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EFFECT OF FLY ASH APPLICATION ON THE GROWTH, NODULATION AND MYCORRHIZATION IN COWPEA (*VIGNA SINENSIS* (L.) VAR. KANCHAN)

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The present study has been undertaken to evaluate the potentiality of AM fungi native to fly ash dumped site, *Rhizobium sp*. and P solubilizer (*Aspergillus niger*) to nullify the adverse effects of fly ash on the growth and yield of Cowpea (*Vigna sinensis* (L.) var. Kanchan). An experiment was set up in pots in green house condition to assess the performance of the crop raised in Agriculture soil of Allahabad amended with organic matter (*Cynodon* 2% w/w) and different concentrations of fly ash (10%, 20%, 30%) and inoculated with all the three bioinoculants.Data show that the high concentrations of fly ash (20% & 30%) without organic matter and microbial inoculants caused a heavy rate of mortality, reduced the root and shoot biomass, nodulation and yield. However, the soil amendment with organic matter and microbial inoculants caused a tremendous improvement in root/shoot biomass, nodulation and yield apart from curtailing the rate of mortality of the plants. Out of three concentrations of fly ash, 10% fly ash gave the best results with organic amendment and microbial inoculations.

Key Words: AM Fungi, *Aspergillus niger*, Cowpea, Fly ash, *Rhizobium*

Fly ash is the inorganic solid waste mineral produced from the combustion of coal during power generation in the thermal power plants. Since more than 70% of the energy today is generated by coal based TPP, enormous amount of fly ash is produced during coal combustion. The current annual production of major coal combustion residues (CCRs) is estimated to be 600 million worldwide, of which about 500 million ton (70-80%) is FA (Ahmaruzzaman 2010). More than 112 million ton of FA is generated annually in India alone, and projections show that the production (including both FA and bottom ash) may exceed 170 million ton per annum by 2015 (Pandey *et al*. 2009, Pandey and Singh 2010). Disposal and utilization of such large quantities of fly ash is an universal problem.

Recently application of fly ash in agriculture is gaining global attention, since it acts as an important source of micronutrients (S, B, Mo, and Ca etc.), improves agronomic properties of soil, and helps in balancing pH and improving moisture retaining capacity of soil. Therefore, large scale utilization of fly ash in soil improvement, wasteland management and reclamation of nutrient deficient soils in terrestrial and agroecosystem is fast emerging at national and international level (Leyval *et al.* 1997, Jamal *et al*. 2002, Zhu *et al*. 2001).

Use of fly ash in agriculture has been shown to increase the yield of cereals, oil seeds, pulses, cotton and sugarcane by 10-15% and vegetables by about 20-40% (Hildebrandt *et al.* 1999).

Besides the pros of fly ash utilization in agriculture, there are several cons associated with long term use of fly ash. Fly ash is deficient in N because it is volatilized during the combustion. It is also deficient in P and is low in microbial activity. Moreover, being rich in trace/heavy metals, long time repeated applications to the soil may result in hyper accumulation of the heavy metals, which may impart toxicity in the soils and hence, in plants (Asokan *et al.* 1996, Saxena *et al.* 1998). Because of these limitations, the sole application of fly ash has been reported to reduce the establishment and germination of transplant plants.

In order to nullify the adverse effects of fly ash and to improve the N and P status of soils and crops there is a need to explore the potentialities of bio-inoculants, especially the nitrogen fixers, phosphate solubilizers and P-scavengers; the arbuscular mycorrhizal fungi. The most fascinating and key role of AM fungi

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played for hosts in fly ash is the amelioration of toxic effects of heavy metals . In fact, it is the AM fungi which protect the host plants from heavy metal toxicity in the flyash.

In view of the above facts present study has been undertaken to evaluate the potentiality of AM fungi native to fly ash dumped site, *Rhizobium* sp. and P solubilizer to improve the growth and yield of cowpea in fly ash amended agricultural soil.

MATERIALS AND METHODS

Site description: For conducting the experiments in the present study, both fly ash and agriculture soil were used. Fly ash was collected from the fly ash dumping sites situated at IFFCO, Phulpur, 25°33'N, 82°6'E near Allahabad, Uttar Pradesh and the agriculture soil from Ganga basin region of Allahabad(Plate1,2). Characteristics of agriculture soil and fly ash used in the experiments are presented in Table-1.

Collection of soil samples: The rhizospheric soil samples were collected from the root region of the plants growing in the vicinity of fly ash damping site of IFFCO, Phulpur, Allahabad. Samples were brought to the laboratory in polythene bags and stored at 5°C until processed.

Isolation of AM fungi: AMF spores were isolated by wet sieving and decanting method (Gerdemann and Nicolson 1963**)**. A known amount of soil was dissolved in water. After thorugh shaking, it was left for some time for the soil particles to settle down. The clear solution was passed through sieve of 500, 350, 210, 150, 90 and 60 micro meters in descending order. The AM spores retained on various sieves were transferred on filter papers. Filter papers were examined under binocular microscope.

Identification of AM fungi: Different AM spores present in the soil were recovered and AM spores were mounted in PVLG and identified to the species level using the synoptic keys of Trappe (1982**),** Schenck and Parez (1990) and INVAM species guide (http//: invam.caf.wvu.edu).

The most dominant indigenous AM fungi were the species of *Acaulospora* and *Glomus viz. Acaulospora denticulate*, *Acaulospora scrobiculata*, *Glomus deserticola*, *Glomus fasiculatum*, *Glomus tortosum, Glomus clarum, Glomus multicaule Glomus intraradices, Glomus mosseae, Glomus multicaule, Gigaspora sp.* etc.

Maintenance of Trap culture: To obtain abundant and healthy spores of different AMF species rhizospheric soils from the plants growing in the vicinity of fly ash dumping site were collected. Shoots were removed at crown and roots were chopped into small fragments. These root segments along with rhizospheric soil were mixed with autoclaved coarse sand soil mixture 1:1 ratio (v/v). These mixtures were then transferred to sterilized earthen pots and seeds of *Trifolium repens* (L.) were sown in each pot. Cultures were grown under greenhouse conditions for three months. After three months spore population was determined in trap cultures. Another set of trap cultures was prepared on *Sorghum bicolor* (L.) using the soil of first set. Mycorrhizal inoculum consisted of soil having 50 AM spores/10 gm. soil, mycelia and infected root fragments (95% root length colonization). This consortium was used as inoculum for the experimental work.

Isolation and Maintenance of Phosphate solubilizing microbes: Soil dilution and plate count method of Timonin (1940**)** was used for isolating/counting of phosphate solubilizing microbes from the rhizospheric soil of the plants growing in the vicinity of fly ash dumping site IFFCO, Phulpur, Allahabad.

All the colonies of phosphorus solubilizing microbes which appeared on the Petri plates and exhibited zone of solubilization were examined carefully, Dominant species were A*spergillus niger*, *Cladosporium sp.*, *Fusarium oxysporum*, *Penicillium sp.* and sub-cultured these dominant species in Pikovskaya's broth media. They were re-examined critically, identified with the help of specific monographs and their phosphate

solubilizing potentiality was estimated. *Aspergillus niger* highest phosphate solubilizing potentiality

.**Isolation and Maintenance of N_2- fixing Bacteria, *Rhizobium leguminosarum*:** *Rhizobium leguminosarum* was isolated from the nodules of cowpea growing in the fields near the fly ash dumping site, IFFCO, Phulpur, Allahabad on Yeast extract Mannitol Agar plates. Large gummy colonies of bacteria that emerged within four or five days were selected, isolated and subsequently transferred on fresh nutrient plates and sub cultured.

Experimental setup: The seeds of Cowpea (*Vigna sinensis* (L.) var. Kanchan) were procured from registered seed shop of Allahabad, which served as the unit of propagation during the experiments.

Experimental Design: An experiment was setup in pots under greenhouse condition to assess the performance of both the crops raised in agriculture soil of Allahabad amended with organic matter (*Cynodon* 2% w/w), different concentration of fly ash (10, 20, 30%) and inoculated with consortium of AM fungi, PSF and *Rhizobium* alone as well as in combination.

The experiment had a complete randomized design in three blocks, eight treatment / block and three replicates / treatment. The treatment were as follows

Block I

Agriculture soil (Control)

Agriculture soil + Phosphate solubilizing fungi (*Aspergillus niger*) (PSF)
Agriculture soil + AM
Agriculture soil+ *Rhizobium* (RHZ)
Agriculture soil + AM+PSF
Agriculture soil+ PSF+RHZ
Agriculture soil+ AM+RHZ
Agriculture soil + PSF+ AM+RHZ

Block II

Agriculture soil + Organic matter (*Cynodon* 2% w/w) (CN)
Agriculture soil + CN + Phosphate solubilizing fungi (*Aspergillus niger*) (PSF)
Agriculture soil + CN + AM
Agriculture soil + CN + RHZ
Agriculture soil + CN + AM +PSF
Agriculture soil + CN+PSF+RHZ
Agriculture soil + CN+ AM + RHZ
Agriculture soil + CN + AM +PSF+RHZ

Block III

Agriculture soil +CN+10% Fly ash
Agriculture soil + Organic matter (CN) + 10% Fly ash + PSF
Agriculture soil + CN + 10% Fly ash + AM
Agriculture soil + CN + 10% Fly ash + RHZ
Agriculture soil + CN + 10% Fly ash + AM + PSF
Agriculture soil + CN+ 10% Fly ash + PSF + RHZ
Agriculture soil + CN + 10% Fly ash + AM + RHZ
Agriculture soil + CN + 10% Fly ash + AM + PSF + RHZ

Block IV

Agriculture soil + CN + 20% Fly ash
Agriculture soil + CN + 20% Fly ash + PSF
Agriculture soil + CN + 20% Fly ash + AM
Agriculture soil +CN + 20% Fly ash + RHZ
Agriculture soil + CN + 20% Fly ash + AM + PSF
Agriculture soil + CN + 20% Fly ash + PSF + RHZ
Agriculture soil +CN + 20% Fly ash + AM + RHZ
Agriculture soil +CN + 20% Fly ash + AM + PSF + RHZ

Block V

Agriculture soil +CN +30% Fly ash
Agriculture soil + CN + 30% Fly ash + PSF
Agriculture soil + CN + 30% Fly ash + AM
Agriculture soil +CN + 30% Fly ash + RHZ
Agriculture soil +CN + AM + 30% Fly ash + PSF
Agriculture soil +CN + 30% Fly ash + PSF + RHZ

Agriculture soil + CN + 30% Fly ash + AM + RHZ

Agriculture soil +CN + 30Fly ash + AM+ PSF + RHZ

Earthen pots were Ôlled with 4 kg soil amended with 2% (w/w) organic matter. All series were supplemented with organic matter except control series. Some sets of experiments were provided with microbial inoculations singly as well as in dual and triple combination.

The above mentioned series were set up in Ôve blocks. In Ôrst block soil in the pots was without any amendments and maintained as control for the experiment. In the second block, soil was amended with *Cynodon*, in the third block soil was amended with 10% Òy ash, whereas in fourth and Ôfth blocks with 20 and 30% Òy ash respectively.

Crops were raised in earthen pots. Seeds were surface sterilised by 3 % (v/v) sodium hypochloride solution for 2-3 minutes and rinsed in sterilized distilled water 2-3 times and dried in shade for 10-15 minutes. In single inoculation series with AM, before sowing the seeds, the mycorrhizal inoculum of AM fungi was separately placed below the seeds by the layering method (Menge *et al*. 1977). The inoculum was spread as a layer at a depth of 3-5 cm in the pot and the seeds were sown just above the inoculum layer. The seeds were covered with a layer of soil to ensure an efÔcient host fungus association. The inoculum consisted of a mixture of infected root pieces and soil with extrametrical spores from cultures of different AM fungi maintained on *Sorghum vulgare* (L.). In single inoculation series with *Rhizobium*, before sowing, the seeds were soaked for 4 hrs in culture suspensions of the isolate of *Rhizobium* (containing approximately 108 cells / ml) prepared from its 8 days old cultures on YEMA liquid medium. For single inoculation series with PSF (*Aspergillus niger*) the seeds were soaked for four hrs in culture suspension (containing approximately 108 conidia / ml) prepared from the 10 days old culture on Pikovskayas liquid medium. For dual inoculation series involving *Rhizobium* and PSF, the crops were raised from seeds treated with a mixture of an equal amount of culture suspensions containing 108 cells or conidia/ml. On the other hand, in dual inoculation series involving *Rhizobium* or PSF and AM fungi, the crops were raised from *Rhizobium* / PSF treated seeds in soil supplemented with inoculum of AM fungi. In triple inoculation series involving *Rhizobium*, PSF and AM fungi, the crops were raised from the seeds treated with *Rhizobium* and PSF supplemented with inoculum of AM fungi. The seeds treated with *Rhizobium* or PSF in single, dual or triple inoculated series were then dried in shade and shown at 10 seeds per pot. Ten seeds per pot were sown and after Ônally emergence and establishment only Ôve seedlings per pot were maintained. Five plants from each treatment series were carefully uprooted at different stages of plant growth *viz*; vegetative, Òowering and fruiting. Samples of roots along with adhering soil were collected and processed for determining the mycorrhizal intensity in the roots and population of AM spores.

Data on dry weight of roots/shoots, number, and dry weight of nodules, number of pods, and dry weight of pods were recorded.

Parameters

Microbiological parameters:

Mycorrhizal Intensity: Mycorrhizal intensity in the roots was processed by the method of Phillips and Hayman (1970).

Mycorrhizal intensity = No. of roots bits infected / Total number of root bits examined 100

AM Spore population: AM spores were isolated by wet sieving and decanting method of Gerdemann and Nicolson (1963). The population of spores in the soil was calculated and expressed in terms of their number per 50g air dried soil.

Growth Parameters:

Five plants per treatment were uprooted at different stages of plant growth to record the data on growth parameters.

Mortality: Ten seeds were sown per pot per treatment. The number of plants that survived out of the total seeds sown was recorded at the emergence stage. The total number of seeds that failed to germinate per treatment was expressed as mortality percentage for each series.

Root and Shoot Biomass: Dry weight of roots and shoots of the plants for each treatment was determined fruiting stage. For recording the dry weight of roots and shoots the samples were oven dried at 70°C for 48 hrs.

Nodulation: Number and dry weight of nodules for each treatment was determined separately at fruiting stage. For recording the dry weight of nodules the samples were oven dried at 70°C for 24 hrs.

Yield: Number of pods and dry weight of pods for each treatment was determined separately at the time of harvest. For recording the dry weight of the seeds the samples were oven dried at 70°C for 48 hrs.

Statistical Analysis: Statistical analysis of all the data by one-way ANOVA using software, SPSS version 16.0 and comparison of the mean valuesbyDuncan'smultiplerangetests($P \leq 0.05$) The graphs were prepared by using Microsoft Excel.

RESULTS AND DISCUSSION

Addition of different concentrations in the agriculture soil caused a significant increase in the mortality of the plants, minimum being in 10% fly ash series while maximum in 30% fly ash added series. The increase in the mortality in plants might be due to the toxic effect of various heavy metals present in fly ash which inhibits seedling germination, survival, establishment and proper and healthy growth of the plants. Pandey *et al.* (2009) also recorded increased rate of mortality of the plants with increasing concentration of fly ash in the soil. They have also observed inhibition in seed germination and post emergence mortality in seedlings of chickpea and lentil in fly ash amended soil. Heavy rate of mortality of tree seedlings during reclamation has been reported by Selvam and Mahadevan (2002) due to deÔciency of essential nutrients (usually N and P), low soil microbial activity, high soluble salt concentrations of trace elements, and the presence of compacted and cement layers on ash disposal sites.

Agriculture soil when amended with organic matter (*Cynodon*) caused appreciable reduction in the rate of mortality in all the treatment series, however, the magnitude of reduction varied with the treatment (Table-3 & Figure 1). Addition of microbial inoculants especially AM fungi, alone as well as in combination caused maximum reduction in the mortality of the plants (Table-3 & Figure 1).It is well known that arbuscular mycorrhizal (AM) fungi play relevant roles for establishment, survival of plant species, and improved soil properties in stressed environments (Ortega-Larrocea *et al.* 2010) by altering the soil microbial communities in rhizosphere directly or indirectly through changes in root exudation patterns (Barea *et al.* 2005) and enhance the soil enzyme activities (Wang *et al.* 2006).The effects of selected isolates of AM fungi play an important role on the plant growth, nutrient uptake, and aggregation of fly ash (Enkhtuya *et al.* 2005, Wu *et al.* 2009). Mycorrhizal fungi, through their mycelia network, accumulate heavy metals from fly ash and retain them within their cells or carry them on their body surface when they form association with the plants. These mycelia threads, along with dense root biomass, assit in binding ash particles. In the present study AM fungi isolated from the plants growing in fly ash pond were used which survived at high concentrations of toxic metals and the nitrogen fixer and P solubilizer played multi-fascinated roles such as P solubilization, heavy metal bioleaching, plant growth promotion, and synergetic effects with mycorrhizal fungi (Medina *et al.* 2006, Yang *et al.* 2009). Kulshreshtha and Khan (1999) studied the impact of fly ash obtained from a thermal power plant at Aligarh, on *Glomus caledonium*

and *Rhizobium sp*. on the roots of *Vigna mungo*. They demonstrated that mycorrhizas and root nodulating bacterium protected the plants from some of the harmful effects caused by fly ash.

Agriculture soil when amended with organic matter and 10% fly ash alone and in combination favoured the mycorrhizal colonization in the roots but addition of 20% and 30% Òy ash had an adverse effect on root colonization (Table-4 & Figure 1). However, signiÔcant increase in root bits infection was recorded in all the treatment series when phosphate solubilizer, nitrogen fixer and AM fungi were inoculated alone as well as in combination, maximum (73%) being in 10% fly ash with all the three inoculants (Table-4 & Figure 1). Same was true for the AM spore population. But for RHZ (*Rhizobium*) and PSF+RHZ (*Rhizobium*) inoculated series where the magnitude of increase in AMF spore population was of a lowest order (Table-5 & Figure 2).Same results were also observed by Garampalli *et al*. (2005) when they studied the effect of fly ash at three different concentrations (10 g, 20 g and 30 g fly ash per kg soil) on the infectivity and effectiveness of arbuscular mycorrhizal fungus (*Glomus aggregatum*) on pigeon pea (*Cajanus cajan* L.) *cv*. Maruti. All the concentrations of fly ash amendment in soil were significantly affected the intensity of AM colonization inside the plant roots. They also reported that higher concentration of fly ash (30 g fly ash per kg soil) suppressed the formation of AM fungal structure. According to Mosse (1975) the soil structure and composition not only affect the spore population but also the biological activity of endophytes. Compaction of the soil reduces the pore size, consequently affecting the sporulation of the fungi. Compaction also decreases development of root system, which in turn affects the development of mycorrhizas (Skujins and Allen 1986).

Root / Shoot dry weights recorded at the harvest, show that addition of 10% fly ash in agriculture soil, amended with organic matter and inoculated with microbial inoculants gave

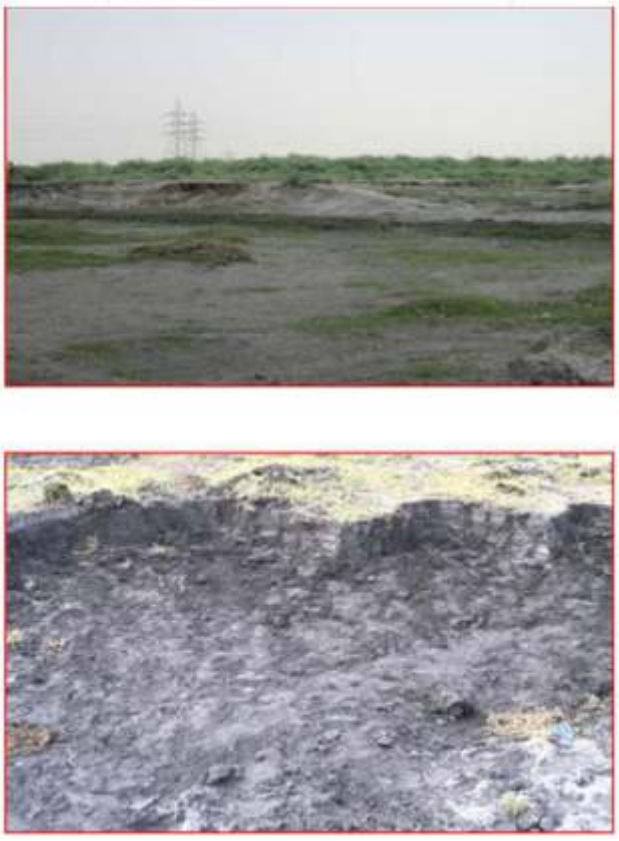
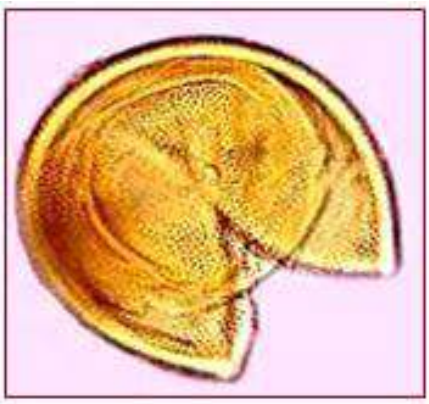


Plate 1: Fly ash dumping site at IFFCO, Phulpur, Allahabd.

Plate 2: Ganga basin area at Allahabad



Acaulospora denticulata

Acaulospora scrobiculata

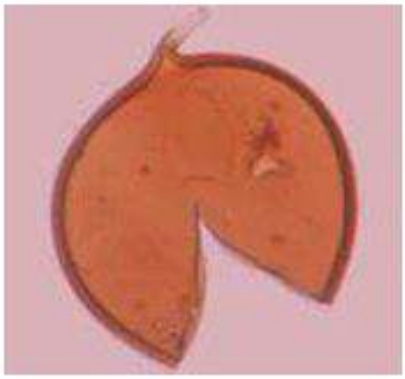
Glomus deserticola

Glomus tortosum

Glomus clarum

Glomus fasciculatum

Glomus intraradices



Glomus mosseae

Gigaspora sp.

Glomus multicaulE

Table 1: Physico-chemical characteristics of agriculture soil and fly ash

	Agriculture soil	Fly ash
Physical		
BD (g cm^{-1})	1.5	<1.0
W.H.C (%)	22-25	35-40
Chemical		
pH	8.1	7.4
Al_2O_3 (PPm)	1.0	18.7
Fe_2O_3 (PPm)	0.37	3.4
CaO (PPm)	0.39	1.54
MgO (PPm)	0.24	0.53
Na_2O (PPm)	0.008	0.05
K_2O (PPm)	232	2.5
SO_3 (PPm)	0.19	0.1
Organic carbon, %	1.2	0.42
Nutrient		
Nitrogen	24	0.030
Phosphorus	29	0.035

Table 2: The phosphate solubilizing potentiality of the isolated phosphate solubilizers

P solubilizing Microbes	P solubilization (ppm)
Aspergillus niger	**0.197**
Cladosporium sp.	0.024
Curvularia sp.	0.072
Fusarium oxysporum	0.124
Penicillium sp. 1	0.091
Penicillium sp. 2	0.026
Penicillium sp. 3	0.062
Penicillium sp. 4	0.026

Table 3: Mortality of Cowpea plan t raised in soil amended with different concentrations of fly ash and *Cynodon* and provided with consortium of AMfungi,PSF andN_2fixer alone as well as in combination

Treatment	Percentage mortality (Pre-emergence)				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	30a	25ab	38a	41a	43a
+PSF	28ab	27a	33b	34b	42ab
+AM	24bcd	22bcd	27cd	29c	36cd
+RHZ	27ab	26ab	32b	35b	38bc
+PSF+AM	22cd	20cd	25de	26cd	33d
+PSF+RHZ	26abc	25ab	30bc	33b	40abc
+AM+RHZ	26abc	23abc	23cde	25cd	27e
+PSF+AM+RHZ	20d	18d	21de	23d	22f
SEMean	±1.1	±1.2	±1.7	±1.8	±2.7

F-critical- 4.47 (n=4), p< 0.05 within each column, means followed by the same letter are not signiÔcantly different by LSD at the 5% level

Table 4: Mycorrhizal intensity in the roots of Cowpea plant raised in agricultural soil amended with 10%, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N fixer as well as in combination

Treatment	Percentage colonization				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	41f	71d	73d	44d	42e
+PSF	62d	73cd	78bc	52c	45e
+AM	72c	75bcd	75cd	64b	55cd
+RHZ	54e	58f	65e	63a	62ab
+PSF+AM	75bc	77bc	79bc	66b	56cd
+PSF+RHZ	56e	62e	67e	64b	53d
+AM+RHZ	77b	79b	81ab	74a	59bc
+PSF+AM+RHZ	82a	83a	85a	76a	65a
SEMean	±4.1	±3.4	±2.7	±2.9	±2.4

F-critical- 2.64 (n=4), p< 0.05 within each column, means followed by the same letter are not significantly different by LSD at the 5% level

Table5:AMsporepopulationinthe rhizospheric soils of Cowpea plant raised in agricultural soil amendedwith10%, 20% and30% fly ash and*Cynodon* and provided with consortium of AM fungi, PSF andN_2 fixer as well as in combination

Treatment	AM spore population (50g air dried soil)				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	24d	36e	38d	27c	25c
+PSF	36c	39de	42c	29c	27c
+AM	38bc	42cd	44bc	35b	31b
+RHZ	25d	25f	29e	26c	25c
+PSF+AM	40bc	45c	47b	36b	32b
+PSF+RHZ	25d	27bf	32e	28c	25c
+AM+RHZ	42b	47b	54a	41a	37a
+PSF+AM+RHZ	47a	55a	57a	44a	39a
SEMean	±3.1	±4	±3.9	±2.5	±2.2

F-critical-2.68 (n=4), p< 0.05 within each column, means followed by the same letter are not signiÔcantly different by LSD at the 5%

Table 6: Dry weight of root of Cowpea raised in agricultural soil amended with 10 %, 20%, 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination.

Treatment	Root dry weight (g) / plant				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	0.431f	0.532a	0.546e	0.543d	0.540e
+PSF	0.521d	0.538a	0.553d	0.550c	0.548d
+AM	0.544cd	0.569a	0.581c	0.576c	0.573c
+RHZ	0.440e	0.470a	0.532g	0.526f	0.518g
+PSF+AM	0.627bc	0.689a	0.846c	0.758c	0.705c
+PSF+RHZ	0.442d	0.472a	0.563f	0.549e	0.534f
+AM+RHZ	0.648b	0.663a	0.861b	0.762b	0.743b
+PSF+AM+RHZ	0.667a	0.682a	0.893a	0.849a	0.818a
SEMean	±0.05	±0.04	±0.15	±0.14	±0.14

F-critical-2.68 (n=4), p< 0.05 within each column, means followed by the same letter are not signiÔcantly different by LSD at the 5%

Table 7: Dry weight of Shoot of Cowpea raised in agricultural soil amended with 10 %, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination.

Treatment	Shoot dry weight (g) / plant				
	AgS	CN	10% FA+ CN	20% FA+ CN	30% FA+ CN
Control	1.580f	1.830a	2.130e	1.910d	1.840e
+PSF	1.630d	1.852a	2.951d	2.390c	2.330d
+AM	1.911cd	3.331a	3.883c	2.540c	2.210c
+RHZ	1.720e	2.632a	2.821g	2.230f	2.190g
+PSF+AM	1.928bc	3.496a	3.910c	2.420c	2.330c
+PSF+RHZ	1.842d	1.858a	2.282f	2.250e	2.240f
+AM+RHZ	1.962b	3.831a	3.460b	2.740b	2.620b
+PSF + AM +RHZ	1.936a	2.115a	3.750a	2.940a	2.790a
SEMean	±0.16	±0.06	±0.26	±0.14	±0.17

F-critical-2.69 (n=4), p<0.05 within each column, mean followed by the same letter are not signiÔcantly different by LSD at the 5% level

Table 8: Number of nodules of Cowpea raised in agricultural soil amended with 10 %, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination.

Treatment	Average Number of nodules / plant				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	22d	25e	37d	33c	30c
+PSF	29c	32de	52c	46c	43c
+AM	53bc	55cd	65bc	59b	48b
+RHZ	43d	45f	54e	49c	36c
+PSF+AM	56bc	59c	67b	62b	44b
+PSF+RHZ	45d	47bf	56e	52c	39c
+AM+RHZ	60b	63b	71a	65a	46a
+PSF+AM+RHZ	62a	65a	76a	68a	50a
SEMean	±4.3	±4.4	±3.5	±3.1	±1.8

F-critical-2.64 (n=4), p<0.05 within each column, mean followed by the same letter are not signiÔcantly different by LSD at the 5% level

Table 9: Dry weight of nodules of Cowpea raised in agricultural soil amended with 10 %, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination.

Treatment	Dry weight of nodules (g) / plant				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	0.029f	0.033a	0.060e	0.058d	0.043e
+PSF	0.036d	0.039a	0.062d	0.059c	0.054d
+AM	0.065cd	0.069a	0.077c	0.062c	0.058c
+RHZ	0.045e	0.049a	0.053g	0.046f	0.041g
+PSF+AM	0.069bc	0.075a	0.089c	0.057c	0.045c
+PSF+RHZ	0.049d	0.053a	0.057f	0.048e	0.043f
+AM+RHZ	0.066b	0.078a	0.092b	0.069b	0.048b
+PSF+AM+RHZ	0.071a	0.089a	0.105a	0.102a	0.094a
SEMean	±0.01	±0.01	±0.04	±0.03	±0.03

F-critical-2.68 (n=4), p<0.05 within each column, mean followed by the same letter are not signiÔcantly different by LSD at the 5% level

Table 10: Average number of pods of Cowpea raised in agricultural soil amended with 10 %, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination

Treatment	Average number of pods / plant				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	4f	5d	9d	7d	5e
+PSF	7d	8cd	13bc	12c	9e
+AM	9c	10bcd	19cd	17b	16cd
+RHZ	5e	6f	12e	10a	8ab
+PSF+AM	6bc	12bc	24bc	22b	20cd
+PSF+RHZ	7e	8e	17e	14b	12d
+AM+RHZ	9b	13b	26ab	24a	15bc
+PSF+AM+RHZ	11a	14a	28a	23a	17a
SEMean	±0.77	±1.1	±2.4	±2.1	±1.6

F-critical-2.68 (n=4), p<0.05 within each column, mean followed by the same letter are not signiÔcantly different by LSD at the 5% level

Table 11: Dry weight of pods of Cowpea raised in agricultural soil amended with 10 %, 20% and 30% fly ash and *Cynodon* and provided with consortium of AM fungi, PSF and N_2 fixer alone as well as in combination.

Treatment	Dry weight of Pods (g) / plant				
	AgS	CN	10% FA + CN	20% FA + CN	30% FA + CN
Control	1.224f	2.175a	4.887e	3.591d	2.480e
+PSF	2.996d	3.800a	7.781d	7.020c	4.932d
+AM	4.635d	5.780b	12.274c	10.625c	9.696c
+RHZ	1.615e	2.550a	7.356g	6.060f	4.736g
+PSF+AM	2.116bc	8.340a	20.208c	16.478c	14.620c
+PSF+RHZ	2.436d	3.640a	11.645f	9.296e	7.476f
+AM+RHZ	4.869b	9.074a	23.114b	18.432b	10.740b
+PSF+AM+RHZ	6.193a	9.870a	25.032a	18.147a	12.665a
SEMean	±6.1	±8.1	±24.1	±17.5	±11.6

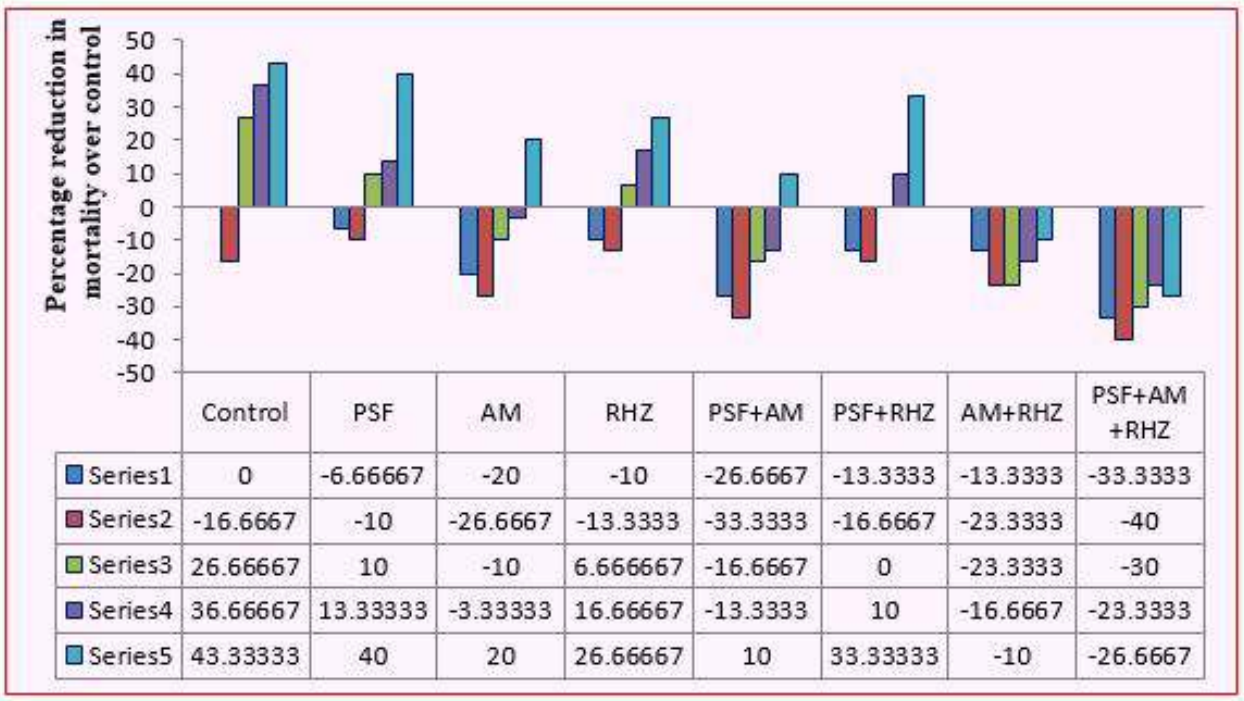
F-critical-2.68 (n=4), p<0.05 within each column, mean followed by the same letter are not signiÔcantly different by LSD at the 5% level

the best performance in comparison to 20 & 30% fly ash added series (Table-6,7 & Figure 2,3). Deleterious effect of high concentrations of Òy ash specially when used in more than 50% levels on plant growth and yield have also been reported by Khan and Khan, (1996) and Raghav *et al*. (2002). However, fly ash amendment with AM inoculation was found to enhance the growth of plants as compared to control plants. Sheela and Sundaram (2003) reported that application of AM fungi with Òy ash increased the plant root and shoot biomass. Plants growing in fly ash inoculated with AM fungi showed a signiÔcant increase in the shoot and root dry weight in comparison to uninoculated plants (Garampalli *et al.* 2005, Juwarkar and Jambhulkar 2007, Ammaiyappan and Ayyamperumal 2002, Kulshreshtha and Khan, 1999, Reddy and Garampalli 2002). Application of Òy ash at 40 t/ha in conjunction with phosphate solubilizer, *Pseudomonas striata* improved the bean yield and did not exert any detrimental effect on the population of *P. striata* in soil (Gaind and Gaur 2002). Juwarkar and Jambhulkar (2007) recorded an increase in the N content of the fly ash when amended with biofertilizers, which helped in biological nitrogen fixation and is a major source of N input. Biologically fixed nitrogen can thus, contribute to the needs of a growing plant, thus contributing its fertility in long run and in a sustainable manner.

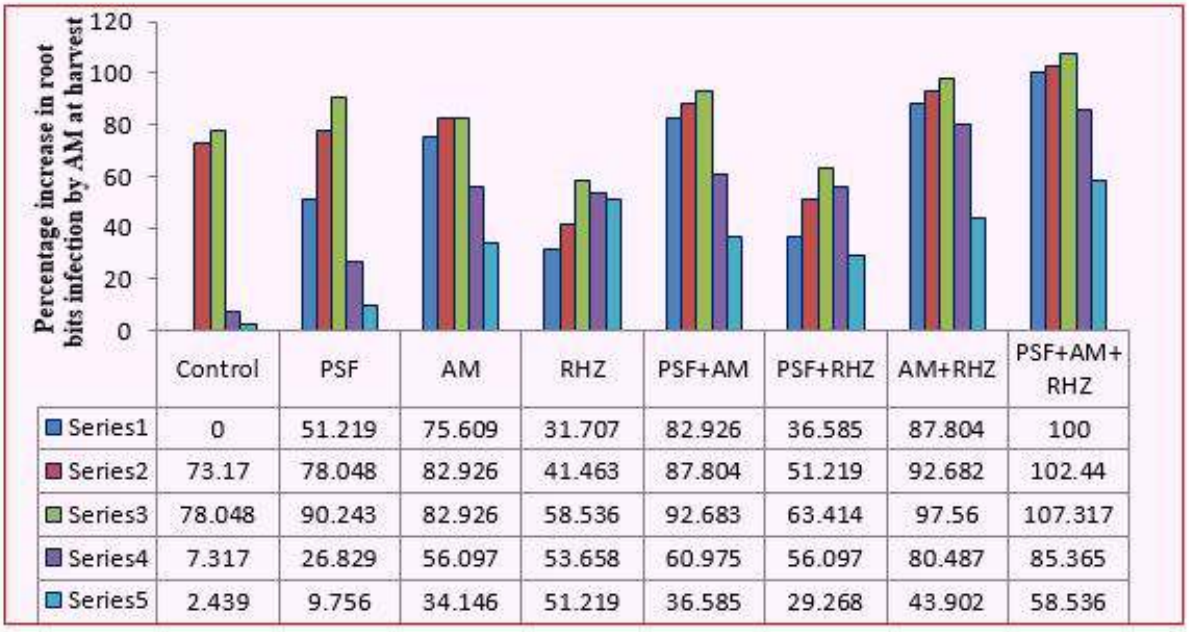
All the microbial inoculants had a favourable effect on nodulation in all the fly ash added series, however in comparison to single or double inoculations, triple inoculation caused the maximum increase in nodule number as well as nodule weight / plant. Same was true for the pod number and dry weight of the seeds (Table-8, 9 & Figure 3, 4). Upto 600% increase over control was recorded in pod number and 1945.08% increase over control in dry weight of pod in 10% fly ash added series, inoculated with all the three microbial inoculants (Table-10, 11 & Figure 4, 5).Maximum nodulation and yield (number of pod, dry weight of pod) in the crops was also recorded in a series where agriculture soil was amended with 10% FA and *Cynodon* and inoculated with all the three microbial inoculants. The results are in conformity with Faizan and Kausar (2010) who were also recorded a signiÔcant increase in nodule number per plant, number of functional nodules per plant and dry weight of the nodules when they added coal ash @ 25%. Singh *et al.* (2011) reported that all the levels of Òy ash suppressed root nodulation signiÔcantly in soybean and suppression gradually increased with the increase of Òy ash in the soil. No nodule was observed at 100% Òy ash level in soybean.

Decline in measured parameters above 10% Òy ash may also be due to reduction in bioavailability of some nutrients due to high pH, high salinity and high content of phytotoxic elements (Pandey and Singh 2010). Some toxic compounds (Helder *et al*. 1983) and metals *viz.*, nickel, arsenic, cadmium, chromium, lead, selenium, zinc, copper etc. (Wadge and Hutton 1987) present in the Òy ash accumulate in plants beyond the threshold level causes reduction in plant growth and yield (Siddiqui *et al.* 2004, Gupta and Sinha 2007, Mishra *et al*. 2007, Yunusa *et al*. 2006).

However, decrease in yield losses of mycorrhizal plants in Òy ash added soils were recorded. The ameliorative effect of the AM fungi can be attributed to the fact that mycorrhizal association improves rooting and root hair production, increases the absorptive surface manifold for the better uptake of nutrients and water, thereby helping in better growth performance of the host plants under stressed conditions. Improvement in plant nutrient uptake, particularly P, due to AM colonization is one of the most important mechanisms of stress tolerance in mycorrhizal plants (Hirrel and Gerdemann 1980). However, the advantages of AM fungi for plant growth and development under stress conditions are not always related to nutrient status. This may be due to increased uptake of nutrients with low mobility, such as P, Zn and Cu (George *et al.* 1994, Marschner and Dell 1994, Ruiz-Lozano *et al.*1995, Al-Karaki and Al-Raddad 1997, Al-Karaki and Clark 1998) and improved water



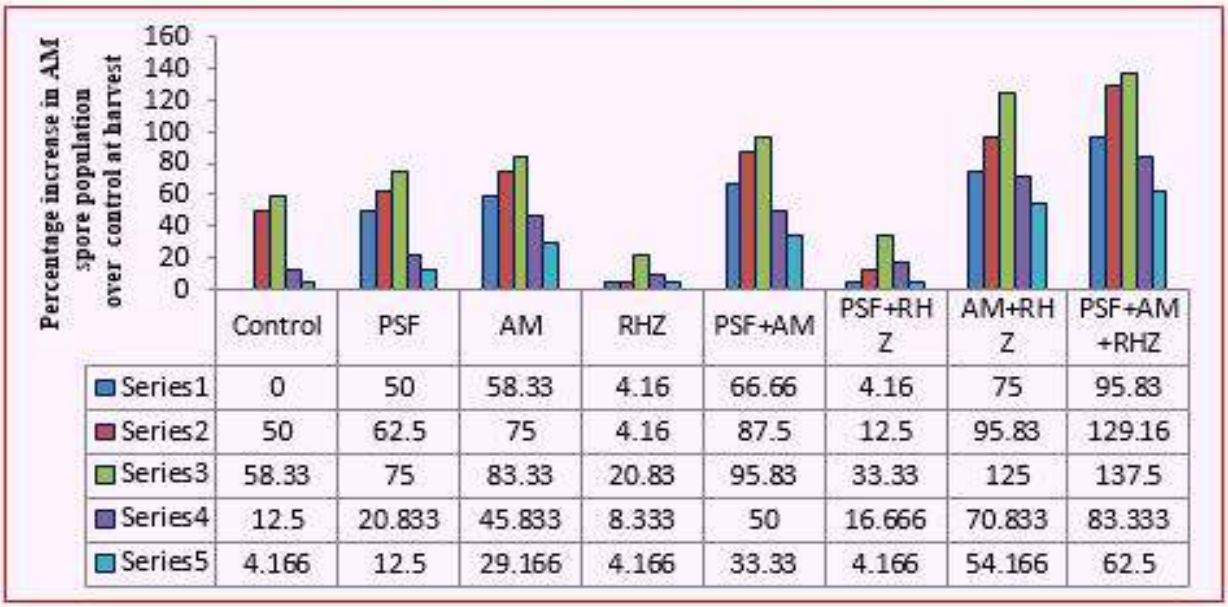
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM +RHZ
Series1	0	-6.66667	-20	-10	-26.6667	-13.3333	-13.3333	-33.3333
Series2	-16.6667	-10	-26.6667	-13.3333	-33.3333	-16.6667	-23.3333	-40
Series3	26.66667	10	-10	6.666667	-16.6667	0	-23.3333	-30
Series4	36.66667	13.33333	-3.33333	16.66667	-13.3333	10	-16.6667	-23.3333
Series5	43.33333	40	20	26.66667	10	33.33333	-10	-26.6667



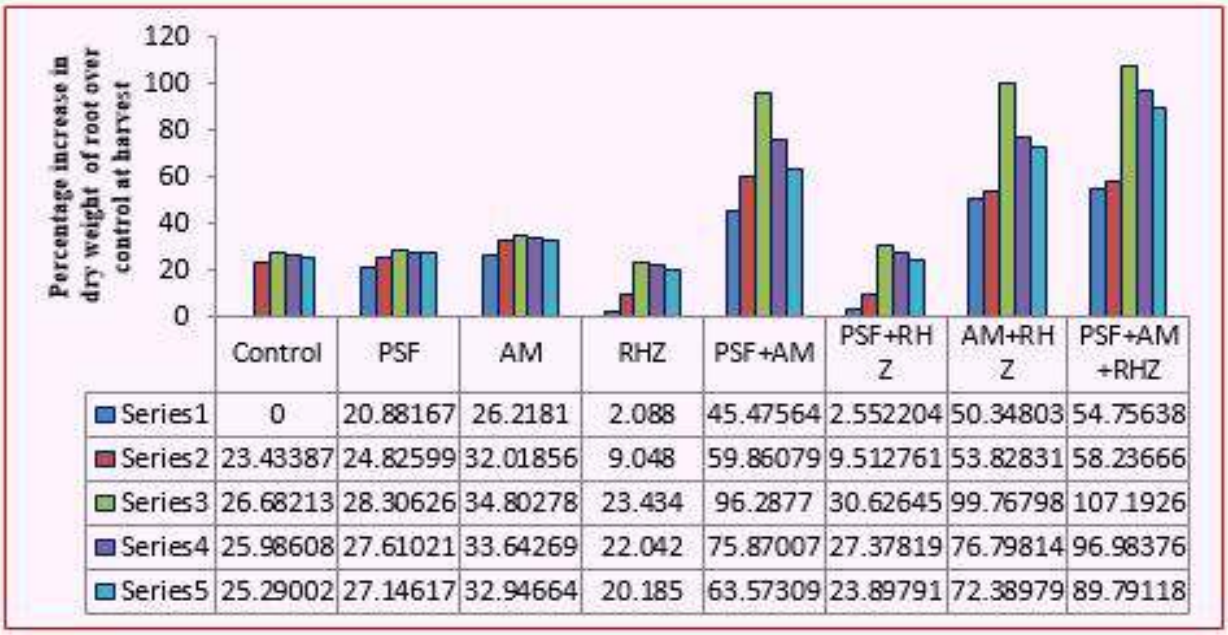
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+ RHZ
Series1	0	51.219	75.609	31.707	82.926	36.585	87.804	100
Series2	73.17	78.048	82.926	41.463	87.804	51.219	92.682	102.44
Series3	78.048	90.243	82.926	58.536	92.683	63.414	97.56	107.317
Series4	7.317	26.829	56.097	53.658	60.975	56.097	80.487	85.365
Series5	2.439	9.756	34.146	51.219	36.585	29.268	43.902	58.536

Series1 : AgS (Agriculture soil)
Series2 : AgS+CN (*Cynodon*)
Series3 : AgS+CN+10%FA (Fly ash)
Series4 : AgS+CN+20%FA
Series5 : AgS+CN+30%FA

Figure 1



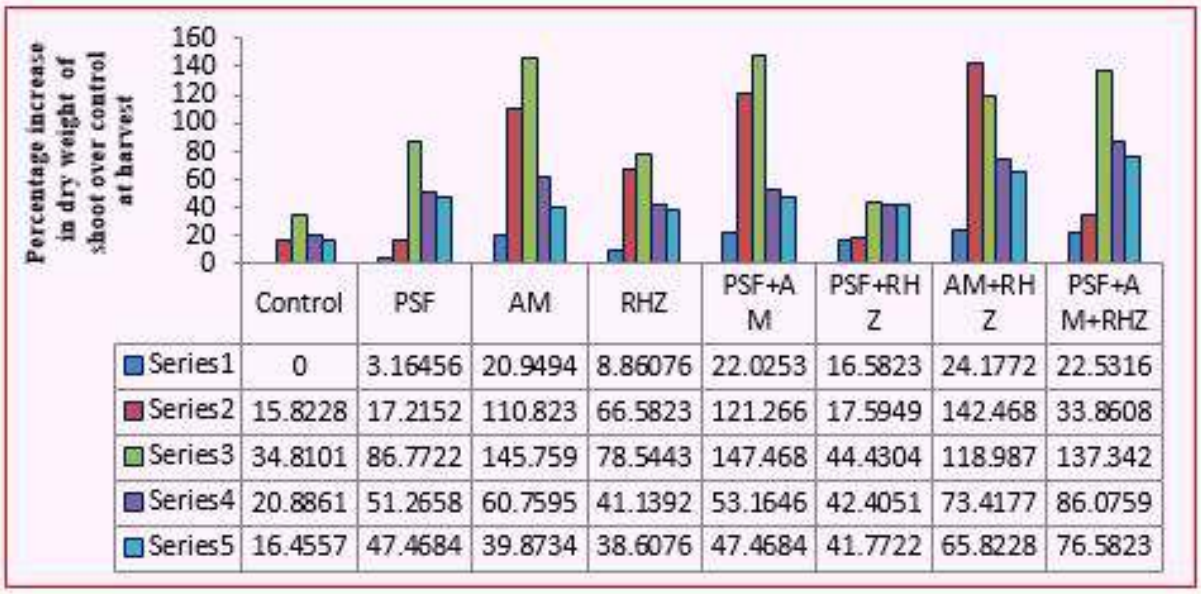
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	50	58.33	4.16	66.66	4.16	75	95.83
Series2	50	62.5	75	4.16	87.5	12.5	95.83	129.16
Series3	58.33	75	83.33	20.83	95.83	33.33	125	137.5
Series4	12.5	20.833	45.833	8.333	50	16.666	70.833	83.333
Series5	4.166	12.5	29.166	4.166	33.33	4.166	54.166	62.5



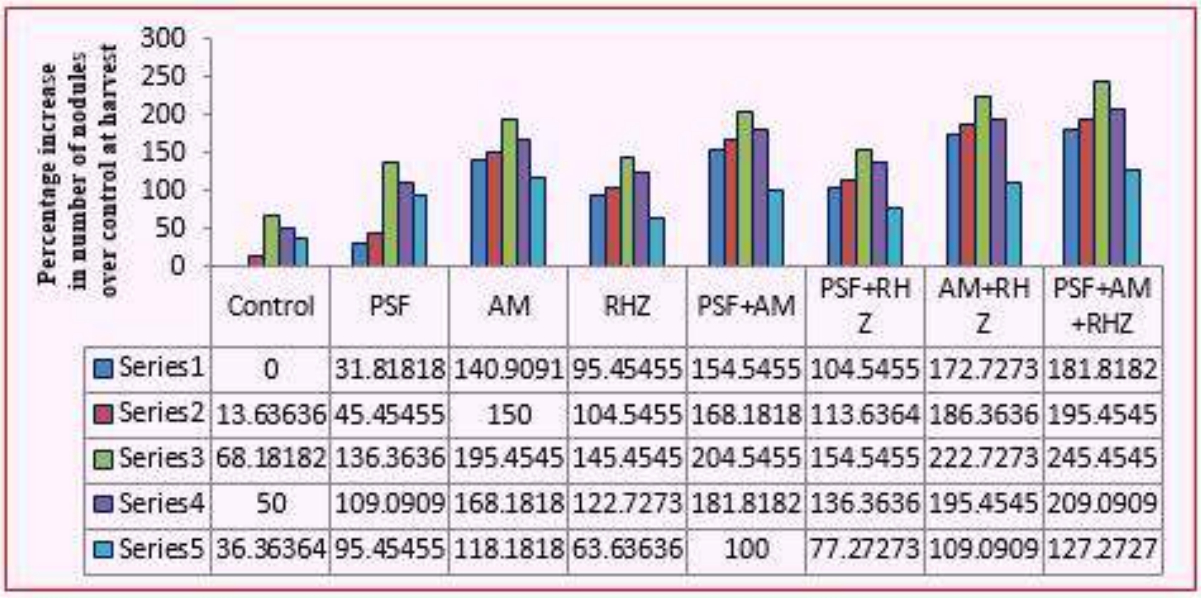
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	20.88167	26.2181	2.088	45.47564	2.552204	50.34803	54.75638
Series2	23.43387	24.82599	32.01856	9.048	59.86079	9.512761	53.82831	58.23666
Series3	26.68213	28.30626	34.80278	23.434	96.2877	30.62645	99.76798	107.1926
Series4	25.98608	27.61021	33.64269	22.042	75.87007	27.37819	76.79814	96.98376
Series5	25.29002	27.14617	32.94664	20.185	63.57309	23.89791	72.38979	89.79118

Series1 : AgS (Agriculture soil)
Series2 : AgS+CN (*Cynodon*)
Series3 : AgS+CN+10%FA (Fly ash)
Series4 : AgS+CN+20%FA
Series5 : AgS+CN+30%FA

Figure 2



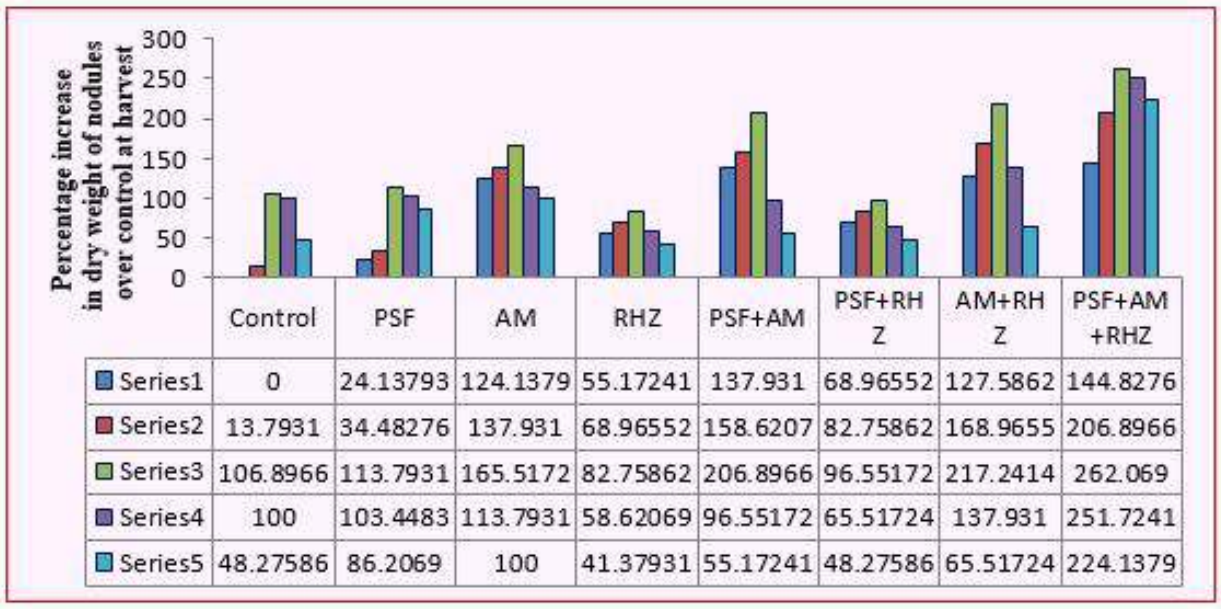
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	3.16456	20.9494	8.86076	22.0253	16.5823	24.1772	22.5316
Series2	15.8228	17.2152	110.823	66.5823	121.266	17.5949	142.468	33.8608
Series3	34.8101	86.7722	145.759	78.5443	147.468	44.4304	118.987	137.342
Series4	20.8861	51.2658	60.7595	41.1392	53.1646	42.4051	73.4177	86.0759
Series5	16.4557	47.4684	39.8734	38.6076	47.4684	41.7722	65.8228	76.5823



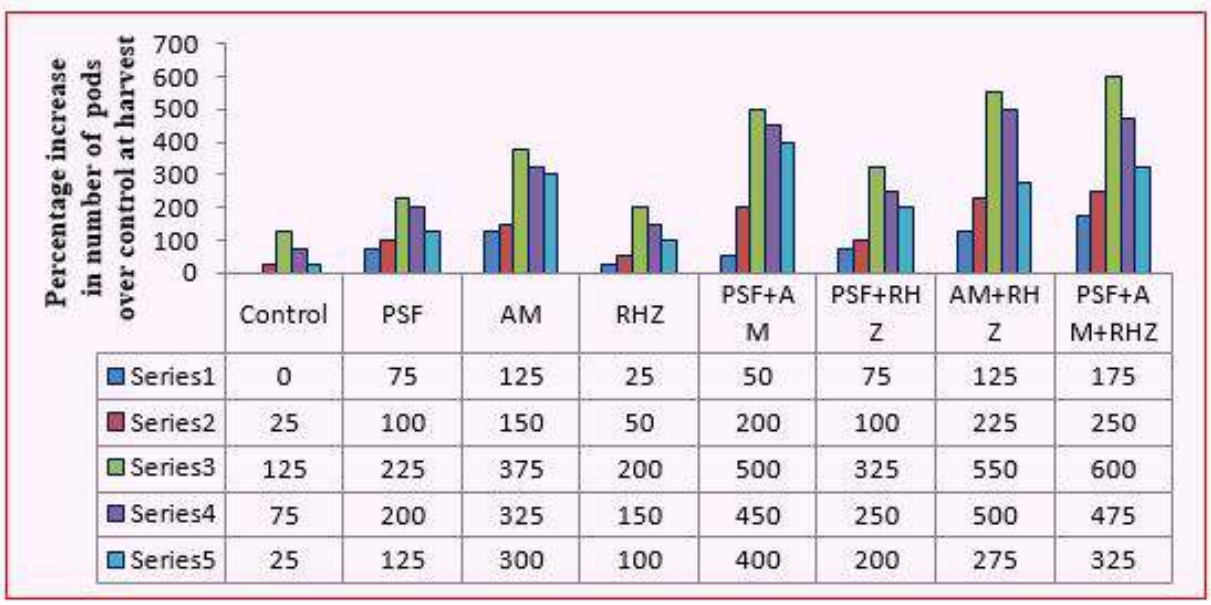
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	31.81818	140.9091	95.45455	154.5455	104.5455	172.7273	181.8182
Series2	13.63636	45.45455	150	104.5455	168.1818	113.6364	186.3636	195.4545
Series3	68.18182	136.3636	195.4545	145.4545	204.5455	154.5455	222.7273	245.4545
Series4	50	109.0909	168.1818	122.7273	181.8182	136.3636	195.4545	209.0909
Series5	36.36364	95.45455	118.1818	63.63636	100	77.27273	109.0909	127.2727

Series1 : AgS (Agriculture soil)
Series2 : AgS+CN (*Cynodon*)
Series3 : AgS+CN+10%FA (Fly ash)
Series4 : AgS+CN+20%FA
Series5 : AgS+CN+30%FA

Figure 3



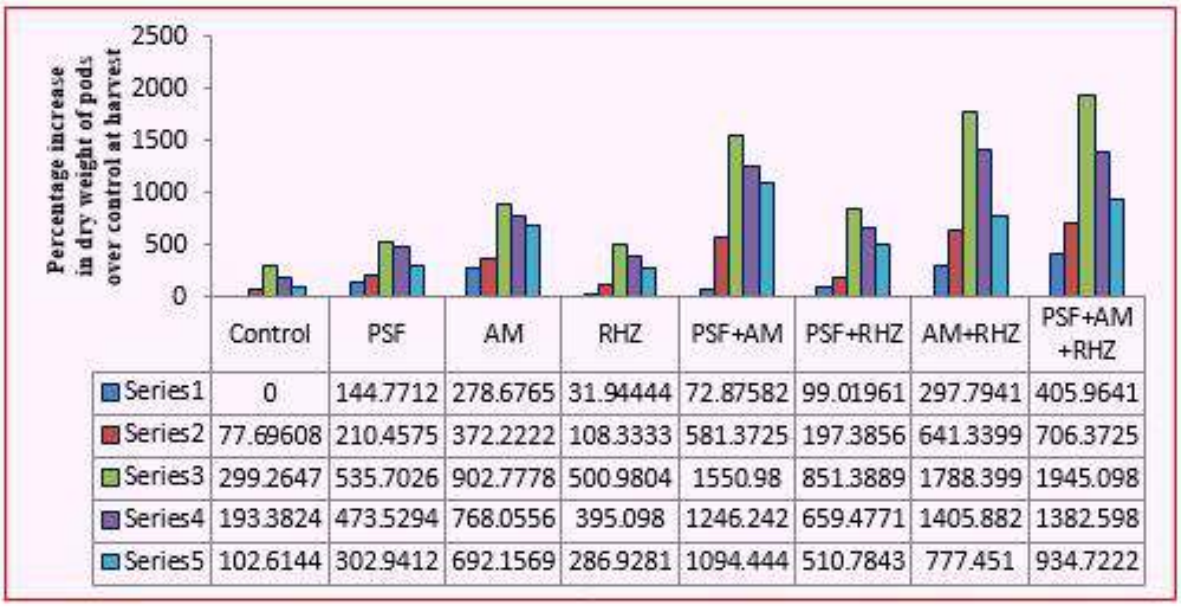
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	24.13793	124.1379	55.17241	137.931	68.96552	127.5862	144.8276
Series2	13.7931	34.48276	137.931	68.96552	158.6207	82.75862	168.9655	206.8966
Series3	106.8966	113.7931	165.5172	82.75862	206.8966	96.55172	217.2414	262.069
Series4	100	103.4483	113.7931	58.62069	96.55172	65.51724	137.931	251.7241
Series5	48.27586	86.2069	100	41.37931	55.17241	48.27586	65.51724	224.1379



	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	75	125	25	50	75	125	175
Series2	25	100	150	50	200	100	225	250
Series3	125	225	375	200	500	325	550	600
Series4	75	200	325	150	450	250	500	475
Series5	25	125	300	100	400	200	275	325

Series1 : AgS (Agriculture soil)
Series2 : AgS+CN (*Cynodon*)
Series3 : AgS+CN+10%FA (Fly ash)
Series4 : AgS+CN+20%FA
Series5 : AgS+CN+30%FA

Figure 4



	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM +RHZ
Series1	0	144.7712	278.6765	31.94444	72.87582	99.01961	297.7941	405.9641
Series2	77.69608	210.4575	372.2222	108.3333	581.3725	197.3856	641.3399	706.3725
Series3	299.2647	535.7026	902.7778	500.9804	1550.98	851.3889	1788.399	1945.098
Series4	193.3824	473.5294	768.0556	395.098	1246.242	659.4771	1405.882	1382.598
Series5	102.6144	302.9412	692.1569	286.9281	1094.444	510.7843	777.451	934.7222

Series1 : AgS (Agriculture soil)
Series2 : AgS+CN (*Cynodon*)
Series3 : AgS+CN+10%FA (Fly ash)
Series4 : AgS+CN+20%FA
Series5 : AgS+CN+30%FA

Figure 5

relations (Bethlenfalvay *et al*. 1988, Sylvia *et al.* 1993, Ruiz-Lozano and Azcon 1995, Al-Karaki and Clark 1988, Ryan and Angus 2003) leading to subsequent dilution of toxic ion effects (Juniper and Abbott 1993).

Best performance in terms of reduced rate of mortality and improved growth, yield and nodulation in cowpea in a soil amended with 10% fly ash and organic matter and inoculated with consortium of AM fungi native to fly ash site with nitrogen fixer and phosphate solubilizer was recorded. Efficiency of AM fungi was increased with the addition of nitrogen fixer which improved the N content of fly ash and phosphate solubilizer which improved the P content of fly ash.

REFERENCES

Ahmaruzzaman M 2010 A review on the utilization of fly ash. Progress in Energy and Combustion. *Science* **36** 327-363.

Al-Karaki GN and Al-Radad A 1997 Effects of arbuscular fungi and rough stres on growth and nutrient uptake of two wheat genotypes difering in their drought resistance. *Mycorrhiza* **7** 83-8.

Al-Karaki GN and Clark RB 1998 Growth, mineral acquisition, and water use by mycorrhizal wheat grown under water stress. *Journal of Plant Nutrition* **21** 263–76.

Ammaiyanppan S and Ayyamperumal M 2002 Distribution of mycorrhizas in an abandoned fly ash pond and mined sites of Neyveli lignite Corporation,Tamil Nadu,India. *Basic and Applied Ecology* **2**(3):277-284.

Asokan P, Saxena, M, Karade, SR, Shrimanth S, Chauhan A and Mandal S 1996 Proceedings of the 3rd International Conference on Power Development

Strategies for 21st Century (Kathmandu, Nepal) **3** 1621-1625.

Barea JM, Pozo, MJ, Azcón R and Azcón-Aguilar C 2005 Microbial co-operation in the rhizosphere. *Journal of Experimental Bot*any **56** 1761–1778.

Bethlenfalvay GJ, Brown MS, Mihara K L and Stafford A E 1988 *Glycine-Glomus-Rhizobium* symbiosis. V. Effects of mycorrhiza on nodule activity and transpiration in soybeans under drought stress. *Journal of Plant Phys*iology **85** 115-119.

Enkhtuya B, Poschl M and Vosatka M 2005 Native grass facilitates mycorrhizal colonization and P uptake of tree seedlings in two anthropogenic substrates. *Water Air and Soil Pollution* **166** 217–236.

Faizan S and Kausar S 2010 Impact of coal ash on growth, yield, biomass and nodulation of lentil (*Lens culinaris*). *Journal of Industrial Pollution Control* **26 (2)** 161-165.

Gaind S and Gaur A C 2002 Impact of fly ash and Phosphate solublising bacteria on soyabean productivity. *Bioresource Technology* **85** 313-315

Garampalli R H, Deene S and Reddy C N 2005 Infectivity and efficacy of *Glomus aggregatum* and growth response of *Cajanus cajan* (L.) mill sp. In fly ash amended sterile soil. *Journal of Environmental Biol*ogy **26(4)** 705-801.

George E, Romheld V and Marschner H 1994 Contribution of mycorrhizal fungi to micronutrient uptake by plants. In: Manthey JA, Crowley DE, Luster DG eds. Biochemistry of metal micronutrients in the rhizosphere. *Lewsi London* 93–109.

Gerdemann J W and Nicholson T H 1963 Spores of mycorrhizal Endogone species extracted from the soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46** 235–244.

Gupta A K, Dwivedi S, Sinhas, Tripathi R D, Rai UN and Singh SN 2007 Metal accumulation and growth performance of *Phaseolus vulgaris* grown in fly ash amended soil. *Bioresource Technology* **98(17)** 3404-7.

Helder T, Stulterheim E and Olte K 1983 The toxicity and toxic potential of fly ash from municipalincinerators assessed by means of fish life stage test. *Chemosphere* **11** 968–972.

Hildebrandt U, Kaldorf M and Bothe H 1999 The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiol*ogy **154** 709–717.

Hirrel M C and Gerdemann J W 1980 Improved growth of onion and bell pepper in saline soils by two vesicular arbuscular mycorrhizal fungi. Proceedings of the soil Science. *Society of America* **44** 654-655.

Jamal A, Ayub N and Usman Mand Khan AG 2002 Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by bean and lentil. *International Journal of Phytoremediation* **4** 205-221.

Juniper S and Abbott L K 1993 Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza* **4** 45–57.

Juwarkar A A and Jambhulkar P H 2007 Restoration of fly ash dump through biological interventions. *Environmental Monitoring Assessment* **139** 355 – 365.

Khan M R and Khan M W 1996 The effect of Òy ash on growth and yield of tomato. *Environmental Pollut*ion **92** 105 – 111.

Kulshreshtha M and Khan M W 1999 Effect of ozone on root colonization by VAM fungi and root nodulation by *Rhizobium* on black gram. Abstracts National Conference on Mycorrhiza, Barkatulla University, Bhopal 64.

Leyval C, Turnau K and Haselwandter K 1997 Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* **7** 139–153.

Marschner H and Dell B 1994 Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* **159** 89–102.

Medina A, Vassileva M, Barea J M and Azcón R

2006 The growth-enhancement of clover by *Aspergillus*-treated sugar beet waste and *Glomus mosseae* inoculation in Zn contaminated soil. *Appllied Soil Ecol*ogy **33** 87–98.

Menge J A, Lembright H and Johnson ELV 1977 Utilization of mycorrhizal fungi in *Citrus* nurseries. *Proceeding International Society of Citriculture* **1** 129.

Mishra M, Sahu RK and Padhy RN 2007 Growth, yield and elemental status of rice (*Oryza sativa*) grown in fly-ash amended soil. *Ecotoxicology* **16** 271–278.

Mosse B 1975 Specificity in VA mycorrhizas. In: Sander FE, Mosse B, Tinker PB eds. *Endomycorrhizas*. Academic Press, London, 469–484

Ortega-Larrocea M P, Xoconostle-Cazares B, Maldonado-Mendoza I, Carrillo-González R, Hernández-Hernández J and Díaz Gardunno M 2010 Plant and fungal biodiversity from metal mine wastes under remediation at Zimapan, Hidalgo, Mexico. *Environmental Poll*ution **158** 1922–1931.

Pandey V C, Abhilash P C, Upadhyay R N and Tewari D D 2009 Application of fly ash on the growth performance, translocation of toxic heavy metals within *Cajanus cajan* L implication for safe utilization of fly ash for agricultural production. *Journal of Hazardous Mater*ial **166** 255–259.

Pandey VC and Singh N 2010 Impact of fly ash incorporation in soil systems. *Agriculture Ecosystems Environment* **136** 16–27.

Phillips JM and Hayman DS 1970 Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Mycological Society* **55** 158–160.

Pikovskaya, RI 1948 Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology* **17** 362–370.

Raghav D and Khan AA 2002 Impact of industrial particulate pollutants applied to soil on growth and yield of tomato. *Thai Journal of Agriculture Sci*ences **35** 187-194.

Reddy C N and Garampalli H R 2002 Effect of Òy ash on VAM formation and growth response of formation and growth response of pulse crops infected with *Glomus aggregatum* in sterile soil. In: *Frontiers in Microbial Biotechnology and Plant Pathology* eds. Manoharachary C, Purohit DK, Ram Reddy S, Singara, Charya MA, Girisham S Jodhpur India, India: ScientiÔc Publisheres (India).

Ruíz-Lozano JM, Azcón R and Gómez M 1995 Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: Physiological and nutritional plant-responses. *Applied Environmental Microbiology* **61** 456-460.

Ryan MH, Angus JF 2003 Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. *Plant Soil* 250 225-239.

Saxena M, Asokan P, Mandal S and Chauhan A 1998 Impact of fly ash phase constituents on Wasteland Soils. *Environmental Energy Conservation* **4(4)** 229–34.

Schenck N C and Perez Y 1990 Manual for the identiÔcation of VAM fungi. University of Florida, Fainesville. *Journal of Agriculture Res*earch **28** 639.

Selvam A and Mahadevan A 2002 Distribution of mycorrhizas in an abandoned fly ash pond and mined sites of Neyveli Lignite Corporation, Tamil Nadu, India. *Basic and Applied Ecology* **3** 277–284.

Shahla F and Saima K 2010 Impact of coal ash on growth, yield, biomass and nodulation of lentil (*Lens culinaris*). *Journal of Indian Pollution Cont*rol **26 (2)** 161-165.

Sheela M A and Sundaram M D 2003 Role of VA-mycorrhizal biofertilizer in establishing black gram (*Vigna mungo* L.) var -T9 in abandoned ash ponds of Neyveli Thermal Power Plant. *Mycorrhiza News* **15** 13–16.

Siddiqui Z A and Singh L P 2004 Effects of soil inoculants on the growth, transpiration and wilt disease of chickpea. *Journal of Plant Diseases and Protec*tion **111** 151-157.

Singh K, Khan A A and Safiuddin 2011 Effect

of fly ash on growth, yield and root-knot disease of soybean. *Nematol medit* **39** 127-131.

Skujins J and Allen MF 1986 Use of mycorrhizae for land rehabilitation. *Mikeem Journal* **2**161-176.

Sylvia D M and Williams S E 1993 Mycorrhizae and environmental stresses. In: Bethlenfalvay GJ and Linderman RG *Mycorrhizae in Sustainable Agriculture*. eds. Madison, WI: ASA Special Publication No. **54** 101-124.

Timonin M I 1940 The interaction of higher plants and soil microorganisms I. Microbial population of the rhizosphere of seedlings of certain cultivated plants. *Canadian Journal of Research* **18** 307-317.

Trappe J M and Schenck N C 1982 Taxonomy of the fungi forming endomycorrhizae : A VAM fungi. In. *Principle of mycorrhizal research* eds. NC Schenck American Phytopathological Society St. PauL 1-9.

Wadge A and Hutton M 1987 The leachability and chemical speciation of selected trace elements in Òy ash from coal combustion and refuse incineration. *Environmental Pollut*ion **48** 85-99.

Wang F Y, Lin XG, Yin R and Wu LH 2006 Effects of arbuscular mycorrhizal inoculation on the growth of *Elsholtzia splendens* and *Zea mays* and the activities of fungal characteristics and soil enzymatic activities in Cd polluted soil. *Environmental Poll*ution **134** 257–266.

Wu F Y, Bi, Y L and Wong MH 2009 Dual Inoculation with an Arbuscular Mycorrhizal Fungus and *Rhizobium* to Facilitate the Growth of Alfalfa on Coal Mine Substrates. *Journal. of Plant Nut*rition **32** 755–771.

Yang J, Wang Q, Wang Q and Wu T 2009 Heavy metals extraction from municipal solid waste incineration Òy ash using adapted metal tolerant *Aspergillus niger*. *Journal of Bio technol*ogy **100** 254–26.

Yunusa I A M, Eamus D, De Silva DL, Murray BR, Burchett MD, Skilbeck GC and Heidrich C 2006. Fly-ash: an exploitable resource for management of Australian agricultural soils. *Fuel* **85** 2337–2344.

Zhu Y G, Christie P and Laidlaw A S 2001 Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere* **42** 193–199.