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Biochemical Analysis and Growth Response of Black Pepper and Sorghum Inoculated with Mycorrhiza from Different Crop Management Systems

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In the present study the biochemical analysis and growth response of Black Pepper and Sorghum inoculated with mycorrhiza from different crop management systems was done. There were three experiments in which the first experiment was on growth response of black pepper to the inoculation of mycorrhiza from different crop management systems. In second experiment black pepper and sorghum were inoculated with mycorrhiza from different crop management systems. In third experiment sorghum was inoculated with mycorrhiza from different crop management systems. Black pepper and sorghum were inoculated with mycorrhiza from the organic soil, inorganic soil, natural soil, *Glomus fasciculatum* and control. Plants inoculated with *Glomus fasciculatum* showed highest phosphorus, potassium and nitrogen content. Plants inoculated with *Glomus fasciculatum* recorded increased biomass than uninoculated control plants. The effect of inoculation of AM fungi on plant height of black pepper and sorghum was significantly higher than the uninoculated control plants. The increase in plant height may be due to increase in higher phosphorus uptake and growth promoting activities by the AM fungi.

Keywords: Arbuscular Mycorrhizal Fungi, Black pepper, Sorghum, *Glomus fasciculatum,* Growth response and Biochemical analysis.

Soil harbours various types of microorganisms. It serves as a source of nutrients and other factors required for the microorganisms. Plants exploit nutrients from soil with the help of beneficial microorganisms such as mycorrhizal fungi, phosphate solubilizing microorganisms etc. Arbuscular Mycorrhiza Fungi (AMF) is an important component of the terrestrial communities.

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Black Pepper (*Piper nigrum* L., Family: Piperaceae), popularly known as "king of spices" is an ancient and important spice crop of India. In India, Pepper is cultivated in an area of 17 lakh hectares, with an annual production of 57,000 tonnes. Major area and production is largely confined to Kerala followed by Karnataka and Tamil Nadu. It earns a foreign exchange to an extent of Rs.416.52 crores on export of 47,000 tonnes annually[1] (Anon, 1998).

The word "Mycorrhiza" refers to any of several types of association between plant roots and soil fungi. 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 inoculant Arbuscular Mycorrhiza Fungi (AMF) into a plant growth system will occur 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[2] (Boddington and Dodd, 2000) or rooting of pepper cuttings[3] (Thanuja *et al*., 2002).

Sorghum (*Sorghum bicolor* (L.) Moench) originated in tropical Africa and later spread to the near and far east. It has been widely used for production of forage and silage for animal feed. Sorghum is a multi-purpose crop yielding 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 grown in several countries of tropics and subtropics. It 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.

Hence glass house study of black pepper and sorghum plants inoculated with mycorrhiza from organic soil, inorganic soil, natural vegetation and *Glomus fasciculatum* and their effects on the plant growth and biochemical changes of these plants in three combinations was carried out. Biochemical and chemical evaluation of pepper and sorghum inoculated with mycorrhiza from different crop management systems was also done.

MATERIAL AND METHODS

The experiments to study the biochemical, chemical analysis and growth response 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.

The planting materials used in this investigation were Pepper cuttings (Panniyur-4) and Sorghum seeds (IS-3443).Pepper cuttings were obtained from Kerala Agricultural University, Kerala. The *Glomus fasciculatum* cultures were mass multiplied on Ragi (*Eleusine coracana)* seedlings using funnel technique and used for inoculation.

Soil samples were collected from pepper fields on organic, inorganic farms and natural fields or undisturbed soil from traditionally pepper growing areas and fallow soils and arbuscular mycorrhiza (*Glomus fasciculatum*). Soil samples on organic farms were collected from Churikad, Thavaligere and Tareu. Soil samples on inorganic farms were collected from Ponnampet, Pallari, Churikad and Tareu. Soil samples on natural vegetation were collected from Bisile forest, Lakkuna and Churikad. At each of 3 sites, 4 replicate plots were selected. From each replicate plot, 4 soil samples were collected randomly with the help of soil corer (Diameter 5 cm, soil depth 25 cm) and pooled to have one composite soil sample per replicate thus, in total 12 composite samples were collected.

Pepper cuttings obtained were raised in plastic containers (5 liter capacity) containing 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 three experimental setups (systems) maintained in the green house which are as follows:

1. Pepper cuttings inoculated with the mycorrhiza from the different crop management systems (i.e., AMF +Pepper system)
2. Pepper and sorghum inoculated with the mycorrhiza from different crop management systems (i.e., AMF +Pepper + Sorghum system)
3. Sorghum inoculated with the mycorrhiza from different crop management systems (i.e., AMF + Sorghum system)

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 soil was inoculated.

Plant Growth Response

In experiment 1 the growth 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*) was studied. The treatments for experiment 1 includes 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 (GKVK) soil as control, $T_{5:}$ Pepper cuttings inoculated with *Glomus fasciculatum.*

In experiment 2 the growth response of black pepper and sorghum plants to the inoculation of 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*) was studied. The treatments for experiment 2 includes-T_1: Pepper cuttings and sorghum inoculated with mycorrhiza from organic soil, T_2: Pepper cuttings and sorghum inoculated with mycorrhiza from inorganic soil, T_3: Pepper cuttings and sorghum inoculated with mycorrhiza from uncultivated traditional (Natural) soil, T_4: Pepper cuttings and sorghum inoculated with mycorrhiza from uncultivated fallow (GKVK) soil as control, $T_{5:}$ Pepper cuttings and sorghum inoculated with *Glomus fasciculatum.*

In experiment 3 the growth 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*). The treatments for experiment 3 include 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 (GKVK) soil as control, $T_{5:}$ Sorghum inoculated with *Glomus fasciculatum.*

Plant height was recorded at 180 days after planting and expressed in cm. The number of leaves was counted at 180 days after planting. For root fresh weight, all roots from the cuttings were removed at 180 days after planting, weighed and expressed in grams. Roots observed for fresh weight were dried in a hot air oven at 65^0 C continuously until a constant weight was attained and then recorded in grams for root dry weight. Fresh weight of shoot was recorded at 180 days after planting and expressed in grams. Shoots used for recording fresh weight were dried in hot air oven at 65^0 C until constant weight is obtained and expressed in grams for shoot dry weight.

Biochemical and chemical analysis of pepper and sorghum

Photo synthetically active leaves were collected from each plant before the harvest of the plants and then the chlorophyll content was estimated by following the Dimethyl sulfoxide (DMSO) method[4](Hiscox and Israedstam, 1979). Total plant sugar was estimated colorimetrically following the phenol sulphuric acid method[5] (Dubios *et al.*, 1996). The total reducing sugars in leaves was estimated by the method of [6]Nelson (1944) as modified by [7]Somogyi (1952). Total soluble proteins were estimated by using the procedure of [8]Lowry *et al.* (1951).

Plant phosphorus concentration was estimated colorimetrically following the vanadomolybdate yellow colour method[9] (Jackson, 1973). The potassium concentration in the plant tissues was estimated by using flame photometer[9] (Jackson, 1973). The total nitrogen concentration estimation was carried out by Microkjeldahal method[10] (AOAC, 1980).

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] (Little and Hills, 1978).

RESULTS

Experiment 1 Plant growth response and biochemical changes in black pepper (*Piper nigrum* L.) due to mycorrhizal inoculation

The main objective behind this experiment was to study the 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*). The results obtained are presented below.

Influence of mycorrhizal inoculation on plant height and number of leaves of black pepper at 180 days after planting is given in table 1. At 180 days after planting maximum plant height was noticed in plant inoculated with mycorrhiza from organic soil (27.7 cm) followed by plant inoculated with *Glomus fasciculatum* (26.2cm), plant inoculated with mycorrhiza from natural soil (25.43 cm) and plant inoculated with mycorrhiza from inorganic soil (23.6 cm) and least plant height was attributed to control (21.27 cm). At 180 days after planting maximum number of leaves was recorded in plants inoculated with mycorrhiza from natural soil (16.33) and least number of leaves was noticed in control (12.67). Plant inoculated with mycorrhiza from organic soil (15.67) was statistically on par with plant inoculated with mycorrhiza from inorganic soil (15.00) and plant inoculated with *Glomus fasciculatum* (15.66).

The data on influence of mycorrhizal inoculation on root fresh weight and root dry weight is presented in table 2. Maximum root fresh weight was noticed in plant inoculated with mycorrhiza from organic soil (2.70g) which was statistically on par with plant inoculated with mycorrhiza from inorganic soil (2.63g) and *Glomus fasciculatum* (2.52g) and lowest root fresh weight was observed in control (1.83g). Maximum root dry weight was noticed in plant inoculated with *Glomus fasciculatum* (0.85g) followed by plant inoculated with mycorrhiza from natural soil (0.53g), plant inoculated with mycorrhiza from organic soil (0.45g) and plant inoculated with mycorrhiza from inorganic soil (0.42g). Lowest root dry weight was observed in control (0.35g).

Maximum shoot fresh weight was noticed in plant inoculated with *Glomus fasciculatum* (12.97g) and lowest shoot fresh weight was observed in control (5.76g).Plant inoculated with mycorrhiza from organic soil (11.97g) was on par with plant inoculated with mycorrhiza from inorganic soil (11.37g).

Maximum shoot dry weight was noticed in plant inoculated with *Glomus fasciculatum* (3.07g) followed by plant inoculated with mycorrhiza from inorganic soil (2.47g), Plant inoculated with mycorrhiza from organic soil (2.40g) and plant inoculated with mycorrhiza from natural soil (2.30g). Lowest shoot dry weight was observed in control (0.72g). Results are shown in table 3.

The data on influence of mycorrhizal inoculation on total chlorophyll content is presented in table 4. Maximum total chlorophyll content was noticed in plant inoculated with *Glomus fasciculatum* (3.00mg/g) which was statistically on par with plant inoculated with mycorrhiza from inorganic soil (2.77mg/g). Least total chlorophyll content was noticed in control (1.89mg/g).

The data pertaining to the influence of mycorrhizal inoculation on Total soluble protein content is presented in table 4. Total soluble protein was high in plant inoculated with mycorrhiza from organic soil (8.47%) which was statistically on par with plant inoculated with mycorrhiza from inorganic soil (8.37%) and plant inoculated with *Glomus fasciculatum* (8.30%). Lowest total soluble protein content was recorded in control (6.17%).

The data on influence of mycorrhizal inoculation on total sugar content is presented in table 5. The total sugar content was high in plant inoculated with mycorrhiza from natural soil (2.62mg/100g) which was statistically on par with plant inoculated with *Glomus fasciculatum* (2.57mg/100g) and the lowest total sugar content was noticed in control (1.80mg/100g).

The data pertaining to the influence of mycorrhizal inoculation on total reducing sugar content is presented in table 5. The total reducing sugar content was high in plant inoculated with *Glomus fasciculatum* (2.75mg/100g) which was statistically on par with plant inoculated with mycorrhiza from natural soil (2.59mg/100g). Lowest total sugar content was noticed in control (1.93mg/100g).

The data pertaining to the influence of mycorrhizal inoculation on phosphorus, potassium and nitrogen content is presented in table 6. Highest phosphorus content was recorded in plant inoculated with *Glomus fasciculatum* (0.222%) and lowest phosphorus content was recorded in control (0.151%). Plant inoculated with mycorrhiza from organic soil (0.182%) was

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

Treatments	Plant Height(cm)				Number of leaves			
	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper+ Sorghum) At Harvest	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper Sorghum) At Harvest
T1	27.70	26.66	49.53	46.13	15.67	12.67	14.33	15.00
T2	23.60	23.86	46.40	43.10	15.00	11.67	13.33	15.67
T3	25.43	26.96	43.83	40.36	16.33	13.67	15.33	14.33
T4	21.26	20.76	38.66	37.00	12.67	11.33	12.00	12.33
T5	26.26	24.90	52.33	42.06	15.67	14.33	15.67	15.33
SEM±	0.643	0.647	1.360	0.871	0.367	0.329	0.387	0.342
CD @ 5%	1.354	1.852	3.814	2.962	2.047	1.557	2.047	1.878

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
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

Table 2. Effect of inoculation of AM on root fresh weight and root dry weight of *Piper nigrum* L. (at 180 days after planting) and *Sorghum bicolor* (L.) Moench (at harvest)

Treatments	Root fresh and dry weight (g)							
	Pepper (AMF+Pepper)		Pepper (AMF+Pepper+Sorghum)		Sorghum (AMF+Pepper+Sorghum)		Sorghum (AMF+Sorghum)	
	RFW	RDW	RFW	RDW	RFW	RDW	RFW	RDW
T1	2.70	0.45	2.80	0.43	4.34	1.43	4.25	1.30
T2	2.63	0.42	2.20	0.48	4.14	1.30	4.27	1.40
T3	2.04	0.53	2.43	0.44	4.36	1.26	4.21	1.43
T4	1.83	0.34	2.06	0.37	3.56	0.60	3.63	0.70
T5	2.51	0.85	3.03	0.97	4.66	1.53	4.70	1.76
SEM±	0.099	0.050	0.104	0.062	0.105	0.094	0.097	0.100
CD @ 5%	0.268	0.236	0.577	0.061	0.397	0.244	0.412	0.322

RFW: Root fresh weight
RDW: Root dry weight
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*
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

statistically on par with plant inoculated with mycorrhiza from natural soil (0.156%).

Potassium content was high in plant inoculated with *Glomus fasciculatum* (2.53%) which was statistically on par with plant inoculated with mycorrhiza from organic soil (2.33%), plant inoculated with mycorrhiza from inorganic soil (2.29%) and plant inoculated with mycorrhiza from natural soil (2.42%). Lowest potassium content was recorded in control (1.83%).

Nitrogen content was higher in plant inoculated with *Glomus fasciculatum* (2.84%) and lowest nitrogen content was observed in control (1.84%).Plant inoculated with mycorrhiza from organic soil (2.00%) was on par with plant inoculated with mycorrhiza from inorganic soil (1.98%).

Experiment 2 Plant growth response and biochemical changes in black pepper and sorghum due to mycorrhizal inoculation

The main objective of this experiment was to study the growth response of black pepper and sorghum plants to the inoculation of 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*). The results obtained are presented below.

Influence of mycorrhizal inoculation on plant height of black pepper at 180 days after planting and sorghum at harvest is given in table 1. At 180 days after planting maximum plant height was noticed in plant inoculated with mycorrhiza from natural soil (26.97 cm) which was statistically on par with plant inoculated with mycorrhiza from organic soil (26.66 cm) and plant inoculated with *Glomus fasciculatum* (24.90 cm). Least plant height was attributed to control (20.77 cm).

At harvest maximum plant height of sorghum was noticed in plant inoculated with mycorrhiza from organic soil (46.13 cm). Plant inoculated with mycorrhiza from inorganic soil (43.10 cm) was statistically on par with plant inoculated with mycorrhiza from natural soil (40.37 cm) and plant inoculated with *Glomus fasciculatum* (42.07 cm). Plant height was recorded least in control (37.00 cm).

Influence of mycorrhizal inoculation on number of leaves of black pepper at 180 days after planting and sorghum at harvest is given in table 1. At 180 days after planting maximum number of leaves of black pepper was recorded in plants inoculated with *Glomus fasciculatum* (14.33) and least number of leaves was observed in control (11.33). At harvest maximum number of leaves of sorghum was noticed in plant inoculated with inorganic soil (15.67) and number of leaves was recorded least in control (12.33).

The data on influence of mycorrhizal inoculation on root fresh weight and root dry weight of black pepper and Sorghum is presented in table 2 and data on influence of mycorrhizal inoculation on shoot fresh and shoot dry weight of black pepper and sorghum is presented in table 3. Maximum root fresh weight of black pepper was noticed in plant inoculated with *Glomus fasciculatum* (3.03g) and lowest root fresh weight was observed in control (2.06g). Maximum root dry weight of black pepper was noticed in plant inoculated with *Glomus fasciculatum* (0.97g) and lowest root dry weight was recorded in control (0.37g). Maximum shoot fresh weight of black pepper was noticed in plant inoculated with *Glomus fasciculatum* (12.47g). Maximum shoot dry weight was noticed in plant inoculated with *Glomus fasciculatum* (3.07g) and lowest shoot dry weight was observed in control (0.71g). Maximum root fresh weight of sorghum was noticed in plant inoculated with *Glomus fasciculatum* (4.67g) and lowest root fresh weight was observed in control (3.56g), maximum root dry weight was noticed in plant inoculated with *Glomus fasciculatum* (1.53mg) and lowest root dry weight was recorded in control (0.60g. Maximum shoot fresh weight of sorghum was noticed in plant inoculated with *Glomus fasciculatum* (32.23g) and lowest shoot fresh weight was observed in control (18.30g). Maximum shoot dry weight of sorghum was noticed in plants inoculated with *Glomus fasciculatum* (6.45g) and lowest shoot dry weight was observed in control (2.03g).

The data on influence of mycorrhizal inoculation on total chlorophyll content in black pepper and sorghum is presented in table 4. Maximum total chlorophyll content of black pepper was noticed in plant inoculated with mycorrhiza from natural soil (2.73mg/g) and lowest total chlorophyll content was observed in control (2.36mg/g). Maximum total chlorophyll content of

Table 3. Effect of inoculation of AM on shoot fresh weight and shoot dry weight of *Piper nigrum* L. (at 180 days after planting) and Sorghum bicolor (L.) Moench (at harvest)

Treatments	Root fresh and dry weight (g)							
	Pepper (AMF+Pepper)		Pepper (AMF+Pepper+Sorghum)		Sorghum (AMF+Pepper+Sorghum)		Sorghum (AMF+Sorghum)	
	SFW	SDW	SFW	SDW	SFW	SDW	SFW	SDW
T1	11.97	2.60	11.23	2.40	26.80	4.50	25.33	4.20
T2	11.37	2.27	12.10	2.47	23.87	4.03	24.67	4.33
T3	10.77	3.00	10.53	2.30	25.03	3.77	23.77	3.97
T4	5.76	0.86	5.17	0.71	18.30	2.03	18.20	1.80
T5	12.97	3.13	12.47	3.06	32.23	6.47	32.43	5.70
SEM±	0.723	0.234	0.766	0.226	1.300	0.411	1.311	0.362
CD @ 5%	0.787	0.213	0.393	0.227	2.127	0.448	2.626	0.427

SFW: Shoot fresh weight
SDW: Shoot dry weight
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
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

Table 4. Effect of inoculation of AM on total chlorophyll content and total soluble protein content of *Piper nigrum* L. and *Sorghum bicolor* (L.) Moench

Treatments	Total chlorophyll content (mg/g fresh weight of sample)				Total soluble protein content (%)			
	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper+ Sorghum) At Harvest	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper Sorghum) At Harvest
T1	2.55	2.60	2.55	2.70	8.47	8.50	7.18	7.27
T2	2.77	2.64	2.73	2.56	8.37	8.56	7.37	7.31
T3	2.63	2.73	2.53	2.36	7.96	7.63	7.17	7.39
T4	1.88	2.36	2.30	2.06	6.17	6.37	6.17	6.33
T5	3.00	2.63	3.03	2.43	8.30	8.49	7.10	7.25
SEM±	0.107	0.035	0.070	0.061	0.247	0.243	0.122	0.112
CD @ 5%	0.352	0.217	0.390	0.285	0.221	0.207	0.650	0.400

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
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

sorghum was noticed in plant inoculated with mycorrhiza from organic soil (2.70mg/g) and lowest total chlorophyll content was observed in control (2.10mg/g).

The data on influence of mycorrhizal inoculation on total sugar content in black pepper and sorghum is presented in table 5. The total sugar content of black pepper was high in plant inoculated with mycorrhiza from natural soil (2.64mg/100g) which was on par with plant inoculated with *Glomus fasciculatum* (2.61mg/100g) and lowest total sugar content was noticed in control (1.80mg/100g).The total sugar content of sorghum was high in plant inoculated with *Glomus fasciculatum* (1.33mg/100g) and the lowest total sugar content was noticed in control (1.01mg/100g). The data on influence of mycorrhizal inoculation on total reducing sugar content in black pepper and sorghum is presented in table 5.The total reducing sugar content of black pepper was high in plants inoculated with mycorrhiza from natural soil (2.71mg/100g) and lowest total sugar content was noticed in control (1.99mg/100g). The total reducing sugar content of sorghum was high in plant inoculated with mycorrhiza from natural soil (2.60mg/100g) which was on par with plant inoculated with *Glomus fasciculatum* (2.54mg/100g) and lowest total reducing sugar content was noticed in control (1.91mg/100g).

The data pertaining to influence of mycorrhizal inoculation on Total soluble protein content in black pepper and sorghum is presented in table 4. Total soluble protein of black pepper was high in plant inoculated with mycorrhiza from high input soil (8.56%) and lowest total soluble protein content was recorded in control (6.37%). Total soluble protein of sorghum was high in plant inoculated with mycorrhiza from natural soil (7.39%) and lowest total soluble protein content was recorded in control (6.33%).

The data on influence of mycorrhizal inoculation on phosphorus, potassium and nitrogen content in black pepper and sorghum is presented in table 6.

Phosphorus content of black pepper was high in plant inoculated with *Glomus fasciculatum* (0.234%) and lowest phosphorus content was recorded in plant inoculated with mycorrhiza from natural soil (0.130%). Phosphorus content of sorghum was high in plant inoculated with *Glomus fasciculatum* (0.322%) and lowest phosphorus content was recorded in control (0.126%). Potassium content of black pepper was high in plant inoculated with *Glomus fasciculatum* (2.64%) and lowest potassium content was noticed in control (1.88%). Potassium content of sorghum was high in plant inoculated with *Glomus fasciculatum* (2.57%) and lowest in control (1.90%). Nitrogen content of black pepper was higher in plant inoculated with *Glomus fasciculatum* (2.80%) and low in control (1.93%). Nitrogen content of sorghum was higher in plant inoculated with *Glomus fasciculatum* (2.46%) and nitrogen content was observed low in control (1.82%).

Experiment 3 Plant growth response and biochemical changes in sorghum due to mycorrhizal inoculation

The main objective of this experiment was to study the 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*). The results obtained are presented below;

Influence of mycorrhizal inoculation on plant height of sorghum at harvest is given in table 1. At harvest maximum plant height was noticed in plant inoculated with *Glomus fasciculatum* (52.33 cm) and control (38.67 cm) showed least plant height. All other treatments were statistically significant.

Influence of mycorrhizal inoculation on number of leaves of sorghum at at harvest is given in table 1. At harvest maximum number of leaves was noticed in plant inoculated with *Glomus fasciculatum* (15.67) which was on par with plant inoculated with mycorrhiza from natural soil (15.33) and control (12.00) showed lowest number of leaves. All other treatments were statistically significant.

The result on the influence of mycorrhizal inoculation on the root fresh weight, root dry weight, shoot fresh weight and shoot dry weight is presented in table 2 and 3. Maximum root fresh weight was noticed in plant inoculated with *Glomus fasciculatum* (4.70g) and lowest root fresh weight was observed in control (3.64g). Maximum root

Table 5. Effect of inoculation of AM on Total sugar content and total reducing sugar content of *Piper nigrum* L. and *Sorghum bicolor* (L.) Moench

Treatments	Total sugar content (mg/100g fresh weight of sample)				Total reducing sugar content (mg/100g fresh weight of sample)			
	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper+ Sorghum) At Harvest	Pepper (AMF+Pepper) 180 DAP	Pepper (AMF+Pepper+ Sorghum) 180 DAP	Sorghum (AMF+ Sorghum) At Harvest	Sorghum (AMF+Pepper Sorghum) At Harvest
T1	1.96	2.05	1.06	1.23	2.35	2.29	2.32	2.29
T2	2.18	2.03	1.49	1.21	2.29	2.44	2.24	2.10
T3	2.62	2.64	1.22	1.44	2.60	2.71	2.64	2.60
T4	1.80	1.79	0.81	0.90	1.93	2.01	1.84	1.91
T5	2.57	2.61	1.38	1.33	2.75	2.60	2.25	2.54
SEM±	0.094	0.097	0.070	0.052	0.080	0.073	0.074	0.074
CD @ 5%	0.126	0.194	0.199	0.280	0.510	0.475	0.384	0.350

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
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

Table 6. Effect of inoculation of AM on percentage of phosphorus, potassium and nitrogen content of *Piper nigrum* L. and *Sorghum bicolor* (L.*)* Moench

Treatments	Percentage of phosphorus (P), potassium (K) and nitrogen (N) content											
	Pepper (AMF+Pepper)			Pepper (AMF+Pepper+Sorghum)			Sorghum(AMF+Pepper+Sorghum)			Sorghum(AMF+Sorghum)		
	P	K	N	P	K	N	P	K	N	P	K	N
T1	0.18	2.33	1.99	0.19	2.10	1.98	0.23	2.22	2.12	0.26	2.22	2.27
T2	0.15	2.29	1.96	0.16	2.34	2.14	0.24	2.43	2.30	0.24	2.31	2.30
T3	0.18	2.43	2.11	0.13	2.53	2.04	0.26	2.50	2.21	0.24	2.40	2.30
T4	0.16	1.92	1.84	0.14	1.88	1.93	0.13	1.88	1.82	0.16	1.72	1.99
T5	0.22	2.53	2.84	0.23	2.64	2.79	0.32	2.57	2.46	0.33	2.47	2.62
SEM±	0.007	0.069	0.102	0.010	0.080	0.091	0.018	0.071	0.061	0.015	0.076	0.057
CD @ 5%	0.036	0.302	0.102	0.026	0.320	0.188	0.030	0.426	0.221	0.028	0.457	0.183

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*
P+S: Pepper and Sorghum simultaneously inoculated with mycorrhiza

dry weight was noticed in plant inoculated with *Glomus fasciculatum* (1.77g) and lowest root dry weight was observed in control (0.70g). Maximum shoot fresh weight was noticed in plant inoculated with *Glomus fasciculatum* (32.43g) and lowest shoot fresh weight was observed in control (18.20g). Maximum shoot dry weight was noticed in plant inoculated with *Glomus fasciculatum* (5.70g) and lowest shoot dry weight was observed in control (1.80g).

The result on the influence of mycorrhizal inoculation on the total chlorophyll content is presented in table 4. Maximum total chlorophyll content was noticed in plant inoculated with *Glomus fasciculatum* (3.03mg/g) and lowest total chlorophyll content was observed in control (2.30mg/g).

The result on the influence of mycorrhizal inoculation on the total reducing sugars content is presented in table 4. Total soluble protein was high in plant inoculated with mycorrhiza from inorganic soil (7.18%) was statistically on par with plant inoculated with mycorrhiza from organic soil (7.18%) and plant inoculated with *Glomus fasciculatum* (7.09%).

The result on the influence of mycorrhizal inoculation on the total sugars content is presented in table 5. The total sugar content was high in plant inoculated with mycorrhiza from inorganic soil (1.48mg/100g) which was statistically on par with plant inoculated with *Glomus fasciculatum* (1.39mg/100g) and the lowest total sugar content was noticed in control (0.82mg/100g).

The result on the influence of mycorrhizal inoculation on the total reducing sugars content is presented in table 5. The total reducing sugar content was high in plant inoculated with mycorrhiza from natural soil (2.64mg/100g) and lowest total sugar content was noticed in control (1.84mg/100g).

The result on the influence of mycorrhizal inoculation on phosphorus, potassium and nitrogen content is presented in table 6.

Phosphorus content was high in plant inoculated with *Glomus fasciculatum* (0.33%) and lowest phosphorus content was recorded in control (0.16%). Plant inoculated with mycorrhiza from natural soil (0.24%) was on par with plant inoculated with mycorrhiza from organic soil (0.23%) and plant inoculated with mycorrhiza from inorganic soil (0.24%). Higher potassium content was recorded in plant inoculated with *Glomus fasciculatum* (2.47%) and potassium content was lower in control (1.72%). Plant inoculated with mycorrhiza from natural soil (2.34%) was on par with plant inoculated with mycorrhiza from inorganic soil (2.31%) and plant inoculated with mycorrhiza from organic soil (2.22%). There was a significant difference in all treatments with respect to potassium content. Nitrogen content was higher in plant inoculated with *Glomus fasciculatum* (2.62%) and lowest nitrogen content was observed in control (1.99%). Plant inoculated with mycorrhiza from natural soil (2.30%) was on par with plant inoculated with mycorrhiza from inorganic soil (2.30%).

DISCUSSION

The results of the investigation on AM fungi (AMF) and other beneficial soil microorganisms, biochemical analysis and their effects on growth of black pepper and sorghum on plant system due to beneficial microorganisms and inoculation of mycorrhiza from different crop management systems is discussed.

The experiment was conducted with three combinations to study the 1) Growth response of black pepper alone, 2) Growth response of both black pepper and sorghum and 3) Growth response of sorghum alone to the inoculation of AM fungi and other beneficial microorganisms from different crop management systems.

In all the three combinations of the effect of inoculation of AM fungi on plant height of black pepper and sorghum was significant. This increase in plant height is due to increase in higher phosphorus uptake and growth promoting activities by the AM fungi. Colonization of the root system by AM fungi confers benefits directly to the host plant growth and development, through the acquisition of phosphate and other mineral nutrients from the soil[12] (Newsham *et al*., 1995) and increased plant height may be due to the increased PGPR activity[13] (Brown,1974).

In the first month number of leaves per plant was not significantly different among the treatments in black pepper and sorghum, but it was more than the control.

There is a significant difference among

the treated plants with respect to biomass .Higher shoot fresh and dry weight was significant among the plants inoculated with AM fungi. The increased biomass of plants inoculated with mycorrhizal fungus was also observed in *Fraxinus* sp[14] (Lamar and Davey, 1988).

[15]Earanna *et al*., (1999) in their study have recorded significantly higher plant biomass in two medicinal plants *Coleus aromaticus* and *Coleus barbatus* on inoculation with *Glomus fasciculatum*.

There was a considerable difference in the chlorophyll content of the leaves of the mycorrhizal inoculated and control in all the three experiments. This may be due to the enhanced nutrient uptake by the plant through AM infected root system. Similar results were observed in [16]Liang *et al*. (1995) on increased chlorophyll content and photosynthetic rates and enhanced nutrient uptake in tree species inoculated with mycorrhizal fungus.

Total sugar content in the treated plants were found to be significant than the control in all the three experiments. This may be due to enhanced depletion of sugars by hydrolysis and their utilization during initiation was reported by earlier workers[17] Adarshabala *et al*. (1970) in *Bryophyllum tubiflorum*, [18]Hegde (1981) in pepper and [19]Prasad *et al*. (1980) in *Clerodendron infortunatum*. An increase in the reducing sugar content was reported in tomato plants inoculated with *Glomus mosseae* + *Meloidogyne incognita*[20] (Suresh and Bagyaraj, 1984).

Generally leaves contain low total sugar content than the spikes. Plants always maintain equilibrium of soluble sugars in the source and whenever the concentration exceeds, it is either converted to polysaccharide starch or interconverted to other primary products or translocated to other organs for the synthesis of secondary compounds. Once it is in the form of starch, further transformations are slow or limited[21] (Raj *et al*., 2001), hence lower amount of sugars is possible. This may be due to increased number of leaves and chlorophyll content of leaves, which led to increase in photosynthetic rates of inoculated plants. Similarly the same results were reported in tomato, inoculated with mycorrhizal fungus[22] (Shivakumara, 2002).

The soluble protein content was significantly varied between the treatments highest soluble protein content was recorded in inoculated plants than the control. Similar results were observed for soluble protein contents in different accessions of *Mucuna pruriens*[23] (Vadivel and Janardhanan, 2000) and in foliage of three species of Curcuma[24] (Niranjana *et al*., 2003).

With respect to chemical composition all the plants inoculated with *Glomus fasciculatum* showed difference in the phosphorus content than the control. This may be due to increased P solubilization and uptake by mycorrhizal mediated nutrient absorption. [25]Lopezaguillon and Garbaye (1989) repoted similar results for increased dry weight and phosphorus content of hybrid poplars inoculated with *Glomus mosseae* and *Paxillus involuthus* than either fungus alone. The extensive proliferation of hyphae increases the surface area of absorption to absorb phosphorus from the soil solution and transport it in to roots[26] (Abbot and Robson, 1982). Similarly, [27]Melin and Nilsson (1952) reported the increased uptake of phosphorus and nitrogen in seedlings of *Pinus sylvestris* inoculated with ectomycorrhiza.

The potassium content was higher in the plants inoculated with *Glomus fasciculatum* than the control. This may be due to the increased absorption of potassium in the mycorrhiza inoculated plant. The overall 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*.

REFERENCES

1. Anonymous, Black-pepper rapid multiplication, *Spice India.*, 1998; **12**(3): 89-90.
2. Boddington, C.L., Dodd, J.C. The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. II. Studies in experimental microcosms. *Plant and Soil.*, 2000; **218:** 145–157.
3. Thanuja, T. V., Hedge, R. V., Sreenivasa, M. N. Induction of rooting and root growth in black pepper cuttings (*Piper nigrum* L.) with inoculation of arbuscular mycorrhizae. Scientia Horti., 2002; **92**(3-4): 339-346.
4. Hiscox, J.D., Israedstam G.F. A method for extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, 1979; **57:** 1332-1334.

5. Dubios, M., Gills, K. A., Hamilton, J. K., Rebers, P. A. Smith, F. M. Colorimetric for the determination of the sugars and related substances. *Anol. Chem*., 1996; **28:** 350-356.
6. Nelson, N. A photometric adoption of the somogyi method for the determination of glucose. *J.biolo.Chem*., 1944; **153:** 375-380.
7. Somogyi, M. Notes on sugar determination.*J. biol. Chem*., 1952; **195:** 19–23.
8. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.*, 1951; **193:** 265-275.
9. Jackson, M.L. *Soil chemical analysis*., 1973. Prentice Hall of India, New Delhi.
10. A.O.A.C., Official methods of analysis, 13th Edn. Washington D.C., The association of official analytical chemists, 1980.
11. Little, T.M., Hills, F. J. , experimentation. John Wiley and sons. Inc., USA, 1978.
12. Newsham, K. K., Fitter, A. H., Watterson, A. R. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J. Ecol.*, 1995; **83**: 991-1000.
13. Brown, M.E. Seed and root bacterization. *Ann. Rev. Phytopath*., 1974; **53**: 181.
14. Lamar, R.T., Davey, C.B. Comparative effectivity of three *Fraxinus pennsylvanica* Marsh and vesicular-arbuscular mycorrhizal fungi in a high phosphorus nursery soil. *New Phytol.*, 1988; **109**: 171-181.
15. Earanna, N., Suresh, C. K., Radhakrishna, D., Farooqui, A. A., Bagyaraj, D. J., Response of VAM inoculation to medicinal plants. XI *Southern Regional Conference on Microbial Inoculants*. 19-20th February. Guntur, India, 1999.
16. Liang, X. T., Brundett, M., Malajczuk, N. Gong Mingqin., Inoculation of forest and fruit trees with vesicular arbuscular mycorrhizal for plantation in Guangix province, china. Mycorrhizas for plantation forestry in Asia: proceedings from an international symposium and workshop, Kapling, Guangdong Province, P.R., China 7-11 November 1995; ACIAR proceedings No. 62: 114-118.
17. Adarshabala, A. N., Anand, V. K. Nanda, K. K. Seasonal changes in rooting response of stem cuttings of *Bryophyllum tubiflorum* and their relationship with biochemical changes. *Ind. J. of Plant physiol*., 1970; **13:** 106-114.
18. Hedge, G.S. 1981, Effect of number of nodes on cuttings and growth regulators on rooting of Panniyur-I pepper (*Piper nigrum* L.) Vine. *M.Sc. (Agri) Thesis*, University of Agricultural Sciences, Dharwad.
19. Prasad, T. K., Mehta, P. M., Dave, Y. S., Suma, T. K. Chemical changes in the regenerating root cuttings of *Clerodendron infortunatum .Indian J. Experimental Biol*., 1980; **18**: 1524-1524.
20. Suresh, C. K., Bagyaraj, D. J. Interaction between a vesicular arbuscular mycorrhiza and a root knot nematode and its effect on growth and chemical composition of tomato. *Nematol. Medit*., 1984; **12**: 31-39.
21. Raj, M.N., Augustin, A. and Nybe, E.V, Biochemical characterisation of medicinal plants in the wild and domestic environments. *J. Med. Arom. Pl. Sci*., 2001; **21**(4A): 623-627.
22. Shivakumara, C., Biological and biochemical variability in *Glomus fasciculatum* and *Bacillus megaterium* isolated from different soil hypes of various agro climatic zones of Karnataka. *M.Sc. (Agri) Thesis* submitted to university of Agricultural Sciences, Bangalore, 2002.
23. Vadivel, V., Janardhanan, K. Preliminary agrobotanical trials and chemical evaluation of *Mucuna pruriens* (Itching beans): A less known food and medicinal legume. *J. Med. Arom. Pl. Sci.*, 2000; **22**: 191-199.
24. Niranjana, A., Dhan Prakash, S.K., Tewari, A., Pande Pushpangadan, P. Chemistry of *Curcuma* species, cultivated on sodic soil. *J. Med. Arom. Pl. Sci.*, 2003; **25**(1): 69-75.
25. Lopezaguillon, R., Garbaye, J. Some aspects of double symbiosis with ectomycorrhizal and VAM fungi. *Agric. Ecosystems Environ.*, 1989; **29**: 263-266.
26. Abbott, L. K., Robson, A. D. The role of vesicular -arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Aust. J. Agric. Res.*, 1982; **33**: 389-408.
27. Melin, F., Nilsson, H. Transfer of labeled nitrogen from glutamic acid to pine seedlings through the mycelium of *Boletus variegates* (SW). *Nature*, 1952; **171**: 434.