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Plant growth promoting activities of soil fungi resistant to synthetic fertilizers

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ABSTRACT

The present study assessed the growth promoting activities of regional soil fungi resistant to chemical fertilizers; besides it also evaluated the resolving sensitivities of the origami optical microscope-Foldscope. Regional agricultural fields derived soil samples were assessed for their physico-chemical parameters and were subsequently processed to isolate the soil fungi. The morphologically identified fungal isolates were also studied microscopically using the Foldscope, a modern microscopic tool. Further, the predominant fungi of the soil were studied for their growth ability on various culture media, different pH levels and temperature regimes. Impacts of various synthetic fertilizers on the growth of the test fungal isolates were also evaluated. The findings showed that *Trichoderma harzianum* and *Aspergillus niger* were the most common isolates from all the sampling sites of Tamilnadu State, India, and were also the most resistant mycoflora against the synthetic fertilizers applied to the agricultural soil. In addition, the plant growth promoting properties of the predominant fungi associated with *Vigna mungo* crop plant were also described.

Keywords: Foldscope, soil fungi, fertilizers tolerance, plant growth promoting activity, *Vigna mungo*.

INTRODUCTION

Among the soil 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 well distributed and represent the primary components in a broad range of habitats principally in soils rich in decaying vegetation (Bridge and Spooner, 2001).

The growth of soil microorganisms is inhibited by injudicious application of chemicals than any other parameters (Baishya, 2015; Karthika *et al.,* 2019). In this context, there is a genuine need to monitor the fertility of the 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 chemical fertilizers. This would also facilitate to understand the role of indigenous microbial population in the plant growth promoting activities.

In the present study, the diversity soil fungi was studied using the origami microscope – Foldscope (Cybulski *et al*., 2014). These were also assessed for their plant growth promoting activities as well as for their toleration capacity against chemical fertilizers.

MATERIALS AND METHODS

Samples: Soil samples were collected from crop cultivated fields of ten districts of Tamilnadu including Perambalur, Ariyalur, Trichy, Cuddalore, Salem, Nagapattinam, Namakkal, Karur, Thanjavur and Thiruvarur. Sterile polyethylene bags were used to collect the soil samples from a depth of 15 cm, and were transported to the laboratory for further analyses.

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

Soil fungi: The soil fungi were isolated by using soil dilution plate method (Hafez, 1982). One gram of each soil sample in 10 mL of sterile distilled water was suspended and 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 dishes. The plates were then incubated at 28°C for 4-7 days (Ratna Kumar *et al*., 2017). After incubation, fungal colonies were counted and recorded. The correlation co-efficient analysis between the physico-chemical parameters and fungal population of the soil was studied using SPSS package. Population density was expressed in terms of propagules per gram of soil with dilution factors. The per cent contribution of each isolate was calculated by the following standard formula:

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

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 were observed under 140X objective of Foldscope (Cybulski *et al*., 2014). Additionally, identity of the fungi was confirmed through conventional light microscopic observation of the mycelia, conidial structures and spore arrangements. Growth effects of predominant fungal isolates on various culture media, namely PDA, Sabouraud dextrose agar (SDA) and Rose Bengal agar (RBA), temperature at 15, 20, 25, 30 and 35 ºC, and pH at 5.0, 5.5, 6.0, 6.5 and 7.0 were studied.

Effect of chemical fertilizers on the growth of predominant fungi: 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 fungal cultures 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:

$$\text{Percentage of inhibition growth} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Total number of propagules of all species}} \times 100$$

Assessment of mycelia dry weight: Potato dextrose broth was prepared with various combinations of chemical fertilizers (non fertilized control, urea, DAP and urea+super phosphate each 0.5, 1.25, 2.5 and 3.25 g/mL) and inoculated with mycelia blocks of test fungi and incubated for 4 days at room temperature. The mycelia mats were collected on 4th day by filtering through the pre-weighed 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:

Weight of dry mycelia = weight of filter paper + weight of mycelium - weight of the filter paper

Pot culture experiment: Pot culture experiment was conducted using *Vigna mungo* plant to study the plant growth promoting activities of *Trichoderma harzianum* (selected as one of the predominant fungus and known biopesticide), biofertilizers, chemical fertilizers, vermicompost and farm yard manure (FYM) with the combinations of soil + *Rhizobium* (N2 biofertilizer) + *Trichoderma*; soil + *Phosphobacterium* (P biofertilizer)+ *Trichoderma*; soil + *Frateuria aurantia* (K biofertilizer) + *Trichoderma*; soil + FYM + *Trichoderma;* soil + vermicompost + *Trichoderma*; soil + urea + *Trichoderma*; soil + super phosphate + *Trichoderma* and soil + DAP + *Trichoderma* and soil alone as control. The doses of synthetic fertilizers, vermicompost, FYM and others were applied as recommended by Latha *et al*. (2014) and Islam *et al*. (2017). The seeds were surface disinfected by immersion in 0.5% bleach solution for 3 min then were rinsed and washed thrice in sterile distilled water and air-dried in a laminar air flow cabinet (Hajieghrari and Mohammadi, 2016). Ten seeds were buried to 2-3 cm depth in each pot used for treatment and then they were placed under green house condition for 75 days. Three replicates were maintained for all the treatments. The seed germination rate, height of the plant, root and shoot length, numbers of leaves, numbers of branches, number of nodules and number of fruits were measured. The nutrients of soil sample collected from the campus (Kurumbalur, Perambalur district, Tamilnadu) were analyzed after cultivation of black gram by pot culture experiment. *Vigna mungo* treated soil nutrients data were analyzed using two-way analysis of variance (ANOVA) for mean comparison test at the significance level of $P<0.05$.

Estimation of leaf chlorophyll content: Chlorophyll content of black gram plant cultivated under pot culture experiment with different growth promoting agents was estimated by Lin *et al*. (2013). Freeze-dried leaf samples were ground with acetone, centrifuged at 13,000 rpm for 5.0 min. Supernatant was collected and spectrophotometrically measured at 663 and 645 nm to analyze chlorophyll a and chlorophyll b.

RESULTS

A total of 1018 fungal colonies were isolated from 38 cultivated field soil samples in ten different districts of Tamilnadu on PDA medium. Maximum fungal propagules (n=195) were isolated from Ariyalur district, followed by Trichy (n=132), Thanjavur (n=125), Cuddalore (n=100), Perambalur (n=93), Namakkal (n=92), Thiruvarur (n=90), Karur (n=78), Salem (n=72) and Nagapattinam (n=41) (**Table 1**). Among 1018 propagules, 8 morphologically distinguished fungal isolates were identified. *Trichoderma harzianum* with 231 propagules and percentage frequency of 22.69% was predominant, followed by *Aspergillus niger* (225 propagules; 22.1%), *A. flavus* (133 propagules; 13.06%), *A. fumigatus* (127 propagules; 12.47%), *Rhizopus* sp. (116 propagules; 11.39%), *Aspergillus terreus* (86 propagules; 8.44%), *Fusarium oxysporum* (66 propagules; 6.48%) and *Cephalosporium* sp. (34 propagules; 3.33%) (**Table 1**). Fungal isolates were identified based on their colony morphology and foldscope observations (**Fig. 1**). Further, identity of the fungi was confirmed through conventional

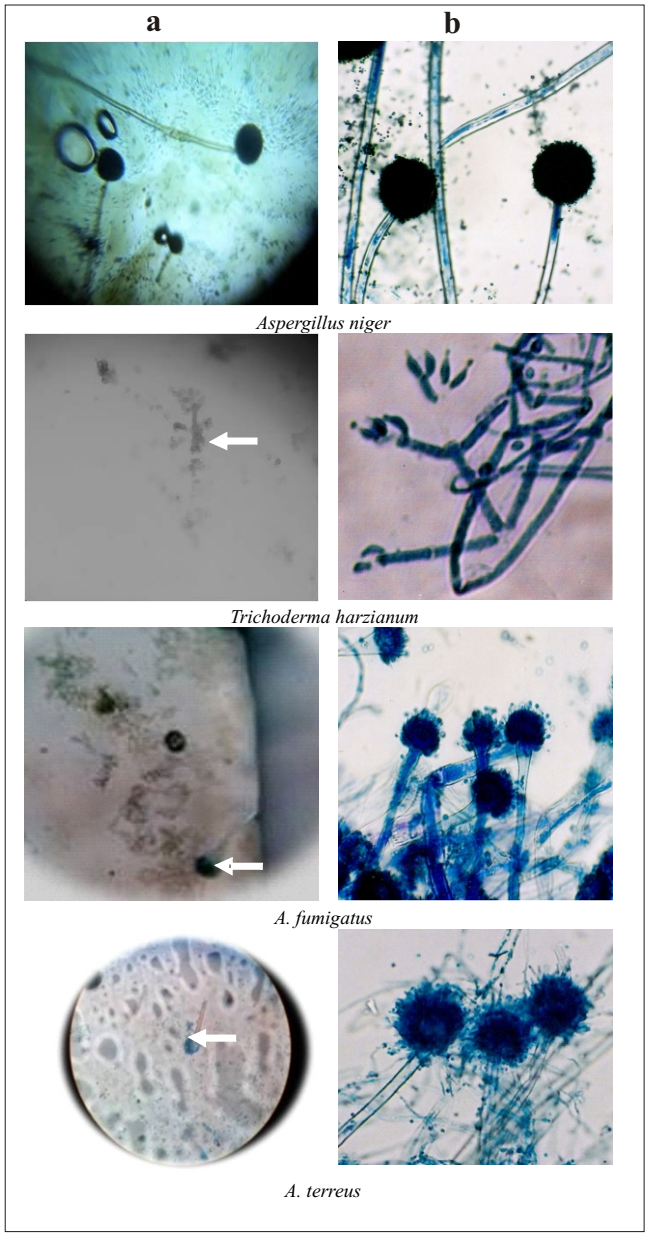

Fig. 1. Microscopic morphologies of the test soil fungal isolates (a) Foldscope (b) Light Microscope

Table 1. Frequency of mycoflora in different sampling stations.

Sampling stations	*T. harzianum*	*A. niger*	*A. flavus*	*A. fumigatus*	*A. terreus*	*Rhizopus* sp.	*F. oxysporum*	*Cephalosporium* sp.	No. of propagules	District –wise Total
Perambalur Dt. Perambalur	-	7	5	4	2	-	3	-	21	93
Alathur	5	6	4	2	1	3	3	2	26	
Kunnam	6	5	2	3	0	3	4	-	23	
Veppanthattai	-	6	3	3	2	4	2	3	23	
Ariyalur Dt. Ariyalur	6	5	4	8	-	7	3	-	33	195
Udayarpalayam	6	5	6	5	4	7	-	3	36	
Sendurai	8	6	4	4	2	6	-	3	33	
Andimadam	10	6	7	8	4	7	3	2	47	
Paluvur	11	7	6	5	5	6	2	4	46	
Trichy Dt. Thuraiyur	4	6	4	4	2	-	-	-	20	132
Musiri	5	6	-	4	3	5	3	-	26	
Lalgudi	5	7	4	3	3	4	2	2	30	
Pullambadi	5	4	3	-	2	3	-	-	17	
Thottiyam	-	4	4	3	-	2	-	-	13	
Pachai Hills	8	6	5	7	-	-	-	-	26	
Cuddalore Dt. Veppur	7	8	5	3	3	4	-	-	30	100
Pennadam	10	10	3	-	2	-	-	-	25	
Virudhachalam	8	6	2	4	2	1	2	-	25	
Thittakudi	6	4	-	2	3	3	2	-	20	
Salem Dt. Gangavalli	6	3	2	3	-	2	2	-	18	72
Athur	5	8	4	4	-	3	2	2	28	
Thalaivasal	4	8	5	3	2	2	2	-	26	
Nagapattinam Dt. Mayiladuthurai	4	5	5	3	3	2	3	-	25	41
Tharangambadi	-	3	-	3	3	4	3	-	16	
Namakkal Dt. Namakkal	4	5	4	3	4	3	2	1	26	92
Rasipuram	8	2	3	-	3	2	-	-	18	
Mohanur	8	8	3	2	2	-	3	2	28	
Paramathi	7	5	-	3	3	2	-	-	20	
Karur Dt. Karur	8	5	2	-	3	4	-	-	22	78
Krishnarayapuram	9	7	4	3	-	2	2	-	27	
Kulithalai	5	8	4	3	2	4	3	-	29	
Thanjavur Dt. Thanjavur	8	3	5	4	2	4	3	2	31	125
Orathanadu	5	10	4	3	4	5	2	3	36	
Thiruvaiyaru	8	5	-	-	3	4	3	2	25	
Pattukottai	7	8	4	4	4	3	3	-	33	
Thiruvarur Dt. Nannilam	10	8	5	5	3	-	2	-	33	90
Muthupet	6	5	6	6	3	2	-	3	31	
Mannargudi	9	5	2	3	2	3	2	-	26	
Total	231	225	133	127	86	116	66	34	1018	
% frequency	22.69	22.10	13.06	12.47	8.44	11.39	6.48	3.33	Average* 26.78%	
*Average propagules of all stations										

Table 2. Physico-chemical properties of soil samples.

Sampling station	Parameters										
	pH	EC (dSm^{-1})	$CaCO_3$ (mg/kg)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	OC (%)
Perambalur Dt. Perambalur	7.7	0.40	High	59.6	11.3	336	2.60	2.30	0.96	0.74	0.33
Alathur	7.8	1.55	High	62.8	12.5	193.5	2.75	1.97	0.87	0.84	0.45
Kunnam	7.94	0.36	High	59.6	8.75	343	2.90	2.20	0.94	0.84	0.40
Veppanthattai	8.07	0.20	High	56.4	6.25	387	2.75	2.15	0.80	0.94	0.39
Pachai Hills	7.32	1.22	Medium	65.7	13.8	288	1.98	1.88	0.97	0.88	0.40
Trichy Dt. Thuraiyur	7.4	1.6	Medium	58.1	12.5	297	2.3	2.1	0.89	0.79	0.56
Musiri	7.2	1.42	Medium	70.2	11.7	312	1.89	2.31	0.91	0.76	0.62
Lalgudi			High	68.8	7.27	287.2	1.80	1.96	0.93	0.67	0.48
Pullambadi	7.9	1.15	High	58.2	8.97	317	2.17	1.87	0.82	0.68	0.51
Thottiyam	7.0	1.28	Medium	63.2	9.7	326	2.89	2.03	0.87	0.91s	0.64
Cuddalore Dt. Veppur	6.9	1.32	High	74.9	9.8	298.7	2.10	1.87	0.94	0.88	0.45
Pennadam	7.2	1.45	High	72.1	10.7	287.1	1.98	1.93	0.77	0.80	0.56
Virudhachalam	7.3	1.29	High	70.2	11.5	269	1.87	2.41	0.81	0.79	0.63
Thittakudi	7.5	1.34	High	71.7	13.1	287	1.89	2.54	0.73	0.81	0.48
Ariyalur Dt. Ariyalur	8.24	1.32	High	58.6	12.1	291.7	2.87	2.18	0.98	0.98	0.30
Udayarpalayam	8.02	1.82	High	61.7	12.9	307.8	2.58	2.08	0.94	0.85	0.32
Sendurai	8.31	1.27	High	60.3	11.7	312	2.78	2.11	0.88	0.78	0.38
Andimadam	8.08	1.30	High	61.4	10.9	327.8	2.45	2.31	0.96	0.82	0.35
Paluvur	7.80	1.23	High	59.7	10.5	299.2	2.12	1.93	0.82	0.79	0.41
Salem Dt. Gangavalli	8.1	0.76	High	60.8	12.7	324.9	1.58	2.30	0.89	0.92	0.52
Athur	8.3	0.90	High	58.3	11.9	318.7	3.87	3.2	1.05	0.99	0.32
Thalaivasal	8.4	0.46	High	59.2	11.2	268.8	4.6	2.98	0.95	0.91	0.62
Nagapattinam Dt. Mayiladuthurai	7.7	0.78	High	53.2	8.2	331.0	3.28	3.11	0.66	0.85	0.58
Tharangambadi	8.2	1.21	High	55.4	9.7	311.8	3.11	2.45	0.82	0.90	0.60
Namakkal Dt. Namakkal	7.2	1.25	High	59.1	10.7	308.0	4.88	1.98	0.93	0.88	0.38
Rasipuram	7.3	1.32	High	60.2	11.2	266.0	3.89	2.31	0.84	085	0.45
Mohanur	7.4	1.14	High	61.7	9.1	263.7	3.2	2.85	0.87	0.76	0.47
Paramathi	7.0	1.38		65.2	9.6	254.0	4.2	3.02	0.79	0.82	0.51
Karur Dt. Karur	7.0	1.41	Medium	66.1	10.1	189.2	3.1	2.55	0.91	0.76	0.46
Krishnarayapuram	7.6	1.39	High	62.3	8.9	198.2	3.22	2.41	0.82	0.81	0.52
Kulithalai	7.7	1.27	Medium	71.0	9.5	200.7	4.5	2.31	0.81	0.72	0.66
ThanjavurDt. Thanjavur	7.2	7.4	Medium	88.3	7.6	285.2	6.5	3.62	0.91	0.82	0.53
Orathanadu	7.5	1.96	Medium	90.2	8.2	263.1	6.85	3.69	0.86	0.78	0.39
Thiruvaiyaru	7.6	1.60	Medium	80.2	10.4	234.2	5.6	3.48	0.90	0.85	0.44
Pattukottai	7.8	1.85	High	95.1	9.8	221.2	6.33	3.71	0.89	0.78	0.37
Thiruvarur Dt. Nannilam	7.1	1.62	Medium	79.2	10.2	228.9	4.5	2.89	0.75	0.75	0.58
Muthupet	8.1	1.69	High	95.7	13.7	296.3	7.66	3.09	0.94	0.98	0.31
Mannargudi	7.6	0.81	Medium	82.0	11.2	255.8	5.6	3.21	0.91	0.68	0.41

light microscope by observing mycelia and conidial structures and spore arrangement. The studied physico-chemical properties of the soil samples are presented in **table 2**. Statistically, relationships between the soil fungi and physico-chemical properties were studied by correlation co-efficient analysis and it was found that there was no significant positive correlation co-efficient between the physico-chemical properties of soil and total fungal population, whereas, significant positive and negative correlation co-efficient were observed between the nutrients of the soil (**Table 3**).

Fertilizer tolerance capacity of the predominant *A. niger* and *T. harzianum* were studied against urea, di-ammonium phosphate (DAP) and super phosphate, and it was found that the radial growth of both the test fungi was significantly affected by urea at 8 g/L (80.3% and 75.7%) followed by the combination of DAP and super phosphate and DAP (72.7% and 63.3% and 70.5% and 60.3% at 8 g/L) (**Fig. 2a-c**). Fertilizer tolerance ability of the test fungi was further determined by the measurement of mycelial dry weight, and it was found that the mycelia dry weight was reduced in the media with fertilizers when compared to control. Among the three chemical fertilizers tested, the mycelia dry weight was significantly reduced at higher concentration of urea and super phosphate combination (0.8 g at 3.75 g/100 mL and 1.0 g at 3.75 g/L for *A. niger* and *T. harzianum*, respectively) (**Fig. 3**). Both the predominant fungi could be grown well at pH 5.5, temperature at 30 ºC on PDA medium during 4th day in comparison to the other media and culture conditions tested.

Effect of chemicals as well as biofertilizers and FYM and vermicompost on the morphometric study of *Vigna mungo* was also studied. All the test seeds (100%) were germinated in all the twenty-four treatments including control. The tallest (39±0.52 cm) black gram plant was observed in the pot treated with *Rhizobium* sp. (T12), maximum root length (15±0.5 cm) was observed in the plant treated with the combination of all the three biofertilizers (T20), namely *Rhizobium* sp., *Phosphobacterium* sp. and *Frateuria aurantia*. Maximum (25.5±0.50 cm) shoot length was observed in both T3 and T21 treatments. Both height and root length of the plant were highly influenced by the combination of NPK biofertilizers, whereas the length of shoot was induced by the T1-T3, T12, T14 and combination of both chemical and biofertilizers.

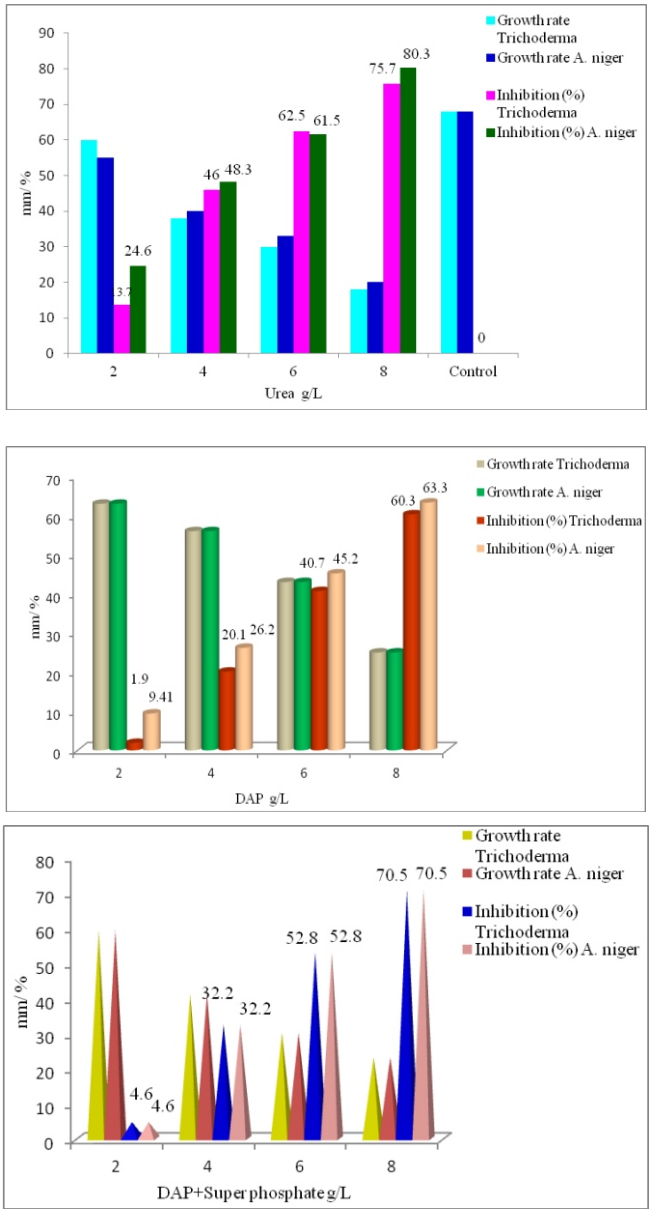

Fig. 2. Effect of various chemical pesticides on the radial growth of *Trichoderma harzianum* and *Aspergillus niger* a) Urea; b) DAP; c) DAP+ Super phosphate.

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

	TFC	Ph	N	P	K	Fe	Mn	Zn	Cu	OC
TFC	1									
pH	-0.3601	1								
N	-0.3682	-0.6815	1							
P	0.02432	-0.9074**	0.9174**	1						
K	0.3590	0.5225	-0.9403**	-0.8297**	1					
Fe	-0.9205**	0.6058	0	-0.3743	0.0340	1				
Mn	0.5710	-0.1424	-0.5317	-0.2701	0.7586*	-0.2954	1			
Zn	0.0774	-0.7104	0.3928	0.5255	-0.0642	-0.1122	0.5487	1		
Cu	-0.3682	0.9339**	-0.5	-0.7412*	0.2478	0.5	-0.4430	-0.8979**	1	
OC	-0.8013**	0.2772	0.4974	0.1521	-0.6662	0.5803	-0.9477**	-0.4582	0.4974	1

* P - 0.05; ** p - 0.01

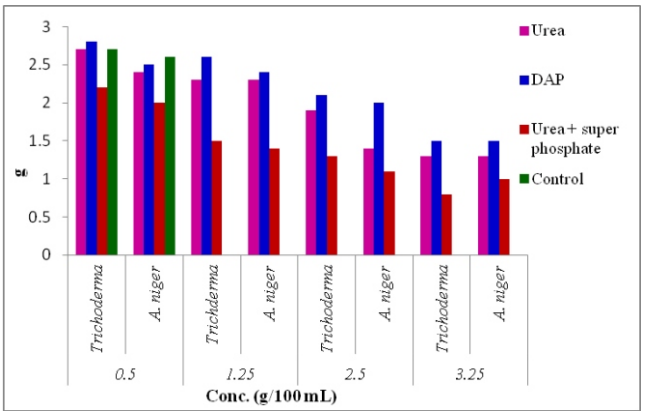

Fig. 3. Effect of various fertilizers on the mycelial dry weights of *Trichoderma harzianum* and *Aspergillus niger*

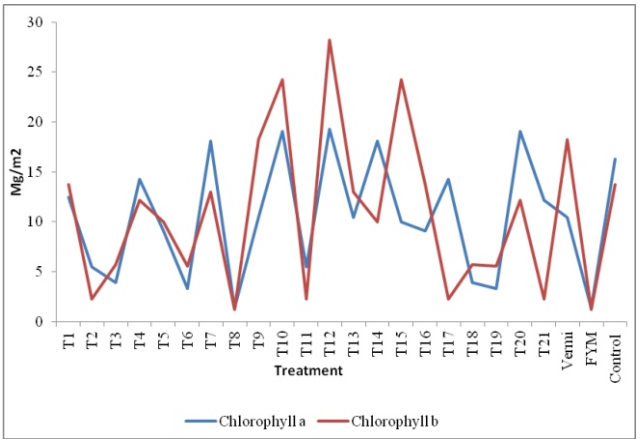

Fig. 4. Effect of various treatments on chlorophyll content of *Vigna mungo*

Number of leaves (n=10), branches (n=6), nodules (n=28±3.21) and fruits (n=5) were recorded in the plant treated using biofertilzers, particularly nitrogen based biofertilizer *Rhizobium* (T12) (**Table 4**). Similarly, maximum contents of both chlorophyll a (19.28 mg/m^2) and b (28.21 mg/m^2) were recorded in T12. Chlorophyll b content was higher than chlorophyll a in almost all the treatments (**Fig. 4**). After treatment, EC content was less than 1.0 dSm^{-1} in all the biofertilizers as well as chemical fertilizers treated soil. Contents of N, P, K, Fe and Mn were slightly increased due to the introduction of NPK based biofertilizers. Whereas heavy

Table 4. Effect of various treatments on the morphometric parameters of *Vigna mungo*

Treatments	Morphometric parameters on 50th day						
	Plant height (cm)	Root length (cm)	Shoot length (cm)	No. of Leaves	No. of. branches	Root nodules	No. of fruits
T1	34 ± 0.76	10.6 ± 0.30	24 ± 0.23	8	3	6 ± 1.0	2
T2	34.5 ± 0.92	10.5 ± 0.30	24 ± 0.41	8	2	5 ± 1.52	2
T3	35 ± 0.47	9.5 ± 0.30	25.5 ± 0.50	9	3	6 ± 1.52	3
T4	33 ± 0.36	11 ± 0.26	22 ± 0.10	8	4	7 ± 1.52	2
T5	34 ± 0.12	12 ± 0.06	22 ± 0.06	9	3	6 ± 2.0	3
T6	34.5 ± 0.90	12.5 ± 0.50	22 ± 0.40	9	4	5 ± 1.0	3
T7	31 ± 0.50	11 ± 0.50	20 ± 0.26	7	3	5 ± 2.0	3
T8	32 ± 1.07	12 ±0.28	20 ± 0.50	6	2	6 ± 1.52	2
T9	32.5 ± 0.78	11 ± 0.28	21.5 ± 0.50	6	3	6 ± 1.0	1
T10	36 ± 0.91	12 ± 0.50	24 ± 0.41	9	4	20 ± 2.0	3
T11	38.5 ± 0.56	14 ± 0.28	24.5 ± 0.28	10	5	24 ± 2.0	5
T12	39 ± 0.52	14 ± 0.32	25 ± 0.20	10	6	28 ± 3.21	5
T13	30 ± 0.61	10 ± 0.26	20 ± 0.41	7	5	9 ± 1.52	4
T14	32 ± 0.86	10 ± 0.50	22 ± 0.36	8	6	8 ± 2.51	3
T15	33 ± 0.60	11 ± 0.20	22 ± 0.40	6	4	9 ± 2.51	2
T16	30 ± 0.50	12 ± 0.32	18 ± 0.60	7	3	9 ± 2.08	3
T17	32 ± 0.40	10 ± 0.10	22 ± 0.30	8	4	6 ± 1.52	4
T18	32.1 ± 0.26	12 ± 0.50	20.1 ± 0.35	7	5	8 ± 1.0	3
T19	36.4 ± 0.35	12 ± 0.18	24.4 ± 0.17	10	5	9 ± 1.0	2
T20	38.5 ± 0.40	15 ± 0.5	23.5 ± 0.35	9	5	25 ± 1.52	4
T21	37.5 ± 0.78	12 ± 0.28	25.5 ± 0.50	11	5	25 ± 1.52	2
Vermicompost	34 ± 0.36	12 ± 0.28	22 ± 0.41	8	5	12 ± 1.0	3
FYM	36 ± 0.47	13 ± 0.28	23 ± 0.50	8	4	13 ± 1.0	3
Control*	28 ± 0.87	9 ± 0.37	19 ± 0.50	6	3	7 ± 1.0	3

T1-Urea 1g; T2 -urea 1.25g; T3 -urea 1.5g; T4 -DAP 1g; T5 -DAP 1.25g; T6 -DAP 1.5g; T7 -Superphosphate 1g; T8 - Superphosphate 1.25g; T9 - Superphosphate 1.5g; T10 -*Rhizobium* 1mL; T11 - *Rhizobium* 2mL; T12 -*Rhizobium* 3mL; T13 - *Phosphobacterium* 1mL; T14*Phosphobacterium* 2mL; T15-*Phosphobacterium* 3mL; T16-*Freuteria aurentica* 1mL; T17 -*Freuteria aurentica* 2mL; T18 -*Freuteria aurentica* 3mL; T19-Combination of chemical fertilizers 1. 25g (each); T20 -Combination of biofertilizers 2mL (each); T21 -Combination of chemical & biofertilizer; *without*Trichoderma* inoculation.

Table 5. Nutrients contents of *Vigna mungo* cultivated soil

Treatment	Parameters											Fungal propagules / g
	pH	EC (dSm^{-1})	$CaCO_3$ (mg/kg)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	OC (%)	
Control pot (Without any supplements)	8.1	1.7	Medium	50.0	4.0	143.8	2.70	2.25	0.90	0.80	0.27	32
Soil + *Rhizobium* + *Trichoderma*	7.2	0.54	High	85.6	10.8	368	2.13	2.89	1.56	0.42	0.38	47
Soil + *Phophobacterium* + *Trichoderma*	7.3	0.72	High	68.9	14.5	374	3.02	2.87	1.32	0.50	0.42	38
Soil + *Frateuria aurantia* + *Trichoderma*	7.5	0.67	High	70.5	14.1	380	2.80	3.10	1.24	0.52	0.44	42
Soil + Farm yard manure + *Trichoderma*	7.2	0.77	Medium	80.2	14.3	365	2.74	2.80	1.09	0.43	0.36	36
Soil + Vermicompost + *Trichoderma*	7.3	0.58	Medium	73.5	12.9	350	2.68	2.64	0.99	0.58	0.39	40
Soil + Urea + *Trichoderma*	8.1	0.55	High	61.2	12.4	340	2.40	2.18	0.89	0.80	0.32	22
Soil + Super phosphate + *Trichoderma*	8.3	0.52	High	52.0	15.1	322	3.01	2.14	0.78	0.70	0.34	26
Soil + DAP + *Trichoderma*	8.0	0.47	High	70.1	15.2	311	2.85	2.24	0.91	0.54	0.28	24

metals like Zn and Cu and OC content was reduced due to utilization of plants. Further, the population of mycoflora of the biofertilizers treated soil also increased when compared to the control and chemical fertilizers treated soil (**Table 5**). Two-way analysis of variance (ANOVA) test was examined between the treatments and nutrient contents of *Vigna mungo* treated soil, and the results are presented in **table 6**. A positive significant ($p<0.05$) variation was observed between the nutrient parameters, but there was no significance between the treatments of *Vigna mungo* treated soil.

Table 6. Two-way ANOVA for the treatments and nutrient content of *Vigna mungo* treated soil.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Rows (Treatments)	5609.41	8	701.1763	1.442245	0.192047
Columns (Nutrient parameters)	851564.2	10	85156.42	175.1577	4.65E-50*
Error	38893.61	80	486.1701		
Total	896067.2	98			
* p <0.05 is statistically significant					

DISCUSSION

The fertility of the soil and water mass is determined by the distribution of nutrients (Bragadeeswaran *et al*., 2007). Soil has many physical and chemical properties, some are changeable, while others are difficult to adjust. Texture, structure, drainage, and organic matter content are the physical properties. Chemical properties including cation exchange capacity and pH generally affect microbial as well as plant growth (Cholarajan and Vijayakumar, 2013). The present study reported the diversity of 8 different soil fungal genera among 1018 isolated fungi from cultivated land soil samples. The frequency of soil fungi from 38 locations of 10 different districts of Tamilnadu revealed that *Aspergillus niger* and *T. harzianum* were the most frequently documented fungi in all the soil samples (**Table 2**). In line with the present observations, Naveenkumar *et al.* (2011) and Chandrashekar *et al*. (2014) also reported the predominance of *Aspergillus* spp. from agricultural and non-agricultural soils of Shimoga and Mysore districts of Karnataka, respectively. Presently, to begin with foldscope was used so as to identify the soil fungi the microscopic details of which was subsequently confirmed by using conventional light microscope. The uniformity in primary observations made and subsequent identification of all the test fungi using foldscope and light microscope brought out the reliability of the origami device – foldscope for making such studies. Using foldscope similar such studies have been made for the identification of *Colletotrichum gloeosporoides*, *C. capsici* and *Fusarium oxysporum* by Gurjar and Kanade (2020), *Penicillium* sp. and *Aspergillus* sp. by Prarthana and Narayana (2020); mycorrhizal fungi by Nivedha *et al*. (2019) and Sabarinathan *et al*. (2019). When physico-chemical properties of the soil were studied, the pH of the soil samples was found to vary from neutral to slightly alkaline in nature. As compared, Pešaković *et al*. (2009) documented acidic (pH 5.9) nature of the alluvial soil in Serbia. Likewise, Nakhro and Dkhar, (2010) documented varied levels of pHs in control (5.310±0.17), organic (5.120±0.14) and inorganic (5.350±0.13) field soil, respectively. However, Lazcano *et al*. (2012) reported slight acidic nature of the soil pH (6.6±0.02). Although it is a well established fact that most of the fungal populations prefer acidic pH ranges, but during the present investigations the documented fungal taxa were isolated from the soil samples having neutral to slight alkaline pH range. In general, it is a known fact that the diversity of native microbial population of a particular ecosystem is regulated by the physical, chemical as well as biological factors. During the present investigations, no positive correlation was observed between the biological and physico-chemical factors of the soil. In a related work, statistical significance between the soil microorganisms and soil nutrients has already been reported using correlation co-efficient studies by Vijayakumar *et al*. (2007).

To understand the impact of use of chemical fertilizers on the occurrence and survivability of population of indigenous microflora in the soil three chemical fertilizers were tested. The mycelial dry weights of the *A. niger* and *T. harzianum* present in the soil was significantly reduced when higher concentration of urea and super phosphate combination was broadcasted in the soil. This may be due to the toxic effect of urea when used in higher concentration. This observation is in

conformity with the earlier such reports by Veverka *et al*. (2007) and Karthika and Vijayakumar (2018). Initial propagule densities of *Trichoderma* spp. were found to be higher in soils amended with swine manure and cotton-gin trash (CGT) in comparison to the soils amended with rye-vetch or synthetic fertilizer. Bulluck III and Ristaino (2002) also documented increased propagule density of the *Trichoderma* spp. over a period in the soils when amended with synthetic fertilizers. Thus, the results of the present study are in conformity with the earlier reports by Bulluck III and Ristaino (2002). Noticeably, the tolerance capability of the test fungi differed between the methods employed and media used. In the solid media, the radial growth of the test fungi was significantly inhibited when the medium was added with urea, whereas mycelial dry weights of the test fungi was reduced at higher concentration of urea and super phosphate combination.

Recently, species of *Trichoderma* have been effectively used as biostimulant for the growth and development of a wide variety of plants (Bhardwaj *et al*., 2014). Reports also suggested that *Trichoderma* produces plant growth promoting phytohormones such as indole acetic acid (IAA) and their analogous (Vinale *et al*., 2012), vitamins, enzymes leading to plant growth, organic acids in rhizosphere such as gluconic, citric, and/or fumaric acids which decrease the soil pH (Harman *et al*., 2004). Hence, the usage of plant growth promoting *Trichoderma* species is becoming one of the important aspects of sustainable eco-friendly approach in agriculture. During the present study, the predominantly documented soil fungus of the studied region *T. harzianum* was selected and incorporated into all the treatments of pot culture experiment, and also it was found that all the plant growth related parameters were higher in fertilizers treated pots. Further, the physico-chemical parameters of the treated soil when analyzed, it was observed that the pH of biofertlizers treated soil changed to neutral condition. In addition, positive significant ($p<0.05$) variation was observed between the nutrient's parameters of *Vigna mungo*, but there was no significance noted between the treatments. Propagule densities of microbial species including total cultivable fungi, thermophilic microorganisms, *Phytophthora* and *Pythium* spp., and numbers of sclerotia were not significantly affected by treatments over the course of the experiment (Bulluck III and Ristaino, 2002). In contrast to this, Islam *et al*. (2017) reported that most of the parameters were significantly increased after treatment except N and S contents. Mathivanan *et al*. (2014) also reported that most of the morphometric data of the groundnut (*Arachis hypogeaea* L.) and nutrient parameters of the soil increased significantly (at $P<0.05$). These changes may be due to the presence of microbial population which is involved in the biogeochemical cycles and addition of NPK chemical fertilizers.

Generally, age and number of leaves and area of leaf surface accounts for the increase in the quantity of chlorophyll content of the plants. In the present study, the chlorophyll a and chlorophyll b were estimated from the test plant obtained from pot culture experiment, and maximum chlorophyll contents was found in the test plant treated with *Rhizobium* bioinoculant. Similarly, Hajieghrari and Mohammadi (2016) reported that no significant reduction was found in the chlorophyll content of the *Trichoderma* fortified wheat plants.

CONCLUSION

The findings of the present study brought out that the *T. harzianum* and *A. niger* were the most predominant fungal taxa of the studied stations. Further, it was found that among three nitrogen fertilizers tested, urea had the most significant effect on the growth of *T. harzianum* and *Aspergillus niger* even at lower concentration. However, the impacts of chemical fertilizers on soil microbial processes and nutrient cycling could be influenced by different factors as well such as crop rotation, soil type, microbial varieties and compost properties.

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