C.Moreau
30. *Colletotrichum falcatum* went
31. *Curvularia geniculata* (Tracy and Earle) Boedijn
32. *C.lunata* (Walker) Boedijn
33. *C.senegalensis* (Speg) Subram
34. *Dimeriella sacchari*

Phycomycetes :

1. *Absidia glauca* Hagen
2. *Rhizopus nigricans* Ehrenberg
3. *R. stolanifer*
35. *Fusarium moniliforme* Sheldon var.minus wollenweber
36. *F.oxysporum* schlechendahl
37. *F.semitectum* Berkeley and Ravenel
38. *F.solani* (Martius) Appel and Wollenweber
39. *Hypocrea virens*
40. *Gliocladium sagariensis* Saksena
41. *Gloeocercospora sorgh*
42. *Helminthosporium* sp. Link
43. *Helminthosporium oryzae* Breda de Hoan
44. *Humicola* sp. Corda
45. *Hyalopus ater* Corda
46. *Masoniella* sp. G.Smith
47. *Marasmiellus sacchari*
48. *Penicillium chrysogenum* Thom
49. *P.candidum*
50. *P.janthinellum* Biourge
51. *P.javanicum* van Beyma
52. *P.japonicum*
53. *P.lanosum* Westling
54. *P.notatum* Westling
55. *P.purpurogenum* Stoll
56. *P.purpurescens* Sopp
57. *P.turbatum* Westling
58. *Pythium* sp. Pringsheim
59. *Sclerospora* sp.
60. *Setosphaeria rostrata* K.J.Leonard
61. *Torula allii* (Harz) Saccardo
62. *Trichoderma glaucum* Abbott
63. *T.harzianum* Rifai
64. *T.koenigii* Oudemans
65. *T.lignorum* (Tode) Harz
66. *T.viride* AA.Gams
67. *Ustilago scitaminea*
68. *Verticillium* sp. Nees

Table.4 Analysis of physico – chemical parameters of sugarcane soil samples from Palakkodu
Correlation

| | TNC | PH | EC | OC | AN | AP | AK | AZ | AC | AI | AM | CEC | C | M | S | P |
|-----|-------|---------------------|--------------------|-------|---------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-------|---|
| TNC | 1 | | | | | | | | | | | | | | | |
| PH | 0.083 | 1 | | | | | | | | | | | | | | |
| EC | 0.319 | 0.618 [*] | 1 | | | | | | | | | | | | | |
| OC | 0.026 | -0.055 | 0.484 | 1 | | | | | | | | | | | | |
| AN | 0.039 | 0.808 ^{**} | 0.500 | 0.109 | 1 | | | | | | | | | | | |
| AP | 0.079 | 0.689 [*] | 0.191 | 0.144 | 0.727 ^{**} | 1 | | | | | | | | | | |
| AK | 0.185 | 0.606 [*] | 0.015 | 0.221 | 0.435 | 0.609 [*] | 1 | | | | | | | | | |
| AZ | 0.121 | 0.788 ^{**} | 0.422 | 0.000 | 0.577 [*] | 0.562 | 0.724 ^{**} | 1 | | | | | | | | |
| AC | 0.010 | 0.872 ^{**} | 0.375 | 0.161 | 0.783 ^{**} | 0.670 [*] | 0.628 [*] | 0.874 ^{**} | 1 | | | | | | | |
| AI | 0.091 | 0.339 | 0.237 | 0.419 | 0.011 | 0.364 | 0.701 [*] | 0.639 [*] | 0.552 | 1 | | | | | | |
| AM | 0.384 | 0.573 | 0.233 | 0.104 | 0.465 | 0.547 | 0.646 [*] | 0.703 [*] | 0.779 ^{**} | 0.702 [*] | 1 | | | | | |
| CEC | 0.238 | 0.248 | 0.234 | 0.292 | 0.122 | 0.492 | 0.621 [*] | 0.415 | 0.484 | 0.812 ^{**} | 0.847 ^{**} | 1 | | | | |
| C | 0.142 | 0.435 | 0.123 | 0.413 | 0.267 | 0.476 | 0.616 [*] | 0.652 [*] | 0.716 ^{**} | 0.866 ^{**} | 0.838 ^{**} | 0.846 ^{**} | 1 | | | |
| M | 0.215 | 0.588 [*] | 0.082 | 0.382 | 0.391 | 0.608 [*] | 0.654 [*] | 0.731 ^{**} | 0.785 ^{**} | 0.793 ^{**} | 0.869 ^{**} | 0.801 ^{**} | 0.960 ^{**} | 1 | | |
| S | 0.114 | 0.494 | 0.000 | 0.135 | 0.313 | 0.580 [*] | 0.752 ^{**} | 0.700 [*] | 0.687 [*] | 0.844 ^{**} | 0.893 ^{**} | 0.910 ^{**} | 0.910 ^{**} | 0.907 ^{**} | 1 | |
| P | 0.052 | 0.428 | 0.635 [*] | 0.166 | 0.256 | 0.173 | 0.015 | 0.507 | 0.281 | -0.053 | -0.043 | -0.369 | 0.003 | 0.165 | 0.045 | 1 |

** Significant correlation at the 0.01 level. * Significant correlation at the 0.05 level.

EC – Electrical Conductivity
 OC – Organic Carbon
 AN – Available Nitrogen
 AP – Available Phosphorus
 AK – Available potassium
 AZ – Available Zinc
 AC – Available Copper
 AI – Available Iron
 /

AM – Available Magnesium
 CEC – Cation Exchange Capacity
 C – Calcium
 M – Magnesium
 S – Sodium
 P – Potassium

Table.5 Analysis of physico – chemical parameters of sugarcane soil samples from Harur
Correlation

| | TNC | PH | EC | OC | AN | AP | AK | AZ | AC | AI | AM | CEC | C | M | S | P |
|-----|--------|---------------------|--------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-------|---|
| TNC | 1 | | | | | | | | | | | | | | | |
| PH | -0.317 | 1 | | | | | | | | | | | | | | |
| EC | -0.297 | 0.937 ^{**} | 1 | | | | | | | | | | | | | |
| OC | 0.278 | 0.182 | 0.218 | 1 | | | | | | | | | | | | |
| AN | -0.508 | 0.535 | 0.656 | -0.257 | 1 | | | | | | | | | | | |
| AP | -0.555 | 0.420 | 0.468 | -0.290 | 0.868 ^{**} | 1 | | | | | | | | | | |
| AK | -0.072 | 0.284 | 0.408 | -0.258 | 0.639 | 0.729 ^{**} | 1 | | | | | | | | | |
| AZ | -0.156 | 0.245 | 0.392 | -0.434 | 0.775 ^{**} | 0.806 ^{**} | 0.902 ^{**} | 1 | | | | | | | | |
| AC | -0.111 | 0.066 | 0.197 | 0.613 [*] | 0.621 [*] | 0.661 [*] | 0.846 ^{**} | 0.897 ^{**} | 1 | | | | | | | |
| AI | 0.199 | -0.357 | -0.204 | -0.335 | 0.065 | 0.264 | 0.703 [*] | 0.594 [*] | 0.683 [*] | 1 | | | | | | |
| AM | -0.053 | -0.002 | 0.172 | -0.344 | 0.512 | 0.648 | 0.903 ^{**} | 0.838 ^{**} | 0.910 ^{**} | 0.829 ^{**} | 1 | | | | | |
| CEC | 0.130 | -0.190 | -0.065 | -0.359 | 0.260 | 0.401 | 0.849 ^{**} | 0.725 ^{**} | 0.775 ^{**} | 0.908 ^{**} | 0.847 ^{**} | 1 | | | | |
| C | 0.187 | -0.084 | 0.065 | -0.282 | 0.395 | 0.528 | 0.870 ^{**} | 0.810 ^{**} | 0.755 ^{**} | 0.843 ^{**} | 0.851 ^{**} | 0.891 ^{**} | 1 | | | |
| M | -0.211 | -0.143 | 0.000 | 0.579 [*] | 0.445 | 0.591 [*] | 0.802 ^{**} | 0.754 ^{**} | 0.825 ^{**} | 0.831 ^{**} | 0.880 ^{**} | 0.837 ^{**} | 0.840 ^{**} | 1 | | |
| S | 0.065 | -0.082 | 0.059 | -0.389 | 0.336 | 0.489 | 0.883 ^{**} | 0.770 ^{**} | 0.839 ^{**} | 0.930 ^{**} | 0.929 ^{**} | 0.946 ^{**} | 0.924 ^{**} | 0.905 ^{**} | 1 | |
| P | -0.457 | 0.430 | 0.463 | -0.189 | 0.776 ^{**} | 0.630 [*] | 0.450 | 0.535 | 0.457 | -0.031 | 0.292 | 0.245 | 0.212 | 0.209 | 0.231 | 1 |

** Significant correlation at the 0.01 level. * Significant correlation at the 0.05 level.

EC – Electrical Conductivity
 OC – Organic Carbon
 AN – Available Nitrogen
 AP – Available Phosphorus
 AK – Available potassium
 AZ – Available Zinc
 AC – Available Copper
 AI – Available Iron

AM – Available Magnesium
 CEC – Cation Exchange Capacity
 C – Calcium
 M – Magnesium
 S – Sodium
 P – Potassium

The dominate species were *Aspergillus conicus*, *A. flavus*, *A. rugulosus* followed by *Fusarium semitectum*, *F. solani*, *Ceratocystis paradoxa*, *Trichoderma* sp *Penicillium* sp and *Curvularia* sp from the sugarcane field soil of Palakodu whereas, in Harur soils the dominate species were *Aspergillus awamori*, *A. funiculosus*, *A. sydowi*, *Cladosporium* sp, *Hypocrea virens* and *Setosphaeria rostrata*, respectively.

Recently Kalaiselvi and Panneerselvam (2011) studied the seasonal and vertical distribution of soil fugal population in Thanjavur District, Tamilnadu viz., Nadur, Orathanadu, Punnainallur and Tholkappiyar square. Totally 30 different species belonging to Ascomycetes and Phycomycetes were isolated using PDA medium. The dominant species were *Aspergillus niger* and *Cunninghamella* sp, followed by *Trichoderma viride*. During rainy season maximum fungal count was recorded in sub soil layer.

Evidently, Madhanraj *et al.*, (2010) reported that 45 soil samples were collected from 8 different stations along the entire Tamilnadu coast and examined by dilution plating method to assess the fungal diversity and population density. Totally 24 fungal species representing 12 genera are recorded. *Aspergillus* was represented by more number (9 species) followed by *Penicillium* (3 species), *Fusarium* and *Monodictys* (2 species each).

References

Anisworth, G.C., Sparrow, F.K., Anisworth, A.S. 1973. The fungi advanced treatise: A taxonomic review with keys in Ascomycetes and Fungi imperfecti, Vol. 4(A). Academic press, New York, 621 Pp.

- Arunachalam, K., Arunachalam, R.S., Tripathi and Pandey, H.N. 1997. Dynamics of microbial population during the aggradation phase of a selectively logged subtropical humid forest in north-east India. *Trop. Ecol.*, 38: 333–341.
- Breure, A.M. 2004. Soil biodiversity: Measurements, indicators, threats and soil functions. *Funct. Ecol.*, 17: 516–525.
- Chung, H., Zak, D.R., Reich, P.B., Ellsworth, D.S. 2007. Declining plant species richness, elevated CO₂, and atmospheric N deposition alter soil microbial community composition and function. *Global change Biol.*, 13: 980–989.
- Ellis, M.B. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute Pub., Kew, Surrey, England.
- Hackel, E., Bachmann, G., Bottenstein, G. 2004. *Zschmeister, Phyton*, 2000, 48: 890.
- Kennedy, N.M., Gleeson, D.E., Connolly, J., Clifton, N.J.W. 2005. Seasonal and management influences on bacterial community structure in an upland soil. *FEMS Microb Ecol.*, 53:329–337.
- Madhanraj, P., Manorajan, S., Nadimuthu, N., Panneerselvam, A. 2010. An investigation of the mycoflora in the sand dune soils of Tamilnadu coast, India. *Adv. Appl. Sci. Res.*, 1(3): 160–167.
- Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T.S., Seema Rawal, d. Johri, B.N. 2005. Fungal biodiversity: Distribution, conservation and prospecting of fungi from India. *Curr. Sci.*, 89: 58–71.
- Raper, K.B., Thom, C. 1949. A manual of Penicillia. The Williams Wilkins Co., Baltimore, 875 Pp.

- Kalaiselvi, S., Panneerselvam, A. Ecology of soil fungi in paddy field of Tamilnadu-Thanjavur District. *Der chemica Sinica*, 2011, 2(2): 9–19.
- Raper, K.B., Fennell, D.I. 1965. The genus *Aspergillus*. The Williams Wilkins Co., Baltimore, 686 Pp.
- Von Arx, J.A. 1974. The genera of fungi sporulating in pure culture. 2nd edn., vадuz Germany: *A.R.Ganter Verlage, K.G.FL- 9491*, 375.
- Warcup, J.H. 1950. The soil-plate method for isolation of fungi from soil. *Nature*, 166:117–118.