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RELATIVE IMPACT OF DIFFERENT FERTILIZER TREATMENTS UPON PHYSICO-CHEMICAL AND MYCOFLORAL DIVERSITY IN AGRICULTURAL SOIL OF WHEAT FIELD IN HARIDWAR, UTTARAKHAND

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ABSTRACT

The present study was undertaken to investigate the effect of different fertilizer treatments on the physico-chemical and mycofloral proportion in agricultural field of wheat in Haridwar during 2015-2017. Three different plots were selected for the present experiment. A total of 21 species of 14 genera were isolated from the agricultural fields of different treatments from which 9 species were observed from control plot (T_1), 12 species were isolated from chemically amended plot (T_2) and the highest fungal diversity with 16 species were recovered from (T_3) plot which was nourished with combined use of organic and inorganic fertilizer. *Aspergillus niger* was dominant species in T_1plot while *Fusarium graminearum* showed its dominancy in T_2 plot which is considered as pathogenic fungi for wheat plant. *Trichoderma harzianum* was the most dominant species in T_3 plot. Some other beneficial fungi like *Trichodema hamatum, Aspergillus flavus, Aspergillus niger* showed positive correlation with some soil physico-chemical properties like Temperature, OC, N, P and K in this plot. Combined use of FYM and NPK resulted in significant increase of OC, N, P and K in T_3plot while reduction of pH, N, P and K were observed in T_2 plot where inorganic fertilizers were applied alone in the farmer field.The highest crop yield were recovered from T_3 plot. Hence, our findings gave an idea that balanced and combined use of inorganic and organic fertilizer may enhance the physico-chemical and mycofloral diversity in agricultural soil and improve soil fertility.

Keywords: Agricultural soil, Farmyard manure,Fertilizer treatment, Mycofloral Diversity

1. INTRODUCTION

In modern agriculture, to achieve high crop yields and gratify the requirement of an increasing population, a significant amount of inorganic fertilizers is applied to croplands. However, long-standing inputs of inorganic amendments are disadvantageous to develop long-term sustainability in agricultural field. Organic inputs are extensively received as one of the sustainable agricultural practices that improve soil fertility and increase its ability to hold water and nutrients. Raising people concern on overuse of chemical fertilizers in agriculture and their negative effects on the soil fertility status of agricultural land has led to researchers to work on balanced and combined use of organic and inorganic fertilizers in the agricultural field which improves soil productivity and its microbiological property.

Haridwar district with geographical area of 2360 km^2 lies in the south-western part of the Uttarakhand state. Wheat and rice are the main agricultural crops of this region.

Wheat is the main crop and accounts for about 50 percent of the total production of food grain in the state. Out of total surveyed agricultural land in Haridwar district, approximately 98 percent farmers use chemical fertilizers in their agricultural fields [1]. This demanding use of agrochemicals definitely compact the microbial diversity, increase in irreversible soil erosion and decrease soil organic matter [2]. To reduce the undesirable effects of chemical fertilizers, farmers are moving towards the combined application of inorganic fertilizer with organic farming. The addition of organic fertilizers in agricultural field significantly increases microbial population of rhizospheric zone than without inorganic fertilizer [3]. Manure application to the agricultural field play a very important role in improving the fertility of soil as they are organic material taken from plant and animals wastes like dung, urine etc. and get extracted. The addition of Farmyard manure as an organic fertilizer in agricultural soil increases the mycofloral proportion [4]. Farmyard manure provides all

major nutrients as well as micronutrients necessary for plant growth. The soil pH, organic matter, and moisture content are the main factors which affect the fungal population and diversity in soil, continuous use of inorganic fertilizers leads to deterioration of soil health.

The combined application of organic manures with chemical fertilizers is increasingly gaining appreciation as a viable approach to address soil fertility status in all over the world. The high application of chemical fertilizer usually improvescrop yield in short term, [5 but it hardly maintains and even decreases SOC and has negative effects on Microbial diversity of soil and nutrient loss. Organic fertilizer application is a widely accepted strategy to sustain or improve crop production and SOC stock and has significant effects on soil fertility sustainability [6, 7]. Among the types of organic fertilizer applications, manure amendments are favoured for increasing SOC and supplying nutrients to crops as they have higher SOC sequestration efficiency [8]) and supply more available nutrients to crops [9, 5]. However increases in crop yields are either marginal or increase at a low rate when organic fertilizers are used alone [10-12]. Alternatively, the combined use of chemical and organic fertilizers has been preferred to meet the requirements of sustaining crop yield and enhancing soil organic carbon at a reasonable rate [13], in agricultural system and hence also improves the soil microbial diversity.

The fungi form an important constituent of soil micro floral population. Among them, some fungal species play key role in increasing nutrient mineralization and improving soil health as they execute significant functions related to water dynamics, nutrient cycling, and disease inhibition. Fungi are important as decomposers in the soil ecosystem, converting hard to digest organic material into usable forms. They also play a vital role in the decomposition of structural polymers of plants such as cellulose, hemicellulose, lignin and thus contributing to the preservation of the global carbon cycle [14]. Removal and consequent cultivation of agricultural land for crop production has a vast effect on soil mycoflora diversity [15]. Several farming practices like ploughing, tillage increased intensity in land use and excessive use of chemical fertilizers may cause an imbalance in soil mycofloral population affecting microbiological properties of soil. Fungi are known to colonize diverse habitats and substrates they are known to play substantial role in improving plant health and productivity besides producing diseases [16]. The purpose of this study aims to investigate the potential of combined use of organic and inorganic fertilizers in irrigated agricultural land to improve physico-chemical characteristics and mycofloral proportion and soil characteristics to obtain higher crop production without the need of excessive inorganic fertilizers.

2. MATERIALS AND METHODS

2.1. Study Area

The agricultural field of Missarpur village situated in Haridwar district had been selected for the present investigation. This is a small village in Bahadarabad Block in Haridwar district of Uttarakhand state, India. The geographical position of the site is at 29°53'21.8"N longitude and 70°08'11.9"E latitude and is situated at an altitude of 314m from sea level. It is one of the developing area in Haridwar district. Most of the people in this area depend on agriculture.

2.2. Experimental Design:

Materials used for the Present Study:

1. Fertilizer used: Urea (46%N), Single Super Phosphate (18% P_2O_5) and Potash were used as NPK sources in agricultural fields. Farmyard manure was used as an organic fertilizer.
2. Planting Material: The crop chosen to establish the objectives of the study washigh yielding variety of (HD-2967) *of Triticum aestivum* was sown in all the three agricultural plots. The variety matured in 130-145 days.
3. Irrigation: Flood irrigation was applied to all the experimental plots through tube well water. Five irrigations were applied at different growth stages of wheat. Irrigation was stopped 20 days before crop maturity.

Three different farmer plots were selected for the present investigation. The first plot (T_1) with size $35\times35m^2$was taken as Control which was maintained with cow dung manure (1ton/hectare) and without the use of anychemical fertilizer. In second plot (T_2) with size $40\times40m^2$, the Chemical fertilizers (NPK) (100:50:25) kg/hectare were applied by the farmers alone to the field. All the dose of Phosphorus and Potash were applied at the time of seed bed preparation along with half dose of Nitrogen. The remaining dose of Nitrogen was applied to the soil at the time of crop development stage of the crop by the farmer. In third plot (T_3), combined application of organic fertilizer (Farmyard manure) with chemical fertilizer (NPK) were given to the soil as per the requirement of the crop. Dose- 5 ton/hectare FYM+

(50:25:12)kg/hectare NPK were applied by the farmer in combined application. Farmyard manure was applied by the farmer 15 days prior to seed sowing and thoroughly mixed with the soil. Full dose of P and half dose of the N fertilizers for the respective inorganic N and P treatments were applied at the time of seed bed preparation. The remaining half of the N fertilizer and full dose of Potash were applied at mid-season of the crop growth stage as per the requirement of crop.

Fig. 1: Crop chosen for the study: *Triticum aestivum*

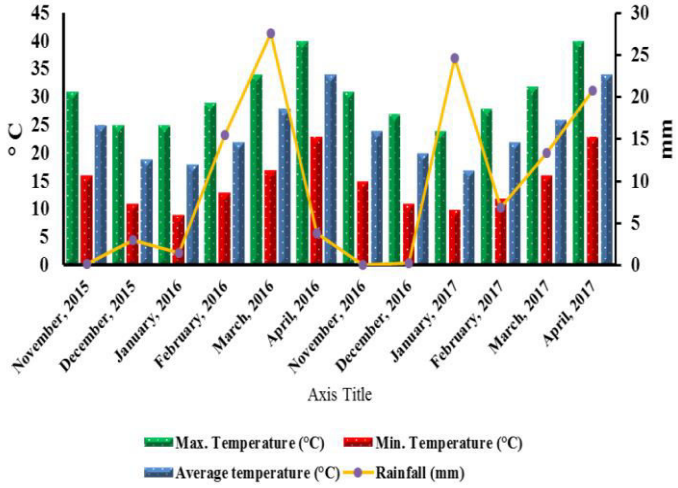

Fig. 2: Meteorological data of Missarpur Village, Haridwar District, Uttarakhand during 2015-17

2.3. Collection of agricultural soil sample

Sampling had been done at 35 days interval from initial stage to Harvest period of crops. Soil sample was also tested before sowing the seeds in the experimental fields. Soil samples were collected from different Experimental plots during the month of November 2015 to April 2016 and November 2016 to April 2017 for two consecutive years (2015-2017).

Three locations were randomly selected from each Experimental Plot from the surface and sub-surface of the soil at various depths viz. 0-15 cm and 15-30 cm respectively. Soil samples of various depths were mixed to make it a composite sample which was brought to the laboratory in sterile polythene bags and further divided into two parts. One part was stored at 4°C and further used for the isolation and identification of Mycofloral Population and the remaining part of the sample was dried and sieved for the determination of Physico-Chemical analysis of soil.

2.4. Physico- Chemical Analysis

Soil temperature was noted through Soil thermometer at the time of Sample collection. Soil texture was determined with the help of USDA soil textural triangle.Moisture content was determined gravimetrically by weighing and drying method. pH was recorded through electronic digital pH meter Organic Carbon and Organic matter was measured by Walkey and Black Method [17]. Kjeldahl Distillation Method, Jackson [18] was used for the determination of Nitrogen. Available Phosphorus was determined by Molybdenum blue method, Trivedy and Goel [19]. Potassium was extracted by Ammonium Acetate and determined by Flame Photometric Method.

2.5. Isolation and Identification of Mycoflora

Serial dilution plate technique described by Aneja [20] was employed to enumerate the soil fungi. Martin's Rose Bengal agar medium supplemented with 1% Streptomycin was used for the isolation of fungi. Three replicates were maintained for dilution 10^{-3} for each soil sample. The inoculated Petri plates were incubated at 25°C for 3-5 days for the growth of fungal colonies. After the incubation Period, the Colony forming units were counted and expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. Identification of mycoflora was made by microscopic examination by using standard procedures, taxonomic guide and relevant literatures.

2.6. Calculation

The numbers of fungal colonies g-1 of dry soil were calculated by applying the following formula.

a. Colony forming unit (CFU)

$$\text{CFU'S (Fungi g}^{-1}\text{ dry soil)} = \frac{\text{Average number of colonies}}{\text{Dry weight of soil}} \times \text{Dilution factor}$$

b. Relative occurrence

$$\text{Occurrence (\%)} = \frac{\text{Average number of colonies of a species}}{\text{Average number of colonies of all the fungal species}} \times 100$$

2.7. Shannon-Wiener index

Diversity indices represent a useful means for quantifying community diversity. The general diversity of total fungal communities were calculated by the generally accepted Shannon Wiener Index by the formula,

$$H = \sum\left[(p_i) \times \ln(p_i)\right]$$

Where,

Pi = Proportion of total sample represented by species i. Divide no. of individuals of species I by total number of samples.

S = Number of species = species richness

H_{max} = in(S) = Maximum diversity possible

$$E = \text{Evenness} = \frac{H}{H_{max}}$$

2.8. Culture characteristics

Different types of fungal isolates produced different characteristics of colonies. Some colonies were coloured, somewhere circular in shape and other were irregular. They were first studied by their colour, shape, colony features and identified microscopically by staining with Lacto phenol cotton blue and observed under microscope byelevation (size) of their conidia, mycelium and other description given by Watanabe [21] and by following mycological literatures and taxonomic guide.

3. RESULTS AND DISCUSSION

3.1. Comparison of physico-chemical parameters of different experimental plots of Wheat field.

The soil textureof all the studied agricultural plots were silt loam. From Table 1-3, we found that a combination of FYM with NPK resulted in considerable changes in soil physico- chemical parameters. After the two years of study, in ($T_{1)}$ plot, the average value of pH was decreased from 6.13 to 6.05. In ($T_{2)}$ plot, the average soil pH was increased upto 6.52 at crop development stage and it was decreased up to 5.93 after the harvesting of the crop. The decrease in soil pH is also dependent on the intensification of the process of decay and decomposition of organic compounds in the soil. Same decreasing pattern of pH was also reported by Nagar *et al.,* [22] after studying the effect of different levels of inorganic fertilizers on physico-chemical properties of agricultural soil in Allahabad City. The decrease in pH might have been resulted from the fact that the key molecules of Nitrogen in term of changes in soil pH are the uncharged Urea molecule, the cation ammonium (NH_4^+) and the anion Nitrate (NO_3^-). The conversion of N from one form to another involves the generation and consumption of acidity and hence pH was decreased. In (T_3) plot, where Farmyard manure was applied integrated with NPK showed decrease in pH from 6.42 to 6.3.

Table 1: Physico-chemical parameters at different growth stages of wheat at T_1 plot during 2015-17.

Physico-chemical Parameters	Before Sowing	Initial Stage	Crop Development	Mid-Season	Ripening Stage	After Harvesting
Temperature (°C)	21.6±0.10	20.7±0.04	18.98±0.07	19.1±0.08	22.8±0.04	24.07±0.08
Moisture Content (%)	9.43±0.06	10.03±0.19	11.50±0.09	11.10±0.14	10.60±0.10	9.37±0.08
pH	6.13±0.02	6.27±0.03	6.33±0.03	6.47±0.03	6.18±0.03	6.05±0.04
Organic Carbon (%)	0.89±0.02	0.92±0.01	0.94±0.01	0.98±0.03	0.96±0.03	0.91±0.02
Organic Matter (%)	1.53±0.03	1.57±0.02	1.62±0.03	1.69±0.04	1.65±0.04	1.57±0.04
Nitrogen (%)	0.27±0.01	0.29±0.01	0.32±0.01	0.35±0.01	0.30±0.01	0.26±0.01
Phosphorus (%)	0.017±0.001	0.019±0.001	0.023±0.001	0.025±0.001	0.022±0.001	0.018±0.001
Potassium (%)	0.086±0.001	0.094±0.001	0.104±0.002	0.102±0.001	0.090±0.001	0.078±0.001

(All values are Mean±S.E. of 6 observations each)

As during the decomposition of Farmyard manure, organic acids and carbon dioxide released into the soil which might decrease the soil pH. Average organic carbon content was slightly increased in T_1 Plot by 2.20% after 2 years of study. In T_2 plot, average organic carbon content was decreased by 8.66% while application of Farmyard manure with NPK increased OC by 7.02% in agricultural soil. The Nitrogen content of pre sowing sample of Plot T_1 and T_2 were decreased by 3.71% and 10.53% respectively in post-harvest soil while the Nitrogen content was increased by 4.88% in post-harvest soil of T_3 Plot. Farmyard manure is known to stimulate the biological nitrogen fixation in soil, which may also have been responsible for the increase in soil nitrogen in T_3 Plot. Percentage of phosphorus was decreased by 8.82% in T_2 plot while it was increased by 5.88% and 7.69% from T_1 and T_3 plot respectively in post- harvest

soils. It may be due to release of organically bound phosphorus produced during decomposition of organic matter. A positive effect of combined application of FYM and inorganic fertilizers on Phosphorus availability in the agricultural soil of Punjab was also reported by Roy *et al*. [23]. The percentage of Potassium was also decreased by 9.30% and 11.11% from plot T_1 and T_2 respectively. There was increase of 8.14% in K content in post-harvest soil of T_3 plot.

Table 2: Physico-chemical parameters at different growth stages of wheat at T_2 plot during 2015-17.

Physico-chemical Parameters	Before Sowing	Initial Stage	Crop Development	Mid-Season	Ripening Stage	After Harvesting
Temperature (°C)	21.48±0.12	20.9±0.07	17.52±0.12	19.25±0.06	22.33±0.08	24.87±0.06
Moisture Content (%)	11.33±0.07	12.6±0.10	13.7±0.04	13.2±0.11	12.5±0.11	11.0±0.09
pH	6.12±0.02	6.25±0.04	6.52±0.03	6.35±0.05	6.12±0.03	5.93±0.04
Organic Carbon (%)	1.27±0.03	1.31±0.03	1.33±0.03	1.28±0.03	1.19±0.03	1.16±0.03
Organic Matter (%)	2.19±0.06	2.25±0.05	2.29±0.05	2.21±0.05	2.06±0.05	2.00±0.13
Nitrogen (%)	0.38±0.02	0.41±0.02	0.48±0.01	0.45±0.02	0.39±0.01	0.34±0.01
Phosphorus (%)	0.034±0.001	0.037±0.001	0.041±0.001	0.039±0.001	0.035±0.001	0.031±0.001
Potassium (%)	0.144±0.004	0.152±0.004	0.170±0.006	0.158±0.008	0.142±0.006	0.128±0.005

(All values are Mean±S.E. of 6 observations each)

Table 3: Physico-chemical parameters at different growth stages of wheat T_3 plot during 2015-17.

Physico-chemical Parameters	Before Sowing	Initial Stage	Crop Development	Mid-Season	Ripening Stage	After Harvesting
Temperature (°C)	20.95±0.07	19.73±0.05	16.9±0.04	18.8±0.08	21.88±0.03	23.83±0.03
Moisture Content (%)	11.47±0.07	12.77±0.11	14.47±0.10	14.20±0.12	13.40±0.10	11.97±0.08
pH	6.42±0.02	6.57±0.04	6.62±0.03	6.75±0.04	6.47±0.02	6.35±0.03
Organic Carbon (%)	1.71±0.06	1.74±0.05	1.83±0.06	1.92±0.06	1.86±0.05	1.83±0.05
Organic Matter (%)	2.95±0.09	3.00±0.09	3.15±0.10	3.31±0.11	3.21±0.09	3.16±0.09
Nitrogen (%)	0.41±0.01	0.42±0.01	0.47±0.02	0.49±0.02	0.45±0.01	0.43±0.01
Phosphorus (%)	0.039±0.002	0.041±0.002	0.044±0.002	0.046±0.002	0.043±0.001	0.042±0.002
Potassium (%)	0.172±0.008	0.178±0.005	0.194±0.006	0.204±0.075	0.190±0.006	0.186±0.006

(All values are Mean±S.E. of 6 observations each)

3.2. Mycofloral diversity in different experimental farmer plots and its correlation with physico-chemical parameters

The total number of 21 species of 14 genera were isolated from different experimental plots of wheat fields of Missarpur Village of Haridwar District, Uttarakhand. (Table 4). From T_1 Plot, a total number of 9 species were identified during 2015-17. The relative occurrence of the identified species were *Aspergillus flavus* (8.81%), *Aspergillus fumigatus* (15.83%), *Aspergillusniger* (19.64%), *Bipolaris australiensis* (4.41%), *Cladosporium sp.* (7.54%), *Fusariumoxysporum* (3.02%), *Penicillium chrysogenum* (16.52%), *Rhizopus oryzae* (6.39 %), *Trichoderma hamatum (*14.52%) and some species were remained unidentified. (3.17 %). The species which showed its dominancy in Control Plot was *Aspergillus niger* followed by *Penicillium chrysogenum*. (Table 6) In T_1 plot, *Aspergillus flavus* showed a negative correlation with Temperature while it was positively correlated with Moisture content. *Aspergillus niger* showed a positive correlation with Temperature, Nitrogen and Potassium but with pH, it was found to be highly negatively correlated. *Cladosporium sp.* was negatively correlated with pH. *Penicillium chrysogenum* showed positive correlation with Temperature and Potassium but negatively correlated with Moisture content. *Trichoderma hamatum* was highly significantly correlated with Organic Carbon. (Table 9).

Table 4: Composition of Fungal species in different ferti-irrigated plots of Wheat field during 2015-17.

Sr. No.	Mycofloral species	Plot 1		Plot 2		Plot 3	
		2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
1.	*Alternaria alternate*	×	×	×	√	×	×
2.	*Aspergillus flavus*	√	√	√	√	√	×
3.	*Aspergilllus fumigates*	√	√	√	×	√	√
4.	*Aspergillus niger*	√	√	√	√	√	√
5.	*Bipolaris australiensis*	×	√	×	×	×	√
6.	*Cladosporium sp.*	×	√	√	√	√	√
7.	*Fusarium graminearum*	×	×	√	√	×	×
8.	*Fusarium oxysporum*	√	×	√	√	×	×
9.	*Monilia sp.*	×	×	√	×	×	×
10.	*Mortierella zychae*	×	×	×	×	×	√
11.	*Mucor circenelloides*	×	×	×	×	×	√
12.	*Mucor hiemalis*	×	×	×	×	√	√
13.	*Penicillium chrysogenum*	√	√	√	×	√	√
14.	*Penicillium citrinum*	×	×	×	×	√	√
15.	*Penicillium lanosum*	×	×	×	√	√	√
16.	*Rhizoctonia solani*	×	×	√	√	×	×
17.	*Rhizopus oryzae*	×	√	×	×	√	√
18.	*Scopulariopsis asperula*	×	×	×	×	×	√
19.	*Trichoderma hamatum*	√	√	×	×	√	√
20.	*Trichoderma harzianum*	×	×	×	×	√	√
21.	*Verticillium sp.*	×	×	√	√	√	√
	Total	**06**	**08**	**10**	**09**	**12**	**15**
		09		**12**		**16**	

Table 5: Values of Colony forming units of Soil Mycoflora in (10^{-3}) dilution under different ferti-irrigated wheat field at different growth stages during 2015-17.

Plot	Before sowing	Initial stage	Crop development	Mid-season	Ripening stage	After harvesting
Plot T_1	1.99±0.07	2.53±0.08	2.90±0.09	3.44±0.09	3.08±0.08	2.35±0.07
Plot T_2	4.49±0.07	5.43±0.10	6.36±0.14	5.05±0.08	4.12±0.09	3.74±0.09
Plot T_3	5.37±0.09	5.92±0.12	6.66±0.17	6.85±0.18	6.11±0.14	5.74±0.11

(All values are Mean±S.E. of 6 observations each)

Plot 1(T_1) = Control; Plot 2 (T_2) = Chemically (NPK) irrigated Plot; Plot 3(T_3) = combined (NPK+FYM) irrigated Plot).

During 2015-17, the total of 12 fungal species were identified from the chemically irrigated plot where NPK were applied alone to the Wheat field. The identified fungal species from different growth stages of Wheat were *Alternaria alternate* (8.17%), *Aspergillus flavus* (5.56%), *Aspergillus fumigatus* (10.43%), *Aspergillus niger* (5.21%), *Cladosporium sp*. (6.70%), *Fusarium graminearum* (16.38%), *Fusarium oxysporum* (5..95%), *Monilia sp.* (6.66%), *Penicillium chrysogenum* (4.82%), *Penicillium lanosum* (6.69%), *Rhizoctonia solani* (11.83%), *Verticillium sp.* (9.68%) and some fungal colonies were unidentified (1.86%). *Fusarium graminearum* followed by *Rhizoctonia solani* were most dominant species in chemically irrigated plot. (Table-7). *Fusarium graminearum* is considered as pathogenic fungi for wheat pant.

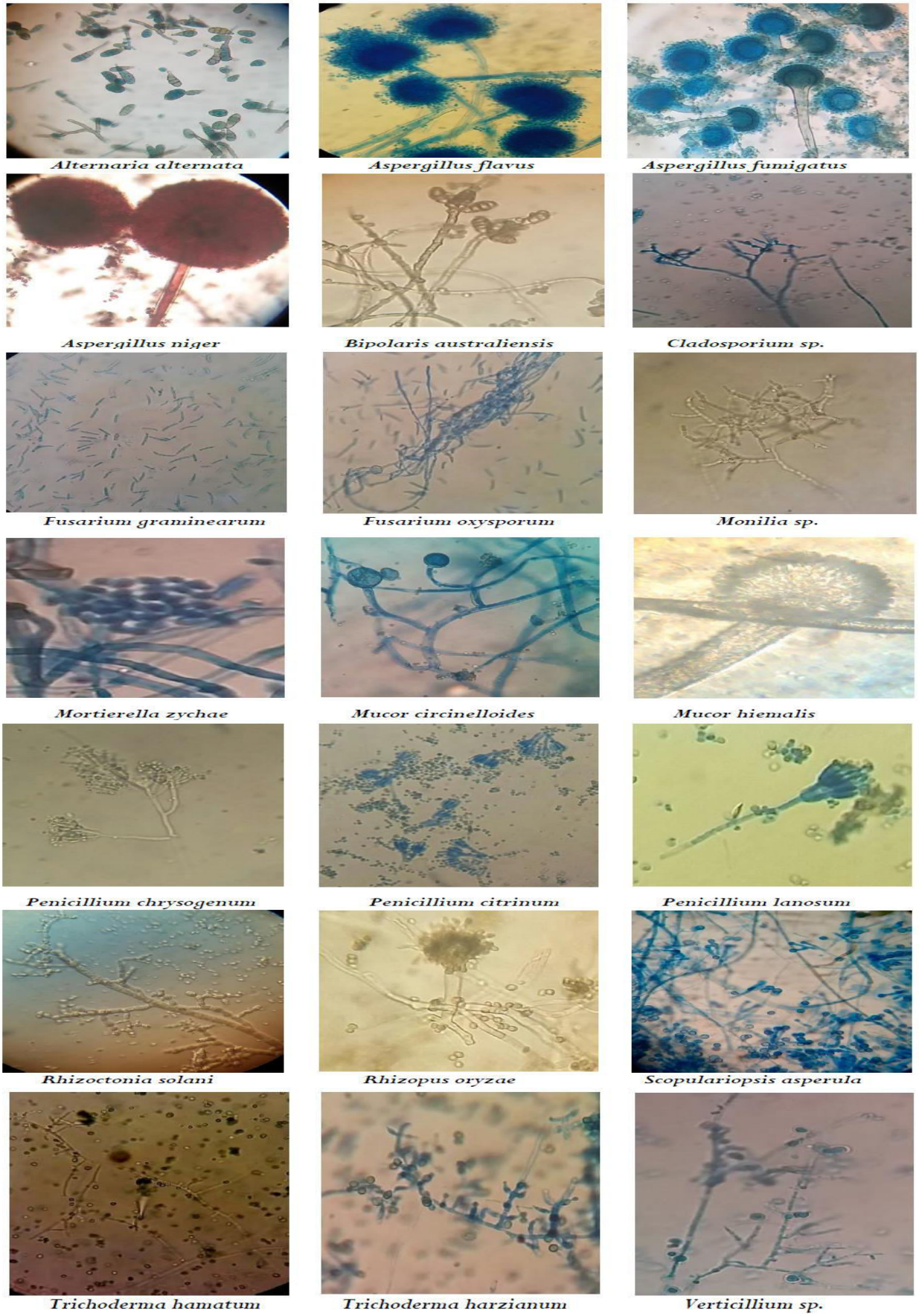

Fig. 3: Microscopic images of total fungal species identified from different Agricultural Plots of Wheat.

Table 6: Relative occurrence of the mycofloral species in T_1 plot at different growth stages of wheat during 2015-2017.

Species	Before sowing	Initial stage	Crop development	Mid-season	Ripening stage	After harvesting	Total Occurrence
Aspergillus flavus	-	17.27±0.61	22.22± 0.67	8.71±0.39	3.26 ±0.16	-	8.81 ±0.31
Aspergillus fumigatus	32.22 ±0.91	-	7.30 ±0.24	11.69±0.43	23.39±0.49	29.83±0.58	15.83±0.44
Aspergillus niger	21.86±0.43	12.91±0.34	11.01±0.21	23.39±0.29	22.64±0.27	25.22±0.33	**19.64±0.48**
Bipolaris australiensis	15.56±0.37	-	-	2.98±0.06	6.26±0.07	4.07±0.04	4.41±0.21
Cladosporium sp.	-	-	3.71±0.08	11.48±0.16	16.14±0.39	7.94±0.12	7.54±0.28
F. oxysporum	10.02± 0.18	4.33 ± 0.06	3.68±0.04	2.93±0.02	-	-	3.02±0.12
Penicillium chrysogenum	-	35.00±0.99	29.80±0.82	17.80±0.62	13.06±0.21	-	**16.52±0.39**
Rhizopus oryzae	-	-	-	15.08±0.31	6.41±0.07	12.29±0.31	6.39±0.11
Trichoderma hamatum	14.87 ±0.23	26.09 ±0.81	18.53±0.63	5.89±0.11	9.59±0.12	16.04±0.32	14.52±0.39
Unidentified sp.	5.40±0.07	4.36±0.05	3.71 ± 0.03	-	3.26±0.04	4.07±0.06	3.17±0.03

(All values are Mean±S.E. of 6 observations each)

Table 7: Relative occurrence of Mycofloral species in T_2 plot at different growth stages of wheat during 2015-17.

Species	Before sowing	Initial stage	Crop development	Mid-season	Ripening stage	After harvesting	Total occurrence
Alternaria alternata	-	-	11.39±0.50	10.18±0.23	12.12±0.52	16.20±0.33	8.17 ±0.19
Aspergillus flavus	-	4.14±0.47	9.41±0.46	12.25±0.39	4.93±0.39	-	5.56±0.18
Aspergillus fumigatus	16.73±0.53	10.68±0.33	7.59±0.37	8.14±0.44	7.37±0.40	14.07± 0.73	10.43±0.28
Aspergillus niger	7.11±0.52	6.25±0.53	7.54±0.56	6.14±0.29	2.44±0.02	-	5.21±0.31
Cladosporium sp.	14.35±0.54	14.94±0.33	5.77±0.43	-	4.87±0.24	-	6.70±0.04
Fusarium graminearum	9.50±0.52	12.84±0.35	17.00±0.27	19.15±0.41	19.56±0.54	21.80±0.55	**16.38±0.29**
Fusarium oxysporum	-	2.14±0.08	3.75±0.26	4.03±0.33	17.06±0.43	10.74±0.36	5.95±0.43
Monilia sp.	-	-	7.46±0.78	14.29±0.62	9.69±0.29	7.90±0.09	6.66±0.39
Penicillium chrysogenum	11.52±0.84	8.46±0.57	3.70±0.39	-	4.86±0.31	-	4.82±0.38
Penicillium lanosum	19.05±0.96	10.67±0.77	3.79±0.41	6.09±0.19	-	-	6.69±0.22
Rhizoctonia solani.	-	10.56±0.90	11.14±0.85	16.33±0.78	14.52±0.42	18.57±0.89	**11.83±0.39**
Verticillium sp.	19.03±0.60	17.70±0.82	9.47±0.63	4.06±0.12	-	8.15±0.21	9.68±0.38
Unidentified sp.	2.39±0.06	2.14±0.08	1.89±0.05	-	2.44±0.04	2.73±0.13	1.86±0.03

(All values are Mean±S.E. of 6 observations each)

Hoorman [24] reported that root-pathogenic fungi, such as *Verticillium, Rhizoctonia, Fusarium graminearum* cause major economic loss in agriculture each year. In T_2 plot, *Alternaria alternate* showed positive correlation with Nitrogen. *Aspergillus fumigatus* showed highly significant positive correlation with Organic Carbon, N, P and K. *Cladosporium sp.* was negatively correlated with Temperature but showed a positive correlation with N

and K. *Fusarium oxysporum* and *Verticillium sp.* were positively correlated with Phosphorus. *Monilia sp.* showed positive correlation with N. *Penicillium chrysogenum* showed negative correlation with Temperature, O.C., N and K but was positively correlated with pH and Phosphorus. *Penicillium lanosum* was positively correlated with Phosphorus and negatively correlated with OC and K. *Rhizoctoniasolani* showed positive correlation with temperature, Moisture Content, N and K. (Table-10).

Table 8: Relative occurrence of mycofloral species in T_3 plot at different growth stages of wheat during 2015-17.

Species	Before sowing	Initial stage	Crop development	Mid-season	Ripening stage	After harvesting	Total Occurrence
Aspergillus flavus	10.56 ± 1.01	13.64±0.22	15.05±0.82	8.76±0.36	4.88±0.17	-	9.02±0.27
Aspergilllus fumigatus	3.70±0.47	3.27±0.31	5.95±0.60	7.33±0.30	14.83±0.89	12.53 ±0.80	7.93 ±0.38
Aspergillus niger	14.77±0.89	15.32±0.19	11.90±0.41	11.58±0.42	11.57±0.82	14.41±0.91	**13.12±0.48**
Bipolaris australiensis	-	-	2.98±0.13	4.32±0.17	6.50±0.43	7.22±0.43	3.54±0.13
Cladosporium sp.	-	6.78±0.48	4.39±0.47	2.88±0.15	-	5.36±0.14	3.26±0.11
Mortierella zychae	3.76±0.74	1.70±0.05	1.50±0.04	4.35±0.19	-	-	2.35±0.13
Mucor circenelloides	-	-	3.00±0.18	5.78±0.32	6.52±0.48	9.06±0.47	4.10±0.26
Mucor hiemalis	9.24±0.23	10.12±0.32	7.41±0.26	-	3.21±0.15	8.83±0.37	6.26±0.37
Penicillium chrysogenum	5.59±0.51	1.70±0.05	4.44±0.47	5.76±0.79	8.00±0.87	-	4.36±0.33
Penicillium citrinum	7.48±0.86	8.50±0.39	10.45±0.80	2.89±0.20	4.92±0.31	7.03±0.26	6.85±0.33
Penicillium lanosum	5.49±0.67	5.00±0.15	-	-	3.26±0.14	3.76±0.14	2.73±0.22
Rhizopus oryzae	7.34±0.54	3.52±0.01	5.94±0.31	10.12±0.76	8.23±0.80	-	6.01±0.26
Scopulariopsis asperula	-	-	2.96±0.16	2.93±0.22	-	3.58±0.11	1.65±0.37
Trichoderma hamatum	12.93±0.98	11.81±0.79	8.98±0.56	13.05±0.75	9.88±0.66	10.68±0.79	11.21±0.49
Trichoderma harzianum	16.66±0.53	16.95±0.81	13.53±0.85	14.50±0.82	13.18±0.86	16.12±0.89	**15.04±0.57**
Verticillium sp.	-	-	-	4.30±0.21	3.27±0.18	-	1.36±0.27
Unidentified sp.	1.86±0.04	1.70±0.05	1.49±0.04	1.46±0.02	1.65±0.05	1.80± 0.08	1.64±0.02

(All values are Mean±S.E. of 6 observations each)

Table 9: Correlation analysis between fungal species and physico-chemical parameters of T_1 Plot of Wheat field

Genera/Species	Parameters						
	Temperature	Moisture Content	pH	Organic Carbon	Nitrogen	Phosphorus	Potassium
Aspergillus flavus	**-0.88****	**0.85***	0.58	0.67	-0.68	0.68	-0.73*
Aspergillus fumigatus	-0.47	0.24	0.38	0.04	0.12	0.11	0.03
Aspergillus niger	**0.89****	-0.13	**-0.89****	-0.71*	**0.84***	-0.73*	**0.82***
Bipolaris australiensis	-0.07	0.25	0.50	0.68	-0.61	0.30	-0.68
Cladosporium sp.	-0.15	0.39	**-0.84***	-0.15	0.08	0.26	-0.02
F. oxysporum	0.32	-0.35	0.03	0.29	-0.44	0.12	-0.28
P. chrysogenum	**0.82***	**-0.84***	-0.70*	-0.78*	0.65	0.91**	**0.83***
Rhizopus oryzae	0.47	**-0.80***	0.03	-0.06	-0.07	-0.28	0.20
Trichoderma hamatum	-0.41	0.08	0.58	**0.88****	-0.69	0.81*	-0.68

$P \leq 0.05$; $P \leq 0.01$***

Table 10: Correlation analysis between fungal species and physico-chemical parameters of T_2 Plot of Wheat field.

Genera/Species	Parameters						
	Temperature	Moisture Content	pH	Organic Carbon	Nitrogen	Phosphorus	Potassium
Alternaria alternate	-0.17	0.05	-0.16	0.27	**0.86****	-0.33	-0.26
Aspergillus flavus	-0.58	0.25	0.37	-0.63	-0.67	-0.48	-0.67
Aspergillus fumigatus	0.77*	0.00	-0.65	**0.98****	**0.95****	**1.00****	**0.97****
Aspergillus niger	-0.15	-0.14	0.47	-0.10	-0.01	-0.07	-0.09
Cladosporium sp.	**-0.87****	**0.86****	0.35	-0.12	-0.22	-0.14	-0.13
Fusarium graminearum	0.23	-0.32	**-0.94****	0.27	**0.92****	0.69	**0.88****
Fusarium oxysporum	0.06	-0.20	-0.15	0.05	-0.02	**0.81***	0.08
Monilia sp.	-0.27	0.62	0.20	-0.62	**0.81****	-0.61	-0.61
Penicillium chrysogenum	**-0.87****	-0.07	**0.80***	**-0.92****	**-0.91****	**0.84****	**-0.94****
Penicillium lanosum	-0.65	-0.11	0.59	**-0.84****	-0.75	**0.85****	**-0.84****
Rhizoctonia solani.	**0.82****	**0.87****	-0.73*	0.43	**0.90****	0.35	**0.86****
Verticillium sp.	0.11	-0.23	0.06	0.51	0.44	**0.84****	0.47

P ≤ 0.05; P ≤ 0.01***

Table 11: Correlation analysis between fungal species and physico-chemical parameters of T_3 Plot of Wheat field.

Genera/Species	Parameters						
	Temperature	Moisture Content	pH	Organic Carbon	Nitrogen	Phosphorus	Potassium
Aspergillus flavus	-0.01	0.54	-0.16	**0.84***	0.28	**0.92****	**0.88****
Aspergilllus fumigatus	-0.11	0.70*	0.64	0.37	**-0.92****	0.42	0.44
Aspergillus niger	**0.93****	0.25	-0.02	**0.91****	**0.84***	**0.81***	**0.84***
Bipolaris australiensis	0.17	-0.14	0.19	0.09	0.02	0.11	0.07
Cladosporium sp.	0.30	0.09	0.17	**0.85****	**0.84***	0.55	**0.84***
Mortierella zychae	0.27	**0.84***	0.45	0.30	0.24	0.26	0.28
Mucor circenelloides	**-0.88****	**0.85****	-0.42	-0.51	-0.47	-0.45	-0.58
Mucor hiemalis	0.21	0.09	**0.84***	-0.38	-0.45	-0.39	-0.40
Penicillium chrysogenum	**-0.87****	-0.62	-0.40	0.33	0.79	0.34	**0.98****
Penicillium citrinum	0.65	0.20	0.32	-0.09	-0.07	-0.12	0.03
Penicillium lanosum	**-0.86****	-0.19	-0.70*	0.33	**-0.98****	0.35	0.21
Rhizopus oryzae	-0.03	**0.84***	0.62	0.23	0.25	0.26	0.27
Scopulariopsis asperula	0.04	0.35	0.59	-0.12	-0.04	-0.07	0.00
Trichoderma hamatum	-0.33	-0.61	**-0.89****	**0.81***	**0.91****	**0.84***	-0.80*
Trichoderma harzianum	0.51	-0.32	-0.45	**0.92****	-0.15	**0.80***	0.11
Verticillium sp.	-0.48	0.34	0.06	0.73*	**-0.85****	0.78*	0.68

P ≤ 0.05; P ≤ 0.01***

The highest fungal diversity with 16 species was identified from the agricultural field of wheat where combined application of Farmyard manure and NPK were given by the farmers during 2015-17. The isolated mycofloral species from this plot were *Aspergillus flavus* (9.02%), *Aspergillus fumigatus* (7.93%), *Aspergillus niger* (13.12%), *Bipolaris australiensis* (3.54%), *Cladosporium sp.* (3.26%), *Mortierella zychae* (2.35%), *Mucor circenelloides* (4.10%), *Mucor hiemalis* (6.26%), *Penicillium chrysogenum* (4.36%), *Penicillium citrinum* (6.85%), *Penicillium lanosum*

(2.73%), *Rhizopus oryzae* (6.01%), *Scopulariopsis asperula* (1.65%), *Trichoderma hamatum* (11.21%), *Trichoderma harzianum* (15.04%), *Verticillium sp.* (1.36%) and some species were unidentified. (1.64%). (Table-8) In T_3 plot, *Aspergillusflavus* showed positive correlation with organic carbon, P and K. *Aspergillus niger* was highly positively correlated with temperature and O.C. and positively correlated with N, P and K. *Cladosporium sp.* showed positive correlation with O.C., N and K. *Mortierella zychae* was positively correlated with moisture Content. *Mucor circenelloides* showed negative correlation with temperature but it was positively correlated with moisture Content. *Mucorhiemalis* showed positive correlation with pH. *Penicillium chrysogenum* showed negative correlation with temperature but was positively correlated with Potassium. *Penicilliumlanosum* was negatively correlated with temperature and it showed significant negative correlation with N. *Trichoderma hamatum* showed negative correlation with pH and was positively correlated with O.C., N and P. *Trichoderma harzianum* showed positive correlation with O.C. and Phosphorus. *Verticillium sp.* was negatively correlated with N. (Table-11) *Trichoderma harzianum* showed its dominancy in the soil where combined application of Farmyard manure and NPK were applied. *Trichoderma sp.* have been known as biocontrol agents for the control of plant diseases for decades. A group of researcher reported that that this species has the ability to solubilize Phosphorus and micronutrients that could be made available to plants [25]. Elicitors released by *Trichoderma sp.* are involved in triggering expressions of defense proteins within the plant to induce plant immunity against pathogen and in turn improve plant growth. The graph (Fig. 4) represented the colony forming units of mycofloral species at different growth stages of wheat in differently irrigated Plots which gave a clear idea that highest CFU of fungal colonies were observed from the soil where combined application of FYM and NPK were applied along with irrigation facility which proved that growth of mycoflora has strong relationship with organic materials as the organic materials added to agricultural soil act as carbon source for microbes. Same pattern of result was also discussed by Gachande and Shaikh [26] while observing the correlation of soil mycoflora and productivity in organic and inorganic farming of *Triticum aestivum*. Shiny *et al.* [27] reported that the organic carbon, N, P and K are important for the growth of fungi. In the absence of any of these nutrients, the growth and sporulation of moulds hampered a lot. This study proved that the combined application of organic and inorganic fertilizer in soil have great capacity to give a good atmosphere for improving soil fertility and the growth of mycoflora when compared with the soil where Inorganic fertilizer were applied alone to the Agricultural field of Wheat.

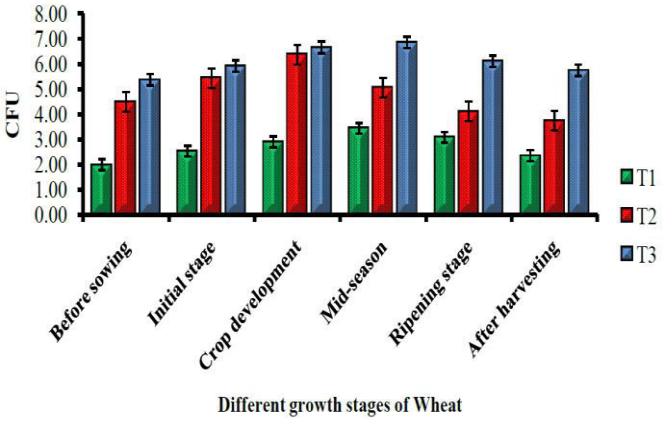

Fig.4: Graphical representation of Colony forming Unit (g^{-1}) of Mycoflora in differently treated Plots of Wheat.

3.3. Mycofloral Diversity Index

Table 12-14 showed the general Shannon Wiener diversity index for total fungal communities present in differently irrigated agricultural plots of wheat which clearly indicated that the highest fungal diversity with value 2.61 was observed in the plot where combination of organic and inorganic fertilizers were applied in judicious manner.

Table 12: Shannon Wiener Index for Mycoflora Diversity in T_1 Plot.

Species	Total no. of colonies	Pi	In(Pi)	(Pi)*pi	H
Aspergillus flavus	14	0.09	-2.42	-0.21	
Aspergillus fumigatus	25	0.16	-1.84	-0.29	
Aspergillus niger	31	0.20	-1.63	-0.32	
Bipolaris australiensis	7	0.04	-3.12	-0.14	
Cladosporium sp.	12	0.08	-2.58	-0.20	
F. oxysporum	5	0.03	-3.45	-0.11	2.13
Penicillium chrysogenum	26	0.16	-1.80	-0.30	
Rhizopus oryzae	10	0.06	-2.76	-0.17	
Trichoderma hamaratum	23	0.15	-1.93	-0.28	
Unidentified sp.	5	0.03	-3.45	-0.11	

Table 13: Shannon Wiener Index for Mycoflora Diversity in T_2 Plot.

Species	Total no. of colonies	Pi	In(Pi)	(Pi)*pi	H
Alternaria alternate	22	0.08	-2.50	-0.20	
Aspergillus flavus	15	0.06	-2.89	-0.16	
Aspergillus fumigatus	28	0.15	-2.26	-0.24	
Aspergillus niger	14	0.05	-2.96	-0.15	
Cladosporium sp.	18	0.07	-2.70	-0.18	
Fusarium graminearum	44	0.16	-1.81	-0.30	
Fusarium oxysporum	16	0.06	-2.82	-0.17	**2.46**
Monilia sp.	18	0.07	-2.70	-0.18	
Penicillium chrysogenum	13	0.05	-3.03	-0.15	
Penicillium lanosum	18	0.07	-2.70	-0.18	
Rhizoctonia solani.	32	0.12	-2.13	-0.25	
Verticillium sp.	26	0.10	-2.34	-0.23	
Unidentified sp.	5	0.02	-3.99	-0.07	

Table 14: Shannon Wiener Index forMycoflora Diversity in T_3 Plot.

Species	Total no. of colonies	Pi	In(Pi)	(Pi)*pi	H
Aspergillus flavus	33	0.09	-2.41	-0.22	
Aspergilllus fumigates	29	0.08	-2.54	-0.20	
Aspergillus niger	48	0.13	-2.03	-0.27	
Bipolaris australiensis	13	0.04	-3.34	-0.12	
Cladosporium sp.	12	0.03	-3.42	-0.11	
Mortierella zychae	7	0.02	-3.96	-0.08	
Mucor circenelloides	15	0.04	-3.19	-0.13	
Mucor hiemalis	23	0.06	-2.77	-0.17	
Penicillium chrysogenum	16	0.04	-3.13	-0.14	**2.61**
Penicillium citrinum	25	0.07	-2.68	-0.18	
Penicillium lanosum	10	0.03	-3.60	-0.10	
Rhizopus oryzae	22	0.06	-2.81	-0.17	
Scopulariopsis asperula	6	0.02	-4.11	-0.07	
Trichoderma hamatum	41	0.11	-2.19	-0.25	
Trichoderma harzianum	55	0.15	-1.90	-0.28	
Verticillium sp.	5	0.01	-4.29	-0.06	
Unidentified sp.	6	0.02	-4.11	-0.07	

Table 15: Comparison between crop productions in all the treated agricultural crops of wheat in Haridwar district (Average value of two years 2015-17).

Treated Plot	Yield (Q/ha)
T_1(Control)	11.1
T_2 (NPK)	20.3
T_3 (FYM + NPK)	23.6

4. CONCLUSION

Soil mycoflora not only plays an important role in decomposition of organic matter and maintaining soil fertility but are also responsible for the prevalence of diseases in the crop fields. Overall results revealed that there is variation in soil mycofloral diversity at each growth stages of wheat plant of all the irrigated plots. The combined use of Farmyard manure with limited amount of NPK enhanced the growth of beneficial fungi like *Trichodermaharzianum*, *Trichoderma hamatum, Aspergillus niger etc* asthese species showed significant positive correlation with soil physico- chemical parameters like Temperature, OC, N, P and K in T_3 plot. The highest crop yield of wheat were recovered from this farmer plot. Hence, it is concluded that soil mycoflora has direct effect on enhancing the crop productivity. There was decrease in soil mycofloral diversity when NPK was applied alone to the agricultural field of wheat and improves the growth of soil borne pathogenic fungi like *Fusarium graminearum, Rhizoctonia solani, Monilia sp., Verticillium sp.,* etc.Soil mycoflora transform mineral nutrients like Nitrogen through symbiotic and non-symbiotic fixation processes. Chemical fertilizers tend to have long persistence in agricultural soil so they are bound to effect the growth of beneficial soil mycoflora and disturb the soil fertility. The present study should enhance the sufficient knowledge to the farmers for the management of soil fertility and soil microbial diversity and the development of sustainable agricultural system.

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6. REFERENCES

1. Charan S. *International Journal of Forestry and Crop Improvement*, 2016; **7(2)**: 177-183.
2. Schiavon M, Perrin-Ganier C, Portal JM. *Agronomie*, 1996; **15(3-4)**: 157-170.
3. Das BB, Dkhar MS. *NeBIO*, 2010; **1**: 1-7.
4. Das BB, Rajak N, Nakhro N, Dkhar M S. *Agricultural Journal*, 2010; **5(3)**: 181-185

5. Zhang WJ, Wang XJ, Xu MG, Huang SM, Liu H, Peng C. *Biogeosciences* Discussions, 2009; **6**:6539-6577.
6. Harris J. *Science*, 2009; **325**:573-574.
7. Liu SL, Huang DY, Chen AL, Wei WX, Brookes PC, Li Y, Wu JS. *Agriculture*, *Ecosystems and Environment*, 2014; **184**:51-58.
8. Wang JZ, Lu CA, Zhang WJ, Feng G, Wang XJ, Xu MG. *Acta Pedologica Sinica*, 2013; **53**:16-27.
9. Zhu P, Ren J, Wang L, Zhang X, Yang X, Mac Travis D. *Journal of Plant Nutrition and Soil Science*, 2007; **170**:219-223.
10. Manna MC, Swarup A, Wanjari RH, Mishra B, Shahi DK. *Soil & Tillage Research*, 2007; **94**:397-409.
11. Seufert V, Ramankutty N, Foley JA. *Nature*, 2012; **485**:229-232.
12. Wei W, Yan Y, Cao J, Christie P, Zhang F, Fan M. *Agriculture*, *Ecosystems and Environment*, 2016; **225**:86-92.
13. Kong XB, Lal R, Li BG, Li KJ. *Agricultural Research*, 2014; **3**:246-256.
14. Guleri S, Saxena S, Sharma P, Malik N, Thapliyal M. *International Journal of Pure and Applied Bioscience*, 2016;**4(1)**:253-264.
15. Gaddeyya G, Niharika PS, Bharathi P, Kumar PR. *Advances in Applied Science Research*, 2012; **3(4)**:2020-2026.
16. Deshmukh P, Shinde S. *International journal of Science and Research*, 2015; **4(2)**:529-533.
17. Walkley A, Black IA. *SoilScience*, 1934; **37**:29-38.
18. Jackson ML. *Soil Chemical Analysis*. Prentis –Hall Inc. Englewood Cliffs, New Jersey, 1958.
19. Trivedy RK, Goel PK. *Environmental Publication*, 1998; Karad, India.
20. Aneja KR. Experiments in microbiology, plant pathology tissue culture and mushroom cultivation (2nd Edition) Wishwa Prakashan (New Age International Private Limited), 2003: ISBN: 81-7328-082-7.
21. Watanabe T. Second Edition, *CRC Press*, 2002: New York.
22. Nagar KC, Meena H, Swaroop N. An Asian Journal of Soil Science, 2005; **10(2)**:222-227.
23. Roy SK, Sharma RC, Trehan SP. J. Agric. Sci., 2001; **137:**271-278.
24. Hoorman JJ.*Agriculture and Natural Resources*, 2008; **4(5)**:1-6.
25. Mastouri F, Bjorkman T, Harman GE. *The American Phytopathological Society*, 2010; **100(11)**:1210-1213.
26. Gachande BD, Shaikh NF. *International Journal of Botany Studies*, 2017; **2(5)**:127-130.
27. Shiny NP, Gaddeya G, Ratna KPK. *International Research Journal of Environmental Sciences*, 2013; **2(9)**:38-44.