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Microbial, Biochemical, Anatomical and Histochemical Analysis of Black Pepper and Sorghum Inoculated with Mycorrhiza

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Summary

Microbial, biochemical and histochemical analysis of Black pepper (*Piper nigrum* L.) and sorghum (*Sorghum bicolor* L. Moench) inoculated with mycorrhiza from the organic soil, inorganic soil, natural soil, control and *Glomus fasciculatum* was studied. Microbial population and activity of different enzymes in soils of pepper and sorghum inoculated with mycorrhiza and control plants were studied. The anatomical studies showed that there were striking differences in plant leaf structures. Sizes of upper epidermis, lower epidermis, xylem cells and spongy layer increased due to inoculation with mycorrhiza from the organic soil, inorganic soil, natural soil, control and *Glomus fasciculatum*. Histochemical analysis of these crop plants were carried out to study the difference in the accumulation of the polysaccharides, proteins and nucleic acids in leaf tissues of control and mycorrhiza inoculated plants. There was increased accumulation of protein, polysaccharide and the nucleic acids in the leaf samples indicating the direct correlation between the Arbuscular Mycorrhizal (AM) fungi and the crop response to inoculation of the AM fungi. The growth parameters studied were stem girth, rooting percentage and sprouting percentage. Mycorrhizal association with pepper and sorghum was also studied.

Key Words: Anatomical analysis, Histochemical analysis, Black pepper, Sorghum, Microbial population and rooting percentage

Introduction

Arbuscular Mycorrhizal Fungi (AMF) is an important component of the terrestrial communities. Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil bound fungi with the roots of higher plants [1]. It has been more than 100 years since Isobel Gallaud published his dissertation that contained detailed descriptions and illustrations of different structures of what are now called arbuscular mycorrhizas [2]. Mycorrhiza offers several benefits to the host plants including faster growth, improved nutrition, greater drought tolerance, protection from soil borne pathogens, greater resistance to invasion by weeds. The main areas in which the benefits of introducing Arbuscular Mycorrhizal Fungi (AMF) into a plant growth system will accrue are those in which they are lacking indigenous inoculum of AMF. These include sterilized soils or post in vitro plant micro-propagation, buried, extremely fertilized, degraded areas [3] or rooting of pepper cuttings [4]. AM fungi are known to increase rooting due to the production of growth hormones such as auxins, gibberellin like substances and cytokinins.

Black pepper (*Piper nigrum* L) belongs to family Piperaceae and is popularly known as "king of spices".It is an ancient and important spice crop of India. Besides its use as a spice in the preparation of savory dishes, pickles and ketchup, its medicinal value as a carminative is also recognized. Where as, Sorghum (*Sorghum bicolor* (L.) Moench) is originally from tropical Africa. It is a short day C_4 plant and has a greater adoptability to a wide range of geographical conditions. It has also been widely used for production of forage and silage for animal feed. Sorghum is used as food in the form of grain, fuel in the form of ethanol from stem juice and fodder from its leaves and bagasse. Sorghum has also been called "a camel among the crops" because of its wide adaptability, its marked resistance to drought and saline-alkaline soils. Sorghum is being cultivated over 43.75 m ha in the world producing 54.15 million tonnes of grain with a productivity of 1238 kg per ha. Several genotypes with the aim of obtaining higher grain yield have been developed in recent years.

Black pepper and sorghum flourishes well in additional inoculation with Arbuscular Mycorrhizal Fungi. Hence in the present study an investigation on microbial profile, biochemical, anatomical and histochemical analysis of black pepper (*Piper nigrum* L.) and sorghum (*Sorghum bicolor* L. Moench) inoculated with mycorrhiza from the organic soil, inorganic soil, natural soil, control and *Glomus fasciculatum* was carried out.

Materials and Methods

The experiments to study the Microbial, Biochemical, Anatomical and Histochemical analysis of Black pepper and Sorghum inoculated with mycorrhiza from different crop management systems was conducted at the Department of Biotechnology, University of Agricultural Sciences, GKVK Campus, Bangalore.

Pepper cuttings (Panniyur-4) and Sorghum seeds (IS-3443) were used for the study. Pepper cuttings were raised in plastic containers (5 liter capacity) with potting substrate. The potting substrate is a mixture of 1:2: 2 V/V of autoclaved Organic matter rich soil: Inert substrate (red soil): Sand. There

were two experiments performed in the green house which are as follows:

1) Pepper cuttings inoculated with the mycorrhiza from crop management systems (i.e., AMF+ Pepper system).

2) Sorghum inoculated with the mycorrhiza from crop management systems (i.e., AMF+ Sorghum system).

Collection of soil samples: Soil samples were collected from different pepper fields on a) organic soil b) inorganic soil c) from undisturbed fields with natural vegetation d) from uncultivated fallow (UAS,GKVK campus) soil.

Spore isolation: The spores were isolated by wet sieving and decanting method **[5]** with the following modifications: 50 g of representative soil sample drawn from each site was suspended in 500 ml of water and stirred thoroughly. The suspension was allowed to stand for 15 minutes and then passed through a series of sieves of size 1 mm, 450µm, 250µm, 105µm and 45 µm arranged in descending order of their mesh size. The spores on the bottom two sieves were transferred to a 100 ml conical flask.

Inoculation of AM: AM fungal inoculum consisting of 1:1 mixture of sterilized sand and soil containing chlamydospores of the fungi and infected root bits having vesicles, arbuscules and hyphae. Inoculation of potting media was done by distributing a thin layer of inoculum @ 25 g per pot at two cm below the surface level so that the base or roots of the pepper cuttings or sorghum should come in contact with the inoculum. For control plants 25 gm of autoclaved UAS, GKVK campus soil was inoculated. The *Glomus fasciculatum* cultures were mass multiplied on Ragi (*Eleusine coracana)* seedlings using funnel technique and used for inoculation.

Experiments 1

Treatment details: T_1: Pepper cuttings inoculated with mycorrhiza from organic soil, T_2: Pepper cuttings inoculated with mycorrhiza from inorganic soil, T_3: Pepper cuttings inoculated with mycorrhiza from uncultivated traditional (Natural) soil, T_4: Pepper cuttings inoculated with mycorrhiza from uncultivated fallow (UAS, GKVK campus) soil as control, T_5: Pepper cuttings inoculated with *Glomus fasciculatum.*

Experiment 2:

Treatment details: T_1: Sorghum inoculated with mycorrhiza from organic soil, T_2: Sorghum inoculated with mycorrhiza from inorganic soil, T_3: Sorghum inoculated with mycorrhiza from uncultivated traditional (Natural) soil, T_4: Sorghum inoculated with mycorrhiza from uncultivated fallow (UAS, GKVK campus) soil as control, T_5: Sorghum inoculated with *Glomus fasciculatum.*

Observations on plant growth parameters for pepper: Stem girth measurements were taken at 180 days after planting and expressed in mm. Representative samples from each treatment and replications were uprooted 30 days after planting. They were thoroughly washed in running water to remove all adhering particles to the roots and the percentage of rooted cuttings was calculated by dividing with the total number of cuttings planted and expressed in percentage. Pepper cutting were observed daily for the emergence of sprout and the duration of the sprouting was recorded as the days taken by pepper cuttings from the day of planting to sprout emergence. One month after planting the number of sprouted cuttings was observed out of the total number of cuttings planted and expressed in percentage.

Observations on growth parameters for sorghum: Stem girth (diameter) measurements were taken at harvest and expressed in mm.

Microbial Count: The Populations of different groups of microorganisms in the soil samples were assessed by standard dilution plate technique **[6]** and the results were expressed in colony forming units (CFU's) per gram of the soil sample.

Mycorrhizal root colonization and Spore count: The rhizosphere soil was subjected to the wet sieving and decanting method [7]. Mycorrhizal root colonization was determined by the gridline intersect method [8]. Fresh root samples were cut into 1cm pieces and placed in screw cap vials. The clearing of the roots was achieved by treating them with 10 per cent potassium hydroxide (KOH) and leaving them overnight. The KOH solution was poured off and the roots were rinsed with tap water. Then the roots were treated with 10 per cent HCL for 10 minutes to neutralize the residual effect of alkali and create an acidic environment required by the stain. Root bits were then stained with 0.05 per cent trypan blue in lacto glycerol (lactic acid, glycerol and water in the ratio (40:40:20 respectively) by boiling them at 90ºC for 30 minutes. Excess stain was decanted and the root samples were immersed in lacto glycerol for destaining [9]. The stained root bits were randomly placed on a grid plate of size 10 X 10cm grid. The horizontal and vertical grid lines were viewed under a stereomicroscope at 40 X magnification to determine the total root bits and the root bits positive for mycorrhizal colonization on grid line intersection. The proportion of roots colonized by mycorrhiza was calculated by the formula:

$$\text{Per cent Colonization} = \frac{\text{Total No. of intersection positive for mycorrhizal colonization}}{\text{Total No. of intersections between roots and grid lines}} \times 100$$

Enzymatic activities: Enzyme activities like acid, alkaline phosphatase and dehydrogenase of soil were analyzed by the assay method **[10]**.

Acid and alkaline Phosphatase: One gram of soil was placed in a 50 ml Erlenmeyer flask and 0.2 ml of toluene,4 ml of modified universal buffer(pH 6.5 for assay of alkaline phosphatase and pH11 for assay of alkaline phosphatase),one ml of P-nitrophenyl phosphate solution (made in same buffer) were added to it. The flasks were stoppered, swirled for a few seconds and incubated at 37ºC in an incubator for 1 hour. After incubation, 1ml of 0.5 M $Cacl_2$ and 4ml of 0.5 NaOH were added to the flask and mixed well for few seconds and the supernatant was filtered through whatman No.2 folded filter paper. The yellow color complex was measured using 1 cm cuvette in the spectrometer (Shimadzu UV-visible) at 420 nm. The amount of P-nitrophenol released was calculated by referring to a calibration graph. Controls were maintained following the same procedure described above but 1 ml of P-nitrophenol was added after the incubation period before filteration.Results were expressed as microgram P-nitrophenol per gram of soil per hour.

Dehydrogenase Activity: Two gram of soil and 0.2 g of $CaCO_3$(AR grade),1ml of 2% 2,3,5 triphenyl tetrazolium chloride(TTC) and a column (1cm) of distilled water was added to screw cap test tubes and incubated at 37^{0}C for 24 hours. After incubation, the tubes were removed and the contents were removed and the contents were filtered (whatman No.2 filter paper) with washings of methanol until a colorless filterate was obtained. Then, the filterate was made upto 100ml with methanol in a volumetric flask and the absorbance units were converted to concentrations of triphenyl formazon (TPF) from a standard curve prepared from TPF and expressed microgram TPF per gram soil per hour.

Statistical analysis: The data obtained from the experiments were subjected to one-way analysis of variance (ANOVA) for completely randomized design (CRD) using MSTAT-C software. The treatment means were separated by Duncan's Multiple Range test (DMRT) a 5% level of significance [11].

Anatomical and histochemical Studies on pepper and sorghum inoculated with AM fungi:

The leaf material of pepper (5 month old) and sorghum (2months old) plants inoculated with AM fungi were considered for the anatomical and histochemical studies. Leaf sections were subjected to micrometry to measure the anatomical changes in the tissue. The plant sections of control and treated were observed for accumulation of total proteins, total insoluble polysaccharides and nucleic acids. The grading for degree of localization of various macromolecular substances were done based on visual observation. The grades were given as absent (-), Low (+), Rich (++) and Intense (+++).

Leaf samples collected were fixed in carnoy's B fixative (6:3:1 ethyl alcohol: chloroform: acetic acid) for 1 hour, dehydrated using n-butanol series and embedded in paraffin wax at 56^{0}C,serial sections of 10 µm(leaf) and 12 µm (root) were taken and subjected to the following procedures.

Staining procedures: Mercuric bromophenol blue (MBB) in absolute alcohol test for insoluble proteins was carried out [12]. The presence of proteins was indicated by deep blue colour, the intensity of which was a measure of amount of proteins in the tissue. For nucleic acids, hydrated sections were stained with 1% aqueous toluidine blue (TB) at 4.1 pH for 10 min as outlined in the method [13]. The nucleic acids such as RNA stained blue/purple colour and DNA appeared green. For polysaccharides,hydrated sections were kept in per-iodic acid for 10-15 min and washed with water.The polysaccharides appeared magenta colour.

Results and Discussion

The ubiquitous nature of vesicular arbuscular mycorrhizal fungi and their effects on plant growth is well known [14]. Arbuscular mycorrhizal fungi (AMF) occur in most vegetation types and constitute an important component of the tropical soil micro flora [15]. Majority of crop plants harbours a wide variety of mycorrhiza in their root zone. It is a symbiotic association between AM fungi and the roots of the plants where plants derive benefits from fungi by the way of nutrient uptake especially Phosphorus.

The results of the investigation on AM fungi (AMF) and other beneficial soil microorganisms, their effects on growth of black pepper and sorghum, enzymatic and anatomical and histochemical changes in plant system due to beneficial microorganisms and inoculation of mycorrhiza from crop management systems was investigated.

Two experiments were conducted, in the first experiment enzymatic, anatomical and histochemical analysis of black pepper to the inoculation of mycorrhiza from crop management systems and *Glomus fasciculatum* and population dynamics of microorganisms was studied. In the second experiment enzymatic, anatomical and histochemical analysis of sorghum to the inoculation of mycorrhiza from crop management systems and *Glomus fasciculatum* and population dynamics of microorganisms was studied.

Experiment 1: Microbial, Biochemical, Anatomical and Histochemical changes in black pepper (Piper nigrum L.) due to mycorrhizal inoculation

This experiment was conducted to study the microbial, biochemical, anatomical and histochemical changes and response of black pepper to inoculation with mycorrhiza from different crop management systems like organic soil, inorganic soil, natural or undisturbed soil from traditionally pepper growing areas , fallow soil and arbuscular mycorrhizal fungi (*Glomus fasciculatum*).

Influence of mycorrhizal inoculation on stem girth: The result on the influence of mycorrhizal inoculation on the stem girth (diameter) is presented in table 1. Maximum stem girth (diameter) was observed in plants inoculated with mycorrhiza from natural soil (7.46 mm) followed by plants inoculated with *Glomus fasciculatum* (6.86 mm), Plant inoculated with mycorrhiza from inorganic soil (6.50 mm) and plant inoculated with mycorrhiza from organic soil (6.40 mm). Lowest stem girth was observed in control (5.80 mm).

Influence of mycorrhizal inoculation on rooting percentage: The data pertaining to rooting percentage as influenced by the inoculation of mycorrhiza is presented in table 1. The maximum percentage of rooting was recorded in plants inoculated with *Glomus fasciculatum* (64.33%) followed by plant inoculated with mycorrhiza from organic soil (57.00%), Plant inoculated with mycorrhiza from natural soil (54.33%) and plant inoculated with mycorrhiza from inorganic soil (53.33%). Lowest rooting percentage was observed in control (48.00%). These results may uphold the ealier observations [16-17]. Thus P being a constituent of phosphonucleotides, which increases cell division and elongation [18] might have increased the rooting percentage.

Table 1: Effect of inoculation of AM on plant height and number of leaves of *Piper nigrum* L. and *Sorghum bicolor* (L.) Moench

Treatments	Stem girth (mm)		Rooting percentage	Duration of Sprouting (Days)	Sprouting percentage
	Pepper (AMF+Pepper)	Sorghum (AMF+Sorghum)	Pepper (AMF+Pepper)	Pepper (AMF+Pepper)	Pepper (AMF+Pepper)
	at harvest	at harvest			
T1	6.40	6.93	57.00	20.33	63.48
T2	6.50	6.60	53.33	21.67	53.17
T3	7.46	7.13	54.33	20.67	64.67
T4	5.80	6.60	48.00	24.67	48.50
T5	6.86	7.56	64.33	20.67	63.40
SEM±	0.158	0.104	1.541	0.461	1.893
CD @ 5%	0.863	0.624	3.044	2.252	2.273

DAP: Days after treatment
DAS: Days after sowing
T_1: Inoculated with mycorrhiza from organic soil.
T_2: Inoculated with mycorrhiza from inorganic soil.
T_3: Inoculated with mycorrhiza from natural soil.
T_4: Control.
T_5: Inoculated with *Glomus fasciculatum*

Table 2: Effect of inoculation of AM on population of other soil microorganisms in root zone soil of *Piper nigrum* L. and *Sorghum bicolor* (L.) Moench

Treatments	Fungi, bacteria and actinomycetes population (CFU's/g of soil)					
	Pepper (AMF+Pepper)			Sorghum (AMF+Sorghum)		
	Fungi	Bacteria	Actinomycetes	Fungi	Bacteria	Actinomycetes
	X 10^3	X 10^4	X 10^2	X 10^3	X 10^4	X 10^2
T1	8.26	16.47	12.22	7.01	16.14	12.20
T2	9.13	14.25	12.62	6.55	14.80	10.90
T3	9.50	20.45	16.60	9.06	18.85	14.81
T4	5.56	7.71	9.07	6.10	11.86	8.15
T5	8.90	19.33	15.70	8.93	18.66	13.20
SEM±	0.407	1.305	0.776	0.356	0.750	0.649
CD @ 5%	1.117	2.483	2.681	1.122	4.410	2.454

T_1: Inoculated with mycorrhiza from organic soil.
T_2: Inoculated with mycorrhiza from inorganic soil.
T_3: Inoculated with mycorrhiza from natural soil.
T_4: Control.
T_5: Inoculated with *Glomus fasciculatum*

Table 3: Effect of inoculation of AM on spore count in root zone soil and percent root colonization of *Piper nigrum* L. and *Sorghum bicolor (*L.*)* Moench

AM spore count in root zone soil (spores/50 gm soil)			Percent root colonization	
Treatments	Pepper (AMF+Pepper)	Sorghum (AMF+Sorghum)	Pepper (AMF+Pepper)	Sorghum (AMF+Sorghum)
T1	75.00	49.53	15.67	14.33
T2	69.33	46.40	15.00	13.33
T3	62.00	43.83	16.33	15.33
T4	13.67	38.66	12.67	12.00
T5	84.67	52.33	15.67	15.67
SEM±	7.149	1.360	0.367	0.387
CD @ 5%	5.518	3.814	2.047	2.047

T_1: Inoculated with mycorrhiza from organic soil.
T_2: Inoculated with mycorrhiza from inorganic soil.
T_3: Inoculated with mycorrhiza from natural soil.
T_4: Control.
T_5: Inoculated with *Glomus fasciculatum*

Table 4: Effect of inoculation of AM on soil dynamics in root zone soil of *Piper nigrum* L.and *Sorghum bicolor (*L*.)* Moench

Dehydrogenase activity* Acid phosphatase and Alkaline phosphatase activity (µg PNP/gm /hour)

Treatments	Pepper (AMF+Pepper)			Sorghum (AMF+Sorghum)		
	Dehydrogenase activity	Acid phosphatase	Alkaline phosphatase	Dehydrogenase activity	Acid phosphatase	Alkaline phosphatase
T1	113.00	53.63	17.61	111.33	50.77	17.44
T2	112.33	48.66	16.16	108.33	47.36	15.17
T3	115.66	60.40	19.04	110.34	53.97	18.37
T4	101.06	47.33	8.13	99.53	45.00	6.57
T5	116.00	57.00	18.96	117.34	53.67	16.44
SEM±	1.577	1.421	1.173	1.663	1.013	1.226
CD @ 5%	7.253	5.860	2.845	3.910	2.056	1.753

T_1: Inoculated with mycorrhiza from organic soil.
T_2: Inoculated with mycorrhiza from inorganic soil.
T_3: Inoculated with mycorrhiza from natural soil.
T_4: Control.
T_5: Inoculated with *Glomus fasciculatum*
* µg of TPF per gm soil per hour

Table 5: Comparison of leaf structure (thickness and size) of inoculated and uninoculated plants of *Piper nigrum* L. and *Sorghum bicolor (*L*.)* Moench

Upper epidermis (µm)	Control	1.89	0.93
	Inoculated	2.92	1.24
Lower epidermis (µm)	Control	1.10	1.2
	Inoculated	1.96	1.67
Xylem cell size(µm)	Control	5.20	3.18
	Inoculated	6.64	4.62
Spongy layer cell size(µm)	Control	3.28	2.10
	Inoculated	3.86	2.97

Influence of mycorrhizal inoculation on duration of sprouting: The data on influence of mycorrhizal inoculation on duration of sprouting is presented in table 1. Longer duration of sprouting was recorded in control (24.67 days) and shorter duration of sprouting was recorded in plant inoculated with mycorrhiza from organic soil (20.33 days). Plant inoculated with mycorrhiza from inorganic soil (21.67 days) was on par with plant inoculated with mycorrhiza from natural soil (20.67 days) and plant inoculated with *Glomus fasciculatum* (20.66 days). This enhancement in the vegetative growth in black pepper may be due to the well proliferated healthier root systems. Earlier studied on these parameters suggests that AM fungi has some growth inducing effects on vegetative growth of the black pepper [19].

Influence of mycorrhizal inoculation on sprouting percentage: The data on influence of mycorrhizal inoculation on sprouting percentage is presented in table 1.Maximum sprouting percentage was noticed in plant inoculated with mycorrhiza from natural soil (64.67%) which was on par with plant inoculated with mycorrhiza from organic soil (63.48%) and plant inoculated with *Glomus fasciculatum* (63.40%) and

lowest sprouting percentage was observed in control (48.50 %).

Influence of mycorrhizal inoculation on abundance of microorganisms in root zone soil: The data pertaining to the influence of mycorrhizal inoculation on abundance of fungi, bacteria and actinomycetes in root zone soil is presented in table 2. With respect to fungal, bacterial and actinomycetes population there was a significant difference among the plants inoculated with the mycorrhiza from different crop management systems than control. Similar results were [20] reported an increase in the population of bacteria in the rhizoplane of mycorrhizal sweet corn and clover compared to the non-mycorrhizal plants.

Fungal population: Highest number of colony forming units (CFU's) for fungi was observed in root zone soil of plant inoculated with mycorrhiza from natural soil (9.50 CFU's/g of soil) and control (5.67 CFU's/g of soil) showed least abundance of fungi in root zone soil. Plant inoculated with mycorrhiza from organic soil (8.26 CFU's/g of soil) was statistically on par with plant inoculated with mycorrhiza from inorganic soil (9.13 CFU's/g of soil).

Bacterial population: Highest number of Colony Forming Units (CFU's) for bacteria was observed in root zone soil of plant inoculated with mycorrhiza from natural soil (20.45 CFU's/g of soil) which was on par with plant inoculated with *Glomus fasciculatum* (19.33 CFU's /g of soil). Control (7.71 CFU's/g of soil) showed least abundance of bacteria.

Actinomycetes population: Highest number of Colony Forming Units (CFU's) for actinomycetes was recorded in root zone soil of plant inoculated with mycorrhiza from natural soil (16.60 CFU's/g of soil) which was statistically on par with *Glomus fasciculatum* (15.71 CFU's /g of soil). Control (9.07 CFU's/g of soil) showed least abundance of actinomycetes.

Mycorrhizal parameters

AM Spore count: The data on AM spore count is presented in table 3. Highest number of AM spores was recorded in root zone soil of plant inoculated with *Glomus fasciculatum* (84.67 spores/50 g soil) followed by plant inoculated with mycorrhiza from organic soil (75.00 spores/50g of soil), plant inoculated with mycorrhiza from inorganic soil (69.33 spores/50 g of soil) and plant inoculated with mycorrhiza from natural soil (62.00 spores/50 g of soil). Control (13.66 spores/50 g of soil) showed least number of spores. Increased root colonization levels in plants inoculated with AM fungi have been observed earlier by several workers [21].

Percent root colonization: The data on percent root colonization is presented in table 3. Highest percentage of root colonization was recorded in plant inoculated with *Glomus fasciculatum* (62.30%) followed by plant inoculated with mycorrhiza from organic soil (60.40%), plant inoculated with mycorrhiza from natural soil (60.13%) and plant inoculated with mycorrhiza from inorganic soil (52.00%). Lowest percent root colonization was observed in control (17.00%). Increased spore count and root colonization was noticed [22] in tomato inoculated with AM fungi.

Soil dynamics: The data pertaining to dehydrogenase activity and acid and alkaline phosphatase is presented in table 4.

Dehydrogenase activity: Highest dehydrogenase activity was recorded in plant inoculated with *Glomus fasciculatum* (116.00µg of TPF /g of soil /hour) which was statistically on par with plant inoculated with mycorrhiza from organic soil (113.00µg of TPF /g of soil /hour), plant inoculated with mycorrhiza from inorganic soil (112.33µg of TPF /g of soil /hour) and plant inoculated with mycorrhiza from natural soil (115.66µg of TPF /g of soil /hour). Lowest was recorded in control (101.06µg of TPF /g of soil /hour).

Acid phosphatase activity: Acid phosphatase activity was highest in root zone soil of plant inoculated with mycorrhiza from natural soil (60.40µg of TPF /g of soil /hour) which was statistically on par with plant inoculated with *Glomus fasciculatum* (57.00µg PNP/g of soil/hour). Lowest activity was recorded in control (47.33µg PNP/g of soil/hour).

Alkaline phosphatase activity: Alkaline phosphatase activity was highest in root zone soil of plant inoculated with mycorrhiza from natural soil (19.04µg of TPF /g of soil /hour) which was statistically on par with plant inoculated with *Glomus fasciculatum* (18.97µg PNP/g of soil/hour). Lowest activity was recorded in control (8.13µg PNP/g of soil/hour).

Experiment 2: Microbial, Biochemical, Anatomical and Histochemical changes in sorghum due to mycorrhizal inoculation

This experiment was conducted to study the microbial, biochemical, anatomical and histochemical changes and response of sorghum to inoculation with mycorrhiza from different crop management systems like organic soils, inorganic soils, natural or undisturbed soil from traditionally pepper growing areas and fallow soils and arbuscular mycorrhiza (*Glomus fasciculatum*).

Influence of mycorrhizal inoculation on stem girth (diameter): The result on the influence of mycorrhizal inoculation on the stem girth (diameter) is presented in table 1.Maximum stem girth was observed in plant inoculated with *Glomus fasciculaum* (7.56 mm) and lowest stem girth was observed in control (6.60mm). Plant inoculated with mycorrhiza from organic soil (6.93mm) was statistically on par with plant inoculated with mycorrhiza from inorganic soil (6.60 mm). These results may uphold the earlier observations [23] on the basis of increased plant height, stem diameter and biomass of the plants inoculated with VAM fungus.

Influence of mycorrhizal inoculation on abundance of microorganisms in root zone soil:

The data pertaining to the influence of mycorrhizal inoculation on abundance of fungal, bacterial and actinomycetes population in root zone soil is presented in table 2.

Fungal population: Highest number of colony forming units (CFU's) for fungi was observed in root zone soil of plant inoculated with mycorrhiza from natural soil (9.01 CFU's/g of soil).Control (6.10 CFU's/g of soil) showed least abundance of fungi. Plant inoculated with mycorrhiza from organic soil (7.01 CFU's/g of soil) was on par with plant inoculated with mycorrhiza from inorganic soil (6.55 CFU's/g of soil).

Bacterial population: Highest number of colony forming units (CFU's) for bacteria was observed in root zone soil of plant

inoculated with mycorrhiza from natural soil (18.85 CFU's/g of soil) which was on par with plant inoculated with *Glomus fasciculatum* (18.67 CFU's /g of soil). Control (11.87 CFU's/g of soil) showed least abundance of bacteria.

Actinomycetes population: Highest number of colony forming units (CFU's) for actinomycetes was recorded in root zone soil of plant inoculated with mycorrhiza from natural soil (14.81 CFU's/g of soil) which was statistically on par with *Glomus fasciculatum* (13.21 CFU's /g of soil) Control (8.15 CFU's/g of soil) showed least abundance of actinomycetes.

Mycorrhizal parameters: The data on influence of mycorrhizal inoculation on mycorrhizal parameters in root zone soil of sorghum is presented in table 3.

AM Spore count: Highest number of AM spores was recorded in root zone soil of plant inoculated with mycorrhiza from organic soil (77.33 spores/50g of soil) plant inoculated with *Glomus fasciculatum* (72.00 spores/50 g soil), plant inoculated with mycorrhiza from natural soil (56.67 spores/50 g of soil) and plant inoculated with mycorrhiza from inorganic soil (53.33 spores/50 g of soil). Control (11.33 spores/50 g of soil) showed least number of spores.

Percent root colonization: Highest percentage of root colonization was recorded in plant inoculated with *Glomus fasciculatum* (69.00%) followed by plant inoculated with mycorrhiza from natural soil (62.00%), plant inoculated with mycorrhiza from organic soil (57.00%) and plant inoculated with mycorrhiza from inorganic soil (55.33.%). Lowest percent root colonization was observed in control (16.00%).

Soil dynamics: The data pertaining to dehydrogenase activity and acid and alkaline phosphatase is presented in table 4.

Dehydrogenase activity: Highest dehydrogenase activity was recorded in plant inoculated with *Glomus fasciculatum* (117.33 µg of TPF /g of soil /hour) followed by plant inoculated with mycorrhiza from organic soil (111.33 µg of TPF /g of soil /hour), plant inoculated with mycorrhiza from natural soil (110.33 µg of TPF /g of soil /hour) and plant inoculated with mycorrhiza from inorganic soil (108.30µg of TPF /g of soil /hour). Lowest was recorded in control (99.53µg of TPF /g of soil /hour).

Acid phosphatase activity: Acid phosphatase activity was highest in root zone soil of plant inoculated with mycorrhiza from natural soil (53.96 µg of TPF /g of soil /hour) followed by plant inoculated with µg of TPF /g of soil /hour), plant inoculated with *Glomus fasciculatum* (53.67 µg PNP/g of soil/hour), plant inoculated with mycorrhiza from organic soil (50.78 µg of TPF /g of soil /hour) and plant inoculated with mycorrhiza from inorganic soil (47.3707µg PNP/g of soil/hour). Lowest activity was recorded in control (45.00 µg PNP/g of soil/hour).

Alkaline phosphatase activity: Alkaline phosphatase activity was highest in root zone soil of plant inoculated with mycorrhiza from natural soil (18.37 µg of TPF /g of soil /hour) followed by plant inoculated with mycorrhiza from organic soil (17.44 µg PNP/g of soil/hour), plant inoculated with *Glomus fasciculatum* (16.43 µg PNP/g of soil/hour) and plant inoculated with mycorrhiza from inorganic soil (15.16 µg PNP/g of soil/hour). Lowest activity was recorded in control (6.57µg PNP/g of soil/hour).

Histochemical changes in black pepper and sorghum due to mycorrhizal inoculation: The histochemical changes in black pepper and sorghum due to mycorrhizal inoculation were studied. The intensity of colour in the leaf tissues of black pepper and sorghum was used to localize the metabolites such as nucleic acids (Plate 1, 2, 7and 8 respectively), polysaccharides (Plate 3, 4, 9 and 10 respectively) and proteins (Plate 5, 6, 11 and 12 respectively).

Total insoluble polysaccharides, proteins and nucleic acids: There was a significant difference between uninoculated and inoculated plants in all the two experimental setup. Higher accumulation of nucleic acid was found in leaf tissue which appeared to be deep blue colour compared to less intense blue colour in leaf tissue of control plant. Similar observations were noticed in the protein accumulation in leaf tissue of inoculated plants. Intense magenta colour was observed in leaf tissue of plants inoculated with mycorrhiza than that of uninoculated plants. The intensity of colour taken by root and leaf tissues was used to localize the metabolites like polysaccharide, protein and nucleic acids. The presence of intense colour observed in Toluidine Blue (TB) and MBB tests indicated the higher concentration of DNA and proteins in the leaf tissues of inoculated plants than that of uninoculated plants respectively. A similar result was observed [24] in *Simarouba glauca* inoculated with *Glomus fasciculatum* along with mycorrhiza helper bacterium *Bacillus coagulans*. The intense colour taken by leaf tissues indicates the presence of higher concentration of polysaccharides in the inoculated plants. This result was similar to the ealier findings [25] for distribution of polysaccharides in eucalyptus mycorrhizas.

Anatomical changes in leaf structure: The date pertaining to the differences in the sizes of upper epidermis, lower epidermis, xylem cells and spongy layer is presented in Table 5.

Upper and lower epidermis: The size of the epidermis in leaf sample of black pepper inoculated with *Glomus fasciculatum* was thicker (2.92 µm) than the control (1.89 µm). Incase of sorghum inoculated with *Glomus fasciculatum* the size of the epidermis (1.24 µm) compared to control (0.93 µm). Thickness of the lower epidermis in leaf sample of black pepper inoculated with *Glomus fasciculatum* was thicker (1.96 µm) than the control (1.10 µm). Incase of sorghum inoculated with *Glomus fasciculatum* the size of the epidermis (1.67 µm) was thick compared to control (1.20 µm). The thickness of the epidermal layer was greater in the plants inoculated with mycorrhiza compared to non-mycorrhizal plants. This is probably because of a part of the polysaccharide gets converted into structural polysaccharides making the cell wall stronger and more compact leading to determine the shape of the cell. The earlier works [26-27] uphold this same observation. The well differentiated cell with thicker cell wall in mycorrhizal plants leads to increased metabolic activity of root which was similar to the findings given by several invetigators [28].

Xylem cells: The size of the xylem cells in leaf sample of black pepper inoculated with *Glomus fasciculatum* (6.64µm) was larger than the control (5.20 µm). Incase of sorghum inoculated with *Glomus fasciculatum* the size of the xylem (4.62 µm) cells was larger than control (3.18 µm). The similar observation of increased thickness of leaves, size of midrib veins, mesophyll cells, motor cells and number of plastids was reported [29] while working in *Eleusine coracana* colonized by VA mycorrhiza. Similarly, few workers [30-31] observed the enlargement and better differentiation of vascular and other tissues in mycorrhizal plants.

Spongy layer: Thickness of the spongy layer in leaf sample of black pepper inoculated with *Glomus* fasciculatum (3.86µm) was comparatively thicker than the control (3.28 µm). Thickness of spongy layer was more incase of sorghum inoculated with *Glomus fasciculatum* (2.97 µm) than control (2.10 µm).

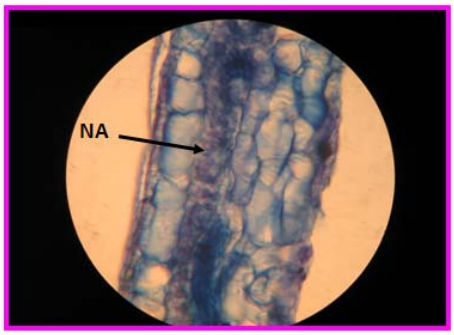

Figure 1: Leaf section of *Piper nigrum* L. control plant showing comparatively less nucleic acid content in tissues

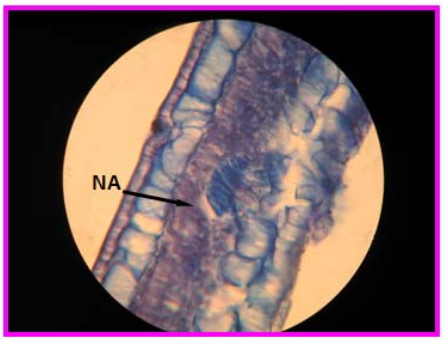

Figure 2: Leaf section of *Piper nigrum* L. plant inoculated with *Glomus fasciculatum* showing comparatively increased nucleic acid content in tissues

*NA-Nucleic acids

** (Observed under 40x magnification)

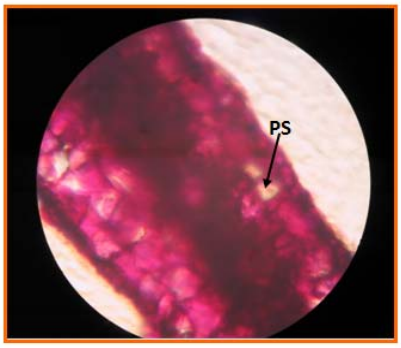

Figure 3: Leaf section of *Piper nigum* L. control plant showing less accumulation of polysaccharides in tissues

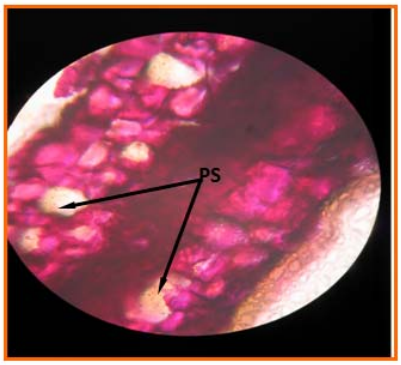

Figure 4: Leaf section of *Piper nigrum* L. plant inoculated with *Glomus fasciculatum* showing comparatively increased polysaccharides in tissues.

* PS- Poly saccharides

** (Observed under 40x magnification)

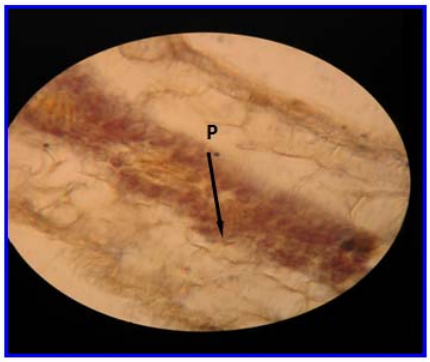

Figure 5: Leaf section of *Piper nigrum* L. control plant showing less accumulation of proteins in tissues

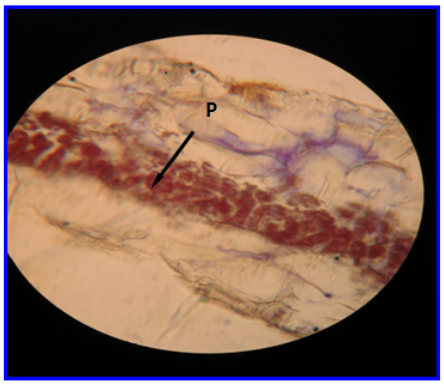

Figure 6: Leaf section of *Piper nigrum* L. plant inoculated with *Glomus fasciculatum* showing comparatively increased accumulation of proteins in tissues.
* P – Proteins
** (Observed under 40x magnification)

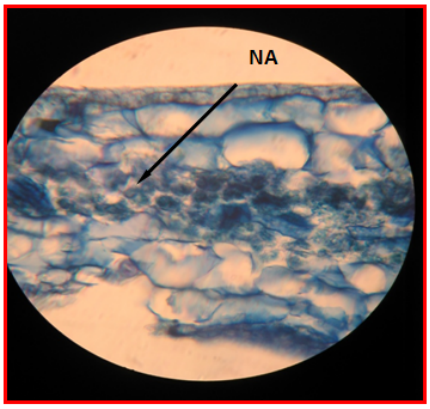

Figure 7: Leaf section of *Sorghum bicolor* (L.) Moench control plant showing comparatively less nucleic acid content in tissues

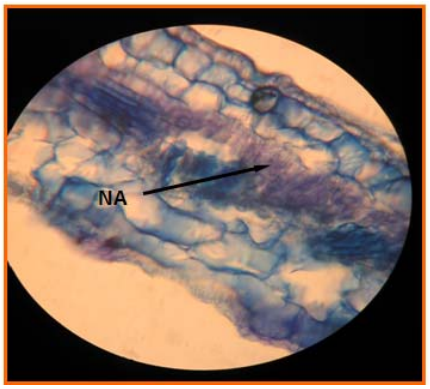

Figure 8: Leaf section of *Sorghum bicolor* (L.) Moench plant inoculated with *Glomus fasciculatum* showing comparatively less nucleic acid content in tissues.
*NA- Nucleic acids ** (Observed under 40x magnification)

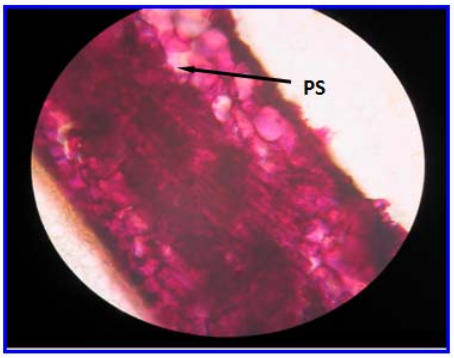

Figure 9: Leaf section of *Sorghum bicolor* (L.) Moench control plant showing less accumulation of polysaccharides in tissues

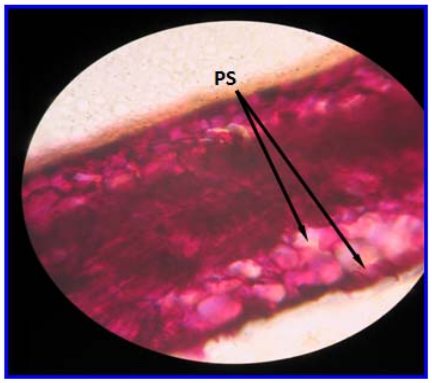

Figure 10: Leaf section of *Sorghum bicolor* (L.) Moench plant inoculated with *Glomus fasciculatum* showing increased accumulation of polysaccharides in tissues.
*PS-Polysaccharides ** (Observed under 40x magnification)

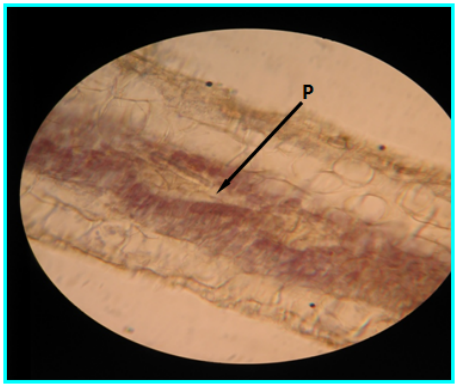

Figure 11: Leaf section of *Sorghum bicolor* (L.) Moench control plant showing less accumulation of proteins in tissues

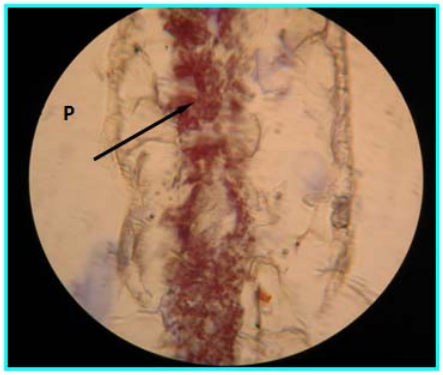

Figure 12: Leaf section of *Sorghum bicolor* (L.) Moench plant inoculated with *Glomus fasciculatum* showing comparatively increased accumulation of proteins in tissues.
*P – Proteins ** (Observed under 40x magnification)

Conclusion

In the present study percentage of rooting and percent root colonization were high in plants inoculated with mycorrhiza from different crop management systems than control. The histochemical studies showed that there were striking differences in plant leaf structures. There was increase in the thickness of upper epidermal cells, lower epidermal cells and size of the xylem cells inoculated with mycorrhiza from different crop management systems. There was a increased accumulation of protein, polysaccharide and the nucleic acids in the leaf samples indicating the direct correlation between the AM fungi and the crop response to inoculation of the AM fungi. The over all view of the studies was that the black pepper (*Piper nigrum* L.) and Sorghum (*Sorghum bicolor* (L.) Moench) benefited from the inoculation of arbuscular mycorrhiza fungi from different crop management systems and *Glomus fasciculatum*.

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