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Foldscope: a simplified tool for the assessment of the soil fungal diversity and synthetic fertilizers tolerance

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R. Vijayakumar* and K. Karthika

Department of Microbiology, Government College of Arts and Science (Formerly Bharathidasan University Constituent College) Perambalur - 621 107, Tamilnadu, India.

Abstract

Foldscopes are the ultra-affordable, origami based paper microscopes designed to be extremely portable and durable, have the potential role to change the way we observe the living things around us. Few years ago the Foldscope was invented by Manu Prakash and Jim Cybulski from Stanford University, USA. These pocket-sized tools are now making their way to remote parts of the world. In the present study, how foldscope is used for the observation and identification of soil fungi and their tolerance capacity against synthetic fertilizers. The samples were collected from agricultural fields of different locations of Ariyalur and Perambalur districts, Tamilnadu, India. The soil samples were analyzed for their physico-chemical parameters. They were serially diluted and inoculated on to potato dextrose agar (PDA) medium by using spread plate technique for the isolation of soil fungi. The isolated fungi were purified and characterized microscopically under light microscope, and Foldscope as an alternative to conventional microscope using lactophenol cotton blue (LCB) technique. The predominant fungi were studied for their ability to grow on various culture media, different pH and different temperature. Effects of various synthetic fertilizers on the growth of predominant fungal isolates were also evaluated. The results showed that *Trichoderma viridae* and *Aspergillus niger* were the most common isolates found in all the sampling sites followed by *Aspergillus fumigates, A.flavus, A.terreus, Cepahalosporium* sp, *Fusarium oxysporum,* and *Rhizopus stolonife.,*

Key words: Fertilizers tolerance, Foldscope, Nutrient analysis,Soil fungi.

INTRODUCTION

In olden days, farming practices were relatively environmental friendly and traditional farms were small-scale and crop yields were depending on internal resources, recycling of organic matter, built-in biological control mechanisms and rainfall patterns, and crop rotation practices were followed to maintain the nutrient contents of soil. In the environmental friendly farming practices, there are different categories of soil microorganisms involved in the nutrient cycles or bio-geo chemical cycles. These microorganisms maintain and increase the fertility and productivity of the soil. Among the microorganisms, fungi play a key role in maintaining the fertility and conversion of complex wastes into the simple nutrients of the soil. They are geographically widely distributed and have been observed in a broad range of habitats principally in soils and decaying vegetation (Bridge and Spooner, 2001).

Now a days the intensive farming, practices involve applying of various external agricultural inputs to agricultural production systems. However, extensive application of synthetic growth promoters including chemical fertilizers and pesticides to agricultural production systems leads to deterioration of soil quality. The presence and bio-availability of these chemical fertilizers in soil cause adverse impacts on plants, soil organisms, animal and human health. The soil microorganisms are more spoiled due to application of chemicals than any other parameters (Baishya, 2015). In this context, there is an urgent need to study about the fertility of soil by observing the diversity of soil fungi and all plant growth promoting microorganisms (PGPM), analysis of soil nutrients, and the survivability of soil microbes against the impact of chemical fertilizers.

Observation and identification of microorganisms, especially fungi, need microscopes. Among the simple microscopes, a recently discovered Foldscope invented by Manu Prakash and his co-workers provides new opportunities for vast users including children around the world who never used microscope (Cybulski *et al.,* 2014). Moreover, the opportunity to make microscopes both cost effective and accessible can inspire the students to examine the biodiversity of our planet. Foldscope can be used for field study and to identify the small living organisms. Foldscopes are an ultra-affordable, origami based paper microscopes designed to be pocket-sized, weightless and durable. The Foldscope gives magnification similar to basic light microscopes

*Corresponding Author :
email: *rvijayakumar1979@gmail.com*

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(Sharma and Nischal, 2019). However, its application potential as a research tool in the area of microbiological research is still in its infant stage. Keeping this in mind, the present study was planned to isolate and identify the soil fungi using the Foldscope from cultivated fields of Perambalur district, Tamilnadu, India and to evaluate the toleration capacity of soil fungi to the chemical fertilizers.

Materials and Methods

Soil sample collection: Soil samples were collected from the cultivated fields of ten different locations of Perambalur and Ariyalur districts.,Tamilnadu,India Soil samples were collected from a depth of 15 cm with the help of a sterilized cork borer pushed horizontally into the ground. The soil within the cork borer was emptied into sterilized polyethylene bags. Each sample bag was labeled appropriately by indicating the site of collection, time, date and place of collection. The samples were transferred to the laboratory in sterile polythene bags.

Analysis of soil properties: The physico-chemical parameters of the soil samples viz., pH, electrical conductivity, organic carbon, organic matter, available nitrogen, phosphorus, potassium, zinc, copper, iron, manganese, calcium, magnesium, sodium and potassium were analyzed by the methods described by Eaton *et al.* (2005) and Gnanasekaran *et al.* (2015).

Isolation of soil fungi: The soil fungi were isolated by using soil dilution plate method (Waksman, 1922). Soil dilutions were made by suspending 1 g of each soil sample in 10 ml of sterile distilled water. 1 ml of the suspension of dilutions of 10^{-2} and 10^{-3} were inoculated on PDA medium added with 1% streptomycin solution for preventing bacterial growth, before pouring into Petri plates. The plates were then incubated at 28°C for 4-7 days (Ratnakumar *et al.*, 2017). After incubation, fungal colonies were counted and recorded. The correlation co-efficient between the physico-chemical parameters and fungal population of the soil was statistically analysed using SPSS package. Population density was expressed in terms of colony forming unit (CFU) per gram of soil with dilution factors. The per cent contribution of each isolate was statistically calculated by using the following standard formula:

$$\% \text{ frequency} = \frac{\text{Total number of CFU of an individual species}}{\text{Total number of CFU of all species}} \times 100$$

Characterization and identification of soil fungi: All the isolated fungal colonies were characterized by the standard methods of (Gillman ,1957) using lactophenol cotton blue (LCB) technique and observed under 140X objective of Foldscope (Cybulski *et al.*, 2014). Growth of predominant fungal isolates on various culture media namely PDA, Sabouraud dextrose agar (SDA) and Rose Bengal agar (RBA); and at temperatures of 15, 20, 25, 30 and 35°C; and at pH of 5.0, 5.5, 6.0, 6.5 and 7.0, were studied.

***In vitro* fertilizer tolerance study:** The fertilizer tolerance capacity of predominant fungal isolates was studied by the methods of Khattabi *et al.* (2004). Various concentrations and combinations of chemical fertilizers namely urea, di-ammonium phosphate and super phosphate were prepared and added into the PDA medium. Then the predominant fungi were inoculated and incubated for 3 days at room temperature. After incubation, radial growth of the fungus was measured, and percentage inhibition of fungal growth was calculated by the standard formula:

$$\text{Percentage of Growth Inhibition} = \frac{\text{Growth in control-Growth in treatment}}{\text{Growth in control}} \times 100$$

Assessment of the mycelial dry weight: Potato dextrose broth was prepared with various concentrations and combinations of chemical fertilizers and inoculated with test fungal blocks and incubated for 7 days at room temperature. The mycelial mat was collected on 3rd and 7th day by filtering through the pre-weighed separate Whatman no.1 filter paper (Kumawat *et al.*, 2016). Then the fungal mycelia were dried in a hot air oven at 50±2°C for 72 h. The actual weight of the dry fungal mycelia was then calculated using the following standard formula:

$$\text{Weight of dry mycelia} = (\text{weight of filter paper} + \text{weight of mycelium}) - \text{weight of the filter paper}$$

RESULTS AND DISCUSSION

The distribution of nutrients determines the fertility potential of soil and water mass (Bragadeeswaran *et al.*, 2007). Soil has many physical and chemical properties. Some are changeable, while others are difficult/ impossible to adjust. Texture, structure, drainage and organic matter content comes under physical properties. Chemical properties that affects microbial as well as plant growth were cation exchange capacity and pH (Cholarajan and Vijayakumar, 2013). In the present study, the soil physico-chemical properties were studied and presented in Table 1. Maximum soil pH (8.31) and EC (1.82 dSm^{-1}) were recorded from the soil samples collected from Sendurai and Udayarpalayam stations of Ariyalur district Calcium carbonate ($CaCO_3$) was high in all the sampling stations except in Pachai Hills. Higher contents of N (65.7 kg/ha) and P (13.8 kg/ha) were found at Pachai Hills station, whereas higher content of K (387 kg/ha) was recorded at Veppanthattai station of Perambalur district. Maximum content of Fe (2.90 ppm) was recorded at Kunnam station of Perambalur district. Maximum content of Mn (2.31 ppm) was

Table 1. Physico-chemical properties of soil samples studied

Parameters	Perambalur district					Ariyalur district				
	Perambalur	Alathur	Kunnam	Veppanthattai	Pachai Hills	Ariyalur	Udayarpalayam	Sendurai	Andimadam	Paluvur
pH	7.7	7.8	7.94	8.07	7.32	8.24	8.02	8.31	8.08	7.80
EC (dSm^{-1})	0.40	1.55	0.36	0.20	1.22	1.32	1.82	1.27	1.30	1.23
$CaCO_3$ (mg/kg)	High	High	High	High	Medium	High	High	High	High	High
N (kg/ha)	59.6	62.8	59.6	56.4	65.7	58.6	61.7	60.3	61.4	59.7
P (kg/ha)	11.3	12.5	8.75	6.25	13.8	5.17	6.9	5.72	5.9	5.65
K (kg/ha)	336	193.5	343	387	288	191.7	207.8	212	227.8	199.2
Fe (ppm)	2.60	2.75	2.90	2.75	1.98	2.87	2.58	2.78	2.45	2.12
Mn (ppm)	2.30	1.97	2.20	2.15	1.88	2.18	2.08	2.11	2.31	1.93
Zn (ppm)	0.96	0.87	0.94	0.80	0.97	0.98	0.94	0.88	0.96	0.82
Cu (ppm)	0.74	0.84	0.84	0.94	0.88	0.99	0.85	0.78	0.82	0.79
OC (%)	0.33	0.45	0.40	0.39	0.40	0.30	0.32	0.38	0.35	0.41

Table 2. Frequency of mycoflora in different sampling stations of the present study

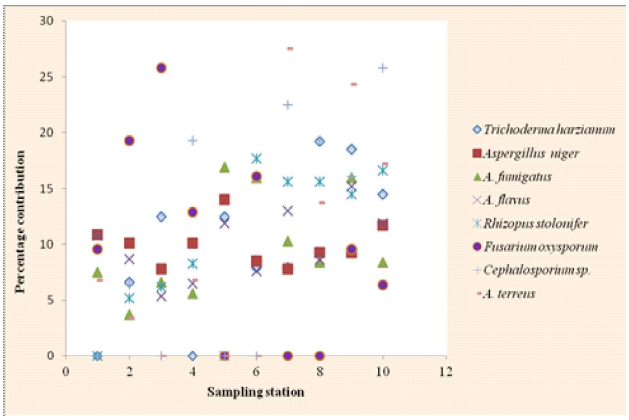
Sampling stations	Average number of individual colonies								Average no. of total CFU
	Trichoderma harzianum	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus terreus*	*Rhizopus stolonifer*	*Fusarium oxysporum*	*Cephalosporium* sp.	
Perambalur	-	14	10	8	2	-	3	-	41
Alathur	10	13	8	4	1	5	6	2	49
Kunnam	19	10	5	7	0	6	8	-	55
Veppanthattai	-	13	6	6	2	8	4	6	45
Pachai Hills	19	18	11	18	-	-	-	-	73
Ariyalur	12	11	7	17	-	17	5	-	62
Udayarpalayam	12	10	12	11	8	15	-	7	75
Sendurai	29	12	8	9	4	15	-	3	80
Animadam	28	12	14	17	7	14	3	5	98
Paluvur	22	15	11	9	5	16	2	8	86
Total	151	128	92	106	29	96	31	31	664
% frequency	22.7	19.3	13.9	15.9	4.4	14.5	4.7	4.7	

Table 3. Correlation co-efficient between physico-chemical properties of soil samples and total fungal colonies

pH	N	P	K	Fe	Mn	Zn	Cu	OC
-0.3601	-0.3682	0.02432	0.3590	-0.9205**	0.5710	0.0774	-0.3682	-0.8013**

**p - 0.01

recorded at Andimadam station of Ariyalur district, and of Zn (0.98 ppm) and Cu (0.99 ppm) contents were recorded at Ariyalur station. Maximum OC content (0.45) was at Alathur station of Perambalur district. A total of 664 fungal colonies (CFUs) were isolated on PDA medium from the cultivated fields of all the 10 stations. Among 664 CFUs, Andimadam station soil contributed a maximum of 98 fungal CFUs, followed by Paluvur (86 CFUs), Sendurai (80 CFUs), Udayarpalayam (75 CFUs), Pachai Hills (73 CFU), Ariyalur (62 CFU), Kunnam (55 CFU), Alathur (49 CFU), Veppanthattai (45 CFU) and Perambalur (41 CFU) (Table 2). From these 664 CFUs, 8 morphologically distinguished fungi were purified and identified based on their colony morphology and Foldscopic (microscopic) properties. The identification of these soil fungi was



1-Perambalur; 2- Alathur; 3- Kunnam; 4- Veppanthattai; 5- Pachai Hills; 6- Ariyalur; 7- Udayarpalayam; 8- Sendurai; 9- Animadam; 10- Paluvur

Fig. 1. Station-wise percent contribution of fungal species

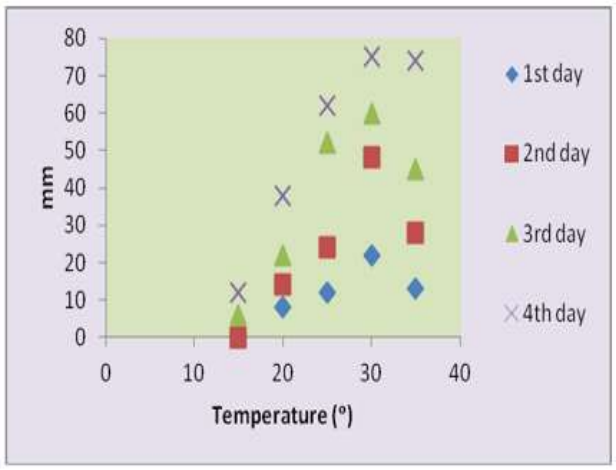


Fig. 2. Effect of temperature on the growth of *T. harzianum*

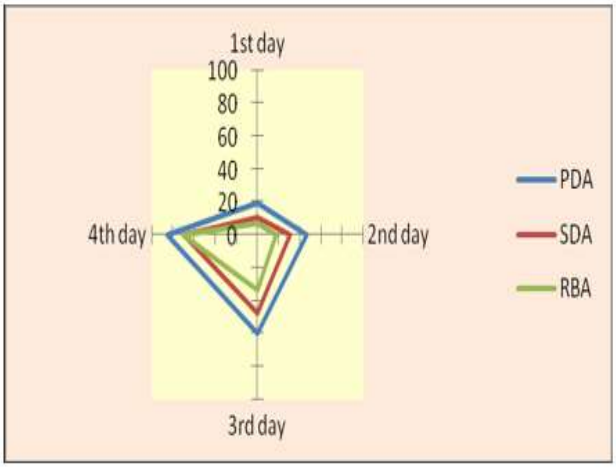


Fig. 3. Effect of different media on the growth of *T. harzianum*

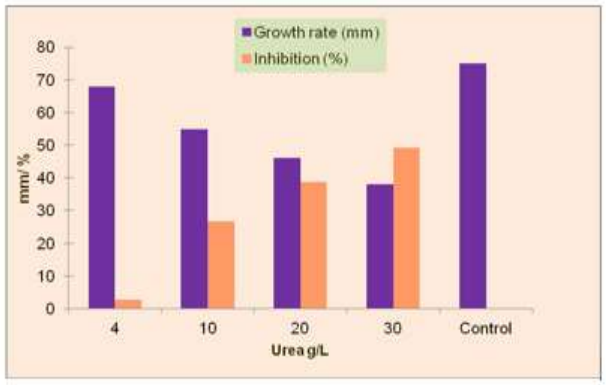


Fig. 4a. Effect of urea on the growth of *T. harzianum*

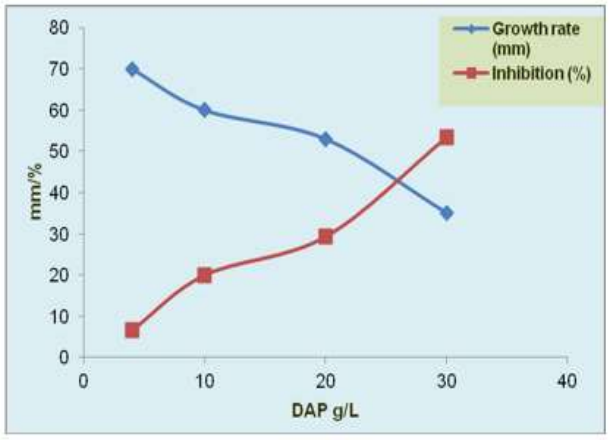


Fig. 4b. Effect of DAP on the growth of *T. harzianum*

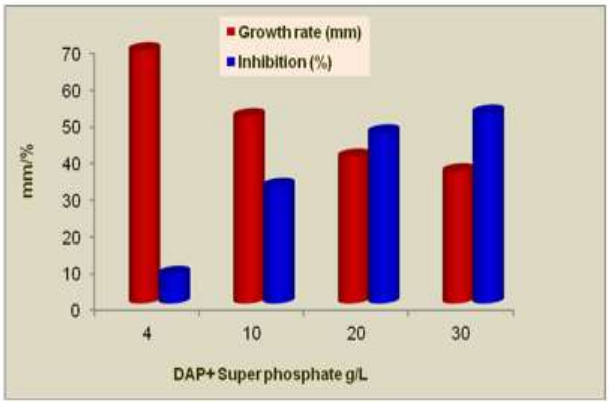


Fig. 4c. Effect of DAP and super phosphate on the growth of *T. harzianum*

done by using the Manual of Soil Fungi by Gillman(1957). Among the 8 different fungi, *Trichoderma harzianum* (151 CFUs; 28.5%) were predominant, followed by *Aspergillus niger* (128 CFUs; 19.27%), *A. fumigates* (106 CFUs; 15.96%), *Rhizopus stolonifer* (96 CFUs; 14.45), *A. flavus* (92 CFUs; 13.85%), both *Fusarium oxysporum* and *Cephalosporium* sp. (each 31 CFUs; 4.66%), and *A. terreus* (29 CFUs; 4.36%) (Table 2). Even though *Trichoderma harzianum* contributed higher percentage of frequency, it was not found in all

Table 4. Effect of fertilizers on the mycelial dry weight of *T. harzianum*

Name of the Fertilizers	Mycelial dry weight (g)				
	4 (g/L)	10 (g/L)	20 (g/L)	30 (g/L)	Control
3rd day					
Urea	1.2	1.0	0.6	0.4	1.8
DAP	1.0	0.9	0.5	0.3	
Urea + super phosphate	0.9	1.2	1.9	0.4	
7th day					
Urea	2.6	2.0	1.3	1.2	2.6
DAP	2.4	1.9	1.8.	1.5	
Urea + super phosphate	2.6	2.1	1.9	1.4	

the sampling stations, whereas three aspergilli namely *A. niger*, *A. flavus* and *A. fumigatus* were found in all the ten sampling stations, followed by *T. harzianum*, *R. stolonifer* and *A. terreus* which were found in eight stations, and *Fusarium oxysporum* and *Cephalosporium* sp. were isolated from seven stations. Further, Andimadam and Paluvur stations of Ariyalur district contributed all the fungal species recorded in this study (Fig. 1).Naveenkumar *et al.* (2011) and Chandrashekar *et al.* (2014) have also reported the predominance of *Aspergillus* spp. from agricultural and non-agricultural soils of Shimoga and Mysore districts of Karnataka respectively.

The soil ecosystem is supported by several interactions among its physical, chemical and biological components (Buscot and Verma , 2005). In the present investigation, it was found that there was no significant positive correlation co-efficient between physico-chemical properties of soil and total fungal population, (Table 3), even though positive correlations between the soil microorganisms and soil nutrients have been reported by Vijayakumar *et al.* (2007).

The predominant fungus *Trichoderma harizanum* was able to grow well at pH 5.5, temperature at 30°C on PDA medium during 4th day of incubation (Figs. 2,3 and 4) when compared to other media and culture conditions tested. Fertilizer tolerance capacity of *T. harzianum* was studied against urea, di-ammonium phosphate (DAP) and super phosphate, and it was found that DAP inhibited up to 53.3% (at 30 g/L) of the growth of *T. harizanum*, followed by 66.2% at 30 g/L of the combination of DAP and super phosphate, and 49.3% at same concentration of urea (Figs. 4a,4b and 4c). The mycelial dry weight response against synthetic fertilizers was carried out, and it was found that the mycelial dry weight was reduced in the culture broth added with synthetic fertilizers when compared to control. Among three chemical fertilizers tested, the mycelial dry weight was significantly reduced in the media prepared with DAP (Table 4). Similarly, the toxic effect of synthetic fertilizers on the growth of soil fungi was already reported by Veverka *et al.* (2007) and Karthika and Vijayakumar (2019).

CONCLUSION

The results of the present study showed that *Trichoderma harizanum* was the most predominant fungal genera in all the soil samples studied. Among the three nitrogen fertilizers tested, DAP had the most significant effect on the growth of *Trichoderma harizanum* even at lower concentration. However, the impacts of chemical fertilizers on soil microbial processes and nutrient cycling could be influenced by different factors such as crop species/rotation, soil type, microbial varieties and compost properties. The present study also suggested that as a chemical fertilizer tolerant strain, *Trichoderma harizanum* could be used as an effective biocontrol and biofertilizer agent.

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