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RESEARCH ARTICLE

EFFECT OF FLY ASH AND MICROBES ON THE GROWTH, NODULATION AND MYCORRHIZATION IN PEA (PISUM SATIVUM (L.) VAR. AP3)

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Abstract

In India electricity generation mainly depend on the coal based thermal power plant. Huge amount of fly ash generation causes serious environmental problem so it necessary to proper utilization of fly ash. Fly ash contain huge amount of toxic element like Cd, Pb etc. but it contains almost all the essential plant nutrients i.e., macronutrients including K, Ca, Mg and Micronutrients like Fe, Mn, Zn, Cu, Co, B etc. So, because of it physical and chemical properties fly ash is beneficial for soil and crop health. The present study has been undertaken to see the effect of fly ash alone and with microbes like AM fungi native to fly ash dumped site, *Rhizobium sp*. and P solubilizer (*Aspergillusniger*) on the growth and yield of Pea (*Pisumsativum*(L.)var. AP3). 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 of the plants. Out of three concentrations of fly ash, 10% fly ash gave the best results with organic amendment and microbial inoculation

Introduction:-

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 a universal problem.

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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 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 fly ash.

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 Pea in fly ash amended agricultural soil.

Material 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.(Plate 1,2) 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 of (Gerdemann and Nicolson 1963**)**. A known amount of soil was dissolved in water. After through 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 *Glomusviz.Acaulospora denticulate, Acaulosporascrobiculata, Glomusdeserticola, Glomusfasiculatum, Glomustortosum,Glomusclarum, Glomusmulticaule, Glomusintraradices, Glomusmosseae, Glomusmulticaule, 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 *Trifoliumrepens* (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*spergillusniger*, *Cladosporium sp*., *Fusariumoxysporum*, *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. *Aspergillusniger* 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 Pea (*Pisumsatium*(L.)var.AP3) 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 (*Aspergillusniger*) (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 (*Aspergillusniger*) (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 + 30% Fly ash + AM + 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 filled 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 five blocks. In first 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% fly ash, whereas in fourth and fifth blocks with 20 and 30% fly ash respectively.

Crops were raised in earthen pots. Seeds were surface sterilized 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 efficient 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 (*Aspergillusniger*) 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 finally emergence and establishment only five seedlings per pot were maintained. Five plants from each treatment series were carefully uprooted at different stages of plant growth *viz*. vegetative, flowering 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 values by Duncan's multiple range tests ($P \leq 0.05$). The graphs were prepared by using Microsoft Excel.

Result 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 deficiency 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-Larroceaet *al.* 2010) by altering the soil microbial communities in rhizosphere directly or indirectly through changes in root exudation patterns (Bareaet *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 (Enkhtuyaet *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 *Glomuscaledonium* and *Rhizobium sp*. on the roots of *Vignamungo*. 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% fly ash had an adverse effect on root colonization (Table-4 & Figure 1). However, significant 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 (*Glomusaggregatum*) on pigeon pea (*Cajanuscajan* 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 the best performance in comparison to 20 & 30% fly ash added series (Table-6,7 & Figure 2,3). Deleterious effect of high concentrations of fly ash specially when used in more than 50% levels on plant growth and yield have also been reported by Khan and Khan, (1996) and Raghavet *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 fly ash increased the plant root and shoot biomass. Plants growing in fly ash inoculated with AM fungi showed a significant 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 fly 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 1050% increase over control was recorded in pod number and 6555% 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 significant 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 fly ash suppressed root nodulation significantly in soybean and suppression gradually increased with the increase of fly ash in the soil. No nodule was observed at 100% fly ash level in soybean.

Decline in measured parameters above 10% fly 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 fly 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 fly 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 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.

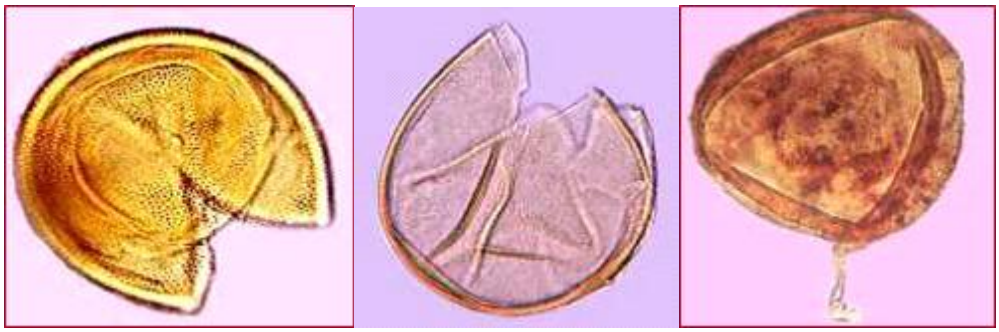
Fly ash dumping site

Plate 1

Ganga basin area

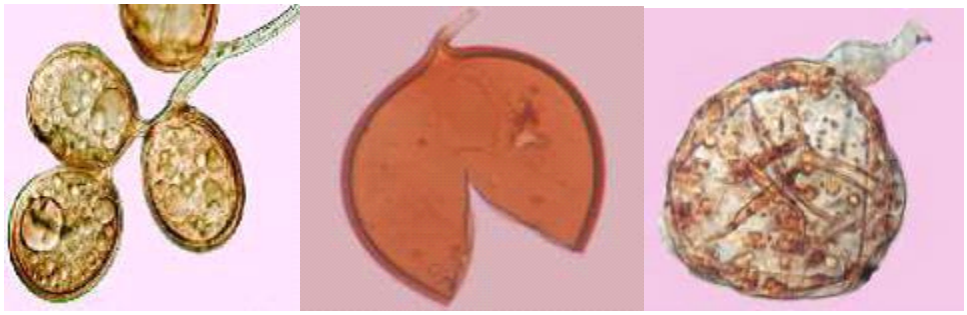
Plate 2

Dominant AMF spores: Fly Ash



Acaulosporadenticulate *AcaulosporascrobiculataGlomusdeserticola*

GlomustortosumGlomusclarumGlomusfasciculatum



GlomusintraradicesGlomusmosseaeGigaspora sp.

Glomusmulticaule

Plate 3

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)**
Aspergillusniger	**0.197**
Cladosporium sp.	0.024
Curvularia sp.	0.072
Fusariumoxysporum	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 Pea plant raised in soil amended with different concentrations of fly ash and *Cynodon* and provided with consortium of AM fungi, PSF andN_2 fixer alone as well as in combination.

Percentage mortality (Pre-emergence)					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	32a	27ab	36a	39a	41a
+PSF	30ab	29a	31b	32b	40ab
+AM	26bcd	24bcd	25cd	27c	34cd
+RHZ	29ab	28ab	30b	33b	36bc
+PSF+AM	24cd	22cd	23de	24cd	31d
+PSF+RHZ	28abc	27ab	28bc	31b	38abc
+AM+RHZ	24abc	22abc	21cde	23cd	25e
+PSF+AM+RHZ	21d	20d	19de	20d	21f

P<0.005

Table 4:- Mycorrhizal intensity in the roots of Pea 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_2 fixer as well as in combination

Percentage Mycorrhization					
Treatment	**AgS**	**CN**	**10% FA +CN**	**20%FA +CN**	**30%FA +CN**
Control	37f	40d	42d	33d	27e
+PSF	41d	45cd	49bc	43c	28e
+AM	48c	52bcd	71cd	63b	53cd
+RHZ	43e	47f	63e	58a	54ab
+PSF+AM	51bc	54bc	76bc	65b	56cd
+PSF+RHZ	45e	48e	66e	59b	56d
+AM+RHZ	57b	67b	77ab	69a	62bc
+PSF+AM+RHZ	63a	69a	81a	73a	65a

$P < 0.05$

Table 5:-AMspore population in the rhizospheric soils of Pea 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_2 fixer as well as in combination

AM spore population (50g air dried soil)					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	31d	38e	40d	37c	34c
+PSF	38c	42de	44c	41c	36c
+AM	41bc	45cd	48bc	39b	37b
+RHZ	35d	37f	42e	40c	32c
+PSF+AM	47bc	49c	52b	44b	41b
+PSF+RHZ	36d	39bf	44e	42c	40c
+AM+RHZ	50b	53b	55a	44a	42a
+PSF+AM+RHZ	52a	55a	58a	48a	46a

$P < 0.05$

Table 6:- Dry weight of root of Pea 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.

Root dry weight (g) / plant					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	0.172f	0.183a	0.197e	0.176d	0.164e
+PSF	0.193d	0.231d	0.249d	0.242c	0.237d
+AM	0.197cd	0.239d	0.285c	0.276c	0.259c
+RHZ	0.185e	0.203d	0.205g	0.178f	0.174g
+PSF+AM	0.195bc	0.304b	0.318c	0.253c	0.246c
+PSF+RHZ	0.189d	0.198a	0.202f	0.183e	0.176f
+AM+RHZ	0.198b	0.359b	0.367b	0.258b	0.253b
+PSF+AM+RHZ	0.201a	0.369b	0.371a	0.269a	0.264a

$P < 0.05$

Table 7:- Dry weight of Shoot of Pea 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.

Shoot dry weight (g) / plant					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	0.342f	0.479a	0.799e	0.697d	0.584e
+PSF	0.653d	0.712a	0.835d	0.780c	0.749d
+AM	0.673cd	0.737a	0.929c	0.869c	0.847c
+RHZ	0.659e	0.711a	0.755g	0.734f	0.667g
+PSF+AM	0.772bc	0.884a	0.916c	0.889c	0.855c
+PSF+RHZ	0.663d	0.718a	0.793f	0.781e	0.728f
+AM+RHZ	0.768b	0.895a	0.949b	0.913b	0.897b
+PSF+AM+RHZ	0.889a	0.915a	0.976a	0.948a	0.937a

P<0.05

Table 8:Number of nodules of Pea 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.

Average Number of nodules / plant					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	6d	8e	12d	10c	8c
+PSF	8c	9de	14c	12c	9c
+AM	14bc	16cd	22bc	19b	17b
+RHZ	12d	13f	14e	10c	7c
+PSF+AM	16bc	18c	24b	21b	20b
+PSF+RHZ	14d	16bf	19e	15c	13c
+AM+RHZ	15b	21b	25a	23a	18a
+PSF+AM+RHZ	18a	24a	29a	26a	21a

P < 0.05

Table 9:- Dry weight of nodules of Pea 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.

Dry weight of nodules (g) / plant					
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	0.005f	0.007a	0.010e	0.008d	0.006e
+PSF	0.006d	0.008a	0.015d	0.011c	0.007d
+AM	0.012cd	0.015a	0.018c	0.016c	0.011c
+RHZ	0.008e	0.010a	0.017g	0.015f	0.013g
+PSF+AM	0.017bc	0.023a	0.029c	0.018c	0.016c
+PSF+RHZ	0.009d	0.013a	0.022f	0.012e	0.009f
+AM+RHZ	0.023b	0.032a	0.038b	0.034b	0.033b
+PSF+AM+RHZ	0.035a	0.037a	0.042a	0.039a	0.038a

P < 0.05

Table 10:Average number of pods of Pea 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.

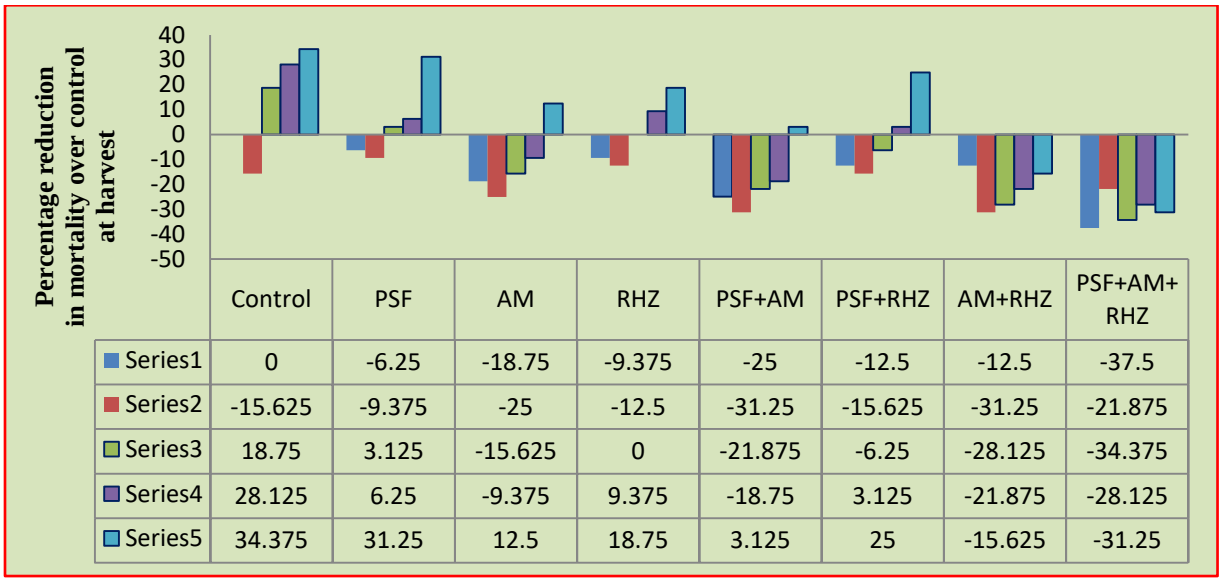
	Average number of pods / plant				
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	2f	5d	4d	3d	3e
+PSF	5d	6c	8bc	7c	6e
+AM	6c	7bcd	10cd	9b	7cd
+RHZ	4e	8f	18e	16a	14ab
+PSF+AM	8bc	13bc	12bc	11b	9cd
+PSF+RHZ	7e	11e	8e	7b	6d
+AM+RHZ	10b	15b	15ab	13a	11bc
+PSF+AM+RHZ	12a	18a	23a	20a	18a

P < 0.05

Table 11:- Dry weight of pods of Pea 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.

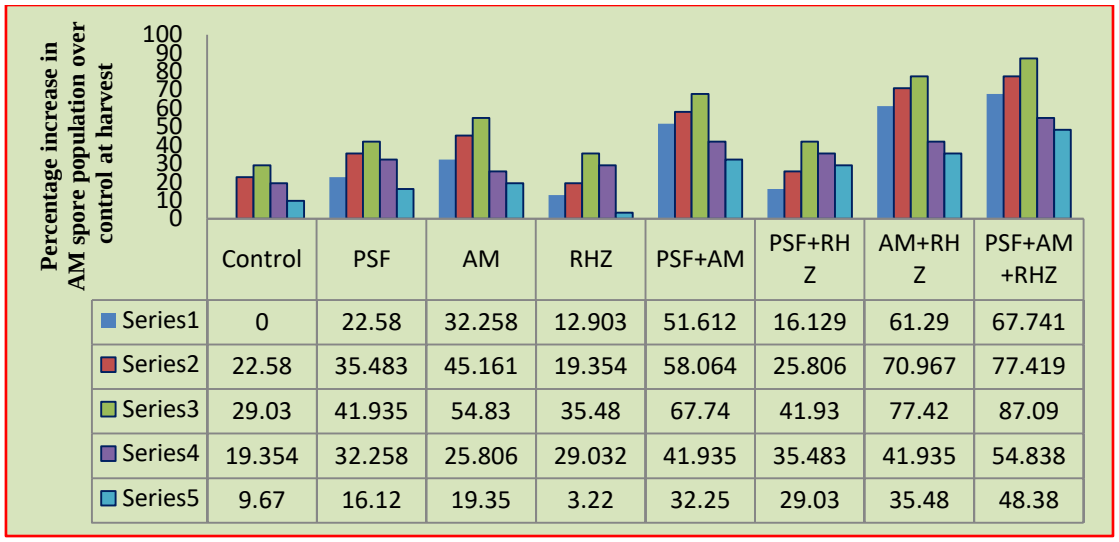
	Dry weight (g) of Pods / plants				
Treatment	**AgS**	**CN**	**10% FA + CN**	**20% FA + CN**	**30% FA + CN**
Control	0.628f	2.090a	2.012e	1.017d	0.456e
+PSF	2.065d	2.562a	6.656d	5.061c	3.366d
+AM	2.514d	3.01b	14.130c	11.493c	8.001c
+RHZ	1.328e	3.368a	13.230g	9.680f	8.358g
+PSF+AM	3.424bc	5.954a	20.532c	14.564c	10.899c
+PSF+RHZ	2.919d	4.807a	10.168f	5.096e	3.828f
+AM+RHZ	4.390b	6.975a	26.970b	17.589b	13.816b
+PSF+AM+RHZ	5.424a	8.478a	41.791a	30.62a	25.002a

P < 0.05

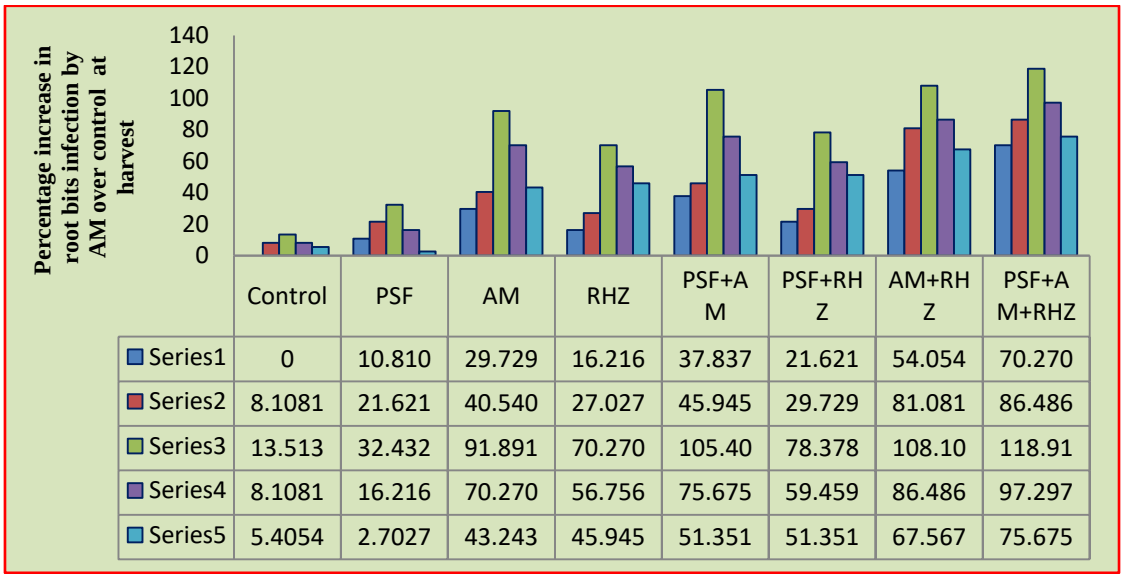


Series1:AgS (Agriculture soil)Series2: AgS+CN (*Cynodon*)Series3: AgS+CN+10%FA (Flyash)Series4: AgS+CN+20%FASeries5: AgS+CN+30%FA

Figure 1



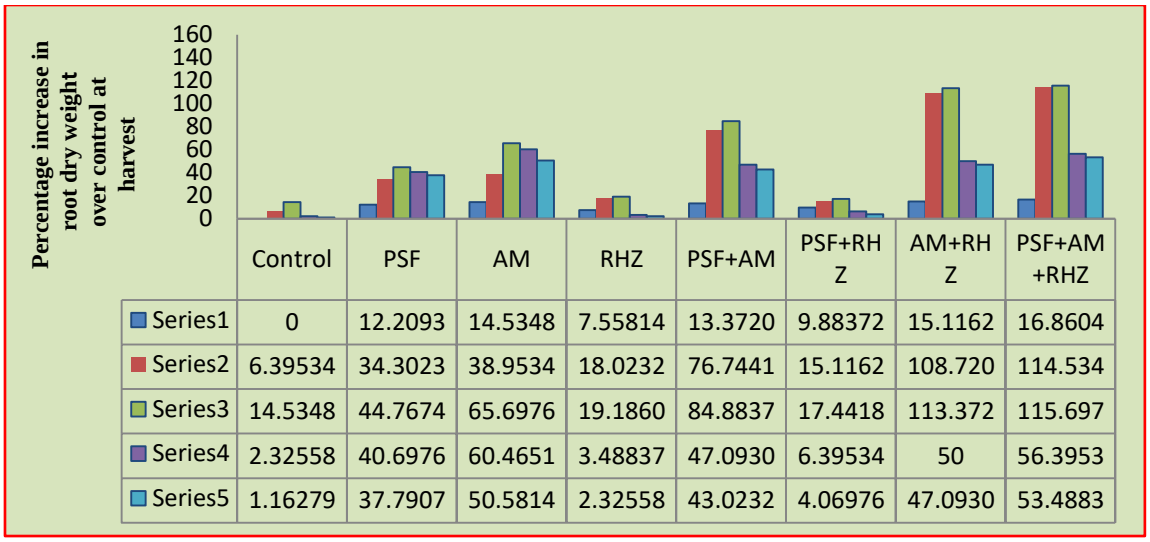
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	22.58	32.258	12.903	51.612	16.129	61.29	67.741
Series2	22.58	35.483	45.161	19.354	58.064	25.806	70.967	77.419
Series3	29.03	41.935	54.83	35.48	67.74	41.93	77.42	87.09
Series4	19.354	32.258	25.806	29.032	41.935	35.483	41.935	54.838
Series5	9.67	16.12	19.35	3.22	32.25	29.03	35.48	48.38



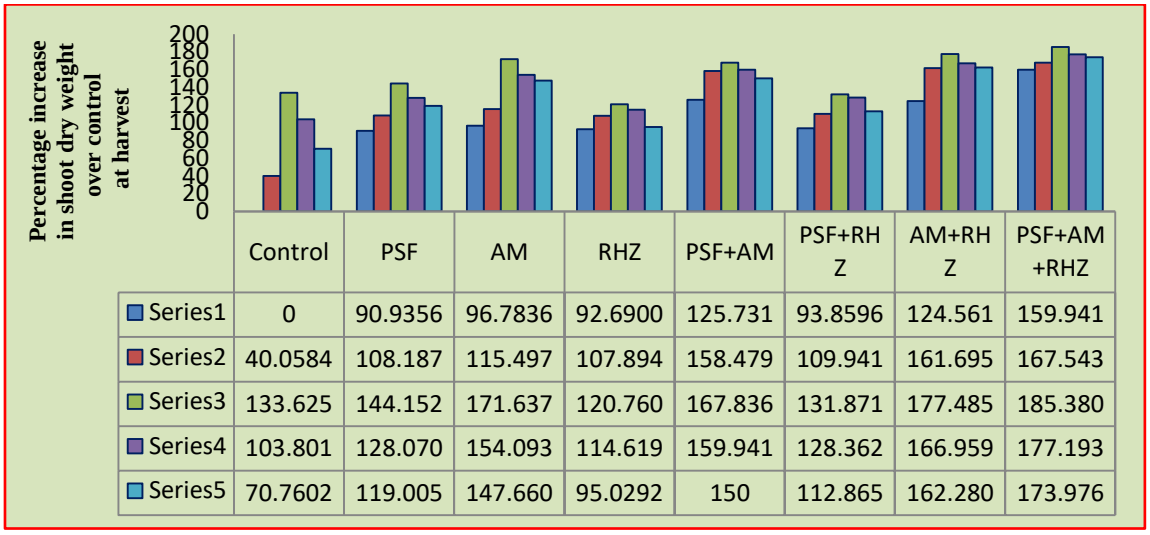
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	10.810	29.729	16.216	37.837	21.621	54.054	70.270
Series2	8.1081	21.621	40.540	27.027	45.945	29.729	81.081	86.486
Series3	13.513	32.432	91.891	70.270	105.40	78.378	108.10	118.91
Series4	8.1081	16.216	70.270	56.756	75.675	59.459	86.486	97.297
Series5	5.4054	2.7027	43.243	45.945	51.351	51.351	67.567	75.675

Figure 2

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



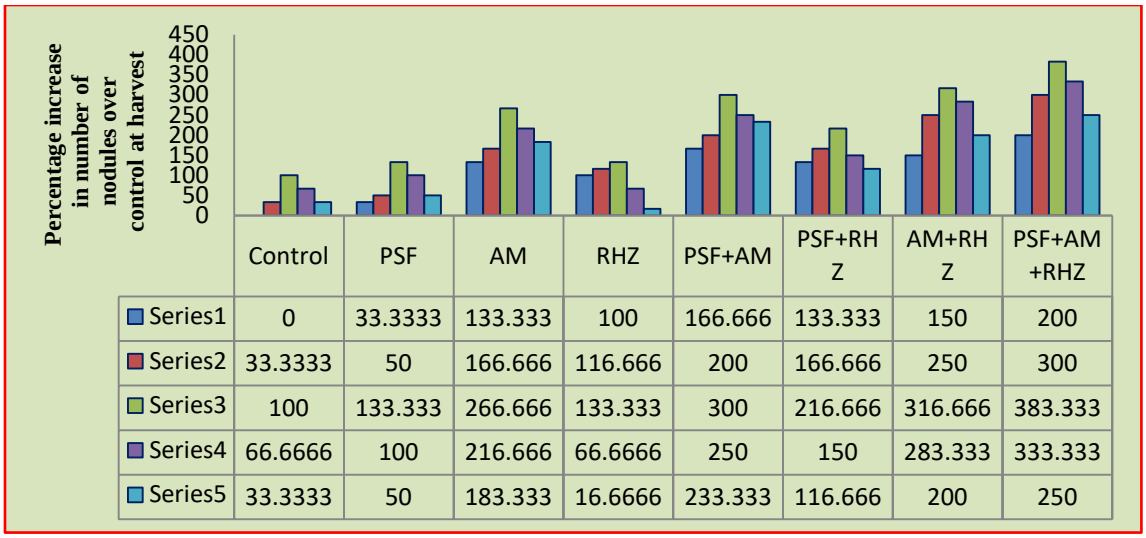
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	12.2093	14.5348	7.55814	13.3720	9.88372	15.1162	16.8604
Series2	6.39534	34.3023	38.9534	18.0232	76.7441	15.1162	108.720	114.534
Series3	14.5348	44.7674	65.6976	19.1860	84.8837	17.4418	113.372	115.697
Series4	2.32558	40.6976	60.4651	3.48837	47.0930	6.39534	50	56.3953
Series5	1.16279	37.7907	50.5814	2.32558	43.0232	4.06976	47.0930	53.4883



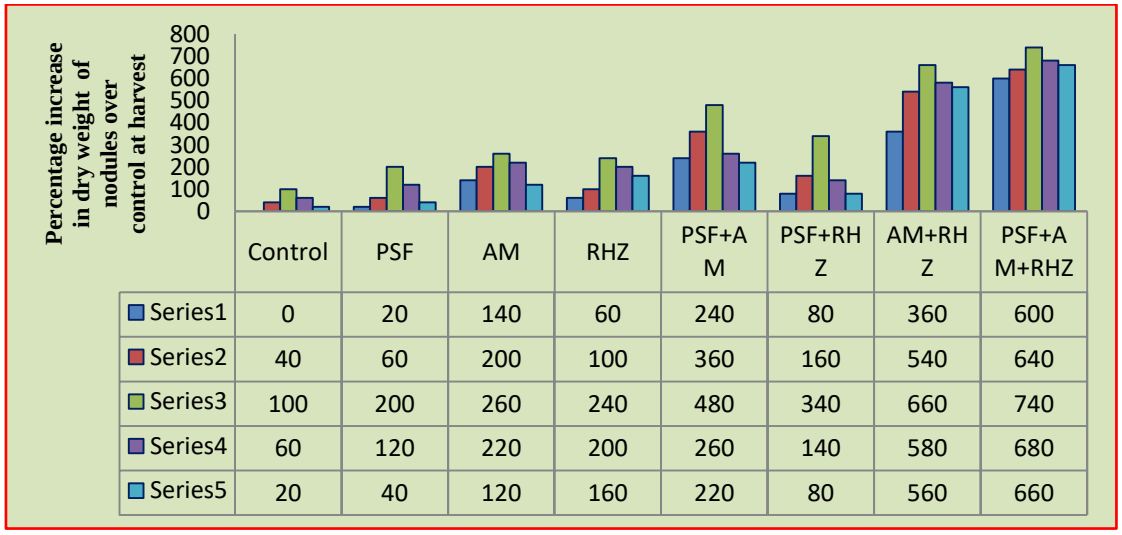
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	90.9356	96.7836	92.6900	125.731	93.8596	124.561	159.941
Series2	40.0584	108.187	115.497	107.894	158.479	109.941	161.695	167.543
Series3	133.625	144.152	171.637	120.760	167.836	131.871	177.485	185.380
Series4	103.801	128.070	154.093	114.619	159.941	128.362	166.959	177.193
Series5	70.7602	119.005	147.660	95.0292	150	112.865	162.280	173.976

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

Figure 3



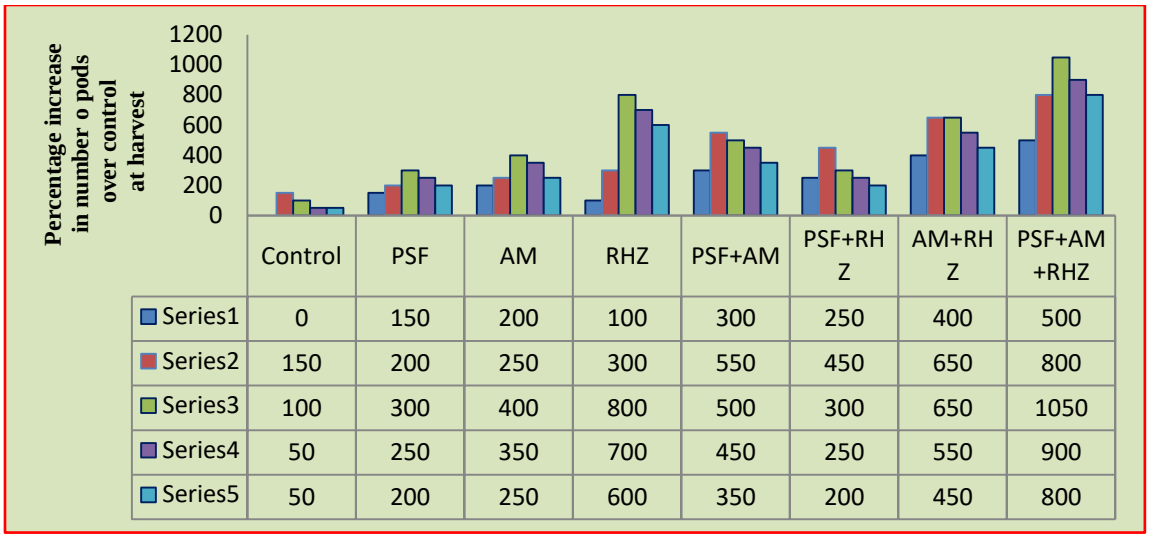
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	33.3333	133.333	100	166.666	133.333	150	200
Series2	33.3333	50	166.666	116.666	200	166.666	250	300
Series3	100	133.333	266.666	133.333	300	216.666	316.666	383.333
Series4	66.6666	100	216.666	66.6666	250	150	283.333	333.333
Series5	33.3333	50	183.333	16.6666	233.333	116.666	200	250



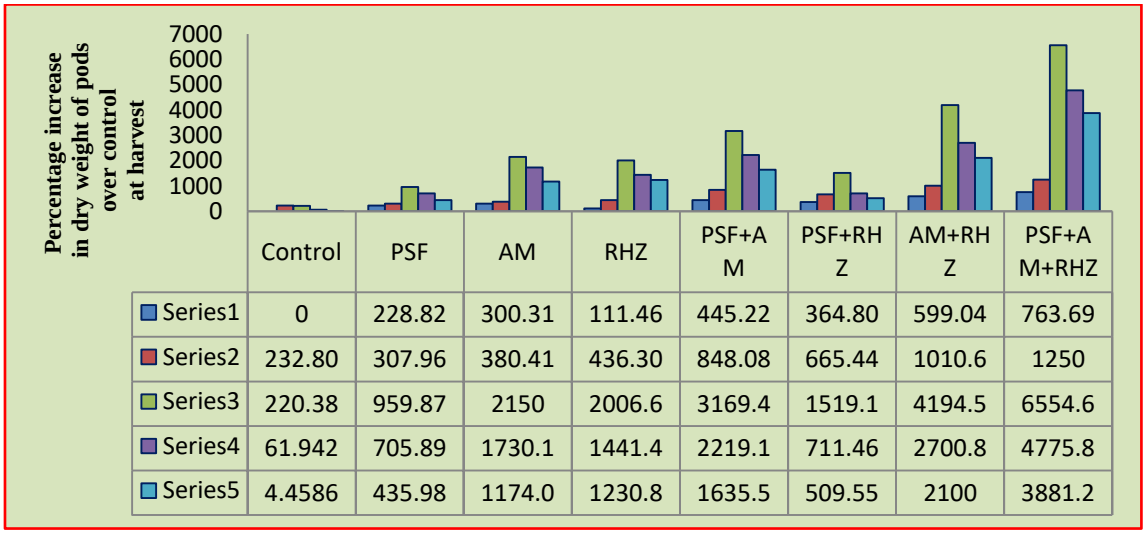
	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	20	140	60	240	80	360	600
Series2	40	60	200	100	360	160	540	640
Series3	100	200	260	240	480	340	660	740
Series4	60	120	220	200	260	140	580	680
Series5	20	40	120	160	220	80	560	660

Figure 4

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



	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	150	200	100	300	250	400	500
Series2	150	200	250	300	550	450	650	800
Series3	100	300	400	800	500	300	650	1050
Series4	50	250	350	700	450	250	550	900
Series5	50	200	250	600	350	200	450	800



	Control	PSF	AM	RHZ	PSF+AM	PSF+RHZ	AM+RHZ	PSF+AM+RHZ
Series1	0	228.82	300.31	111.46	445.22	364.80	599.04	763.69
Series2	232.80	307.96	380.41	436.30	848.08	665.44	1010.6	1250
Series3	220.38	959.87	2150	2006.6	3169.4	1519.1	4194.5	6554.6
Series4	61.942	705.89	1730.1	1441.4	2219.1	711.46	2700.8	4775.8
Series5	4.4586	435.98	1174.0	1230.8	1635.5	509.55	2100	3881.2

Figure 5

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

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