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Effect of Fly Ash and AM Fungus (*Glomus Fasciculaum*) on Growth of *Crotalaria Juncea L* Plant.

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ABSTRACT

Fly ash contains many essential plant nutrients and their availability to the plant may be problematic. However, inoculation of AM fungi supports the growth and nutrient uptake of plants and aggregation of fly ash. The AM fungi may enhance plant P nutrition and increase the plant growth by diluting metal effect in host plant or by binding of the metal to the fungal mycelium and immobilize them in rhizosphere or roots.

1. Introduction

Fly ash is a particulate residue of thermal power plants. The safe disposal, effective and economic utilization of fly ash voluminously generating from various coal based thermal power stations and strict compliance of the environmental regulation are of immense concern. The chemical properties of fly ash are influenced to a great extent by those of the coal type burned and the technologist used for handling and storage. As a soil pollutant fly ash is highly alkaline and rich in salts (Adriano *et al.,* 1998). Fly ash affected the growth, productive and reproductive abilities of a number of plants (Satyanarayan and Pushpalata, 1991; Saquib and Khan, 1999). A large amount of elements (C, K, Ca, Mg, Cu, and Zn) (Raularay *et al.,* 2003, Lee *et al.,* 2006 and Tiwari *et al.,* 2008) get in to the soil as a result of fly ash used at different doses and may probably change the physiochemical properties of soil, which in turn may determine the biological properties soil, which in turn determine the biological properties irrespective of the crop (Dhankar, 2003; Kumar *et al.,* 1999, Srivastva *et al.,* 2002). Fly ash studies show that one can grow commercially important plant species in fly ash covered area (Adholeya, 2000).

Arbuscular mycorrhizal fungi, through their mycelia net work accumulate heavy metals from fly ash and retain them with in their cells or carry them on their body surface when they form association with the plants. These mycelia threads, along with dense root biomass assist in binding ash particles. AM fungi have been used as bioremediation agents (Leyval and Haselwander, 1997) and biofertilizers for agricultural, horticultural and silvicultural plant species in polluted area (Anonymous 2001; Lakshman, 2009). AM fungi helps in binding the fine particles of ash and arrests the uptake of heavy metals by host plants (Sarangi and Mishra, 1998) Experiments were conducted to study the effect of fly ash with AM fungi inoculation on growth response of *Crotalaria juncea* L., Root colonization, and spore count of AM fungi in plant rhizosphere was also consideration. Kalra *et al*., 1997; Singh *et al*., 1997, reported that agricultural utilization of fly ash has been reported because of its considerable content of K, Ca, Mg, S and P Fly ash addition generally increases plant growth and nutrient uptake (Aitken *et al*., 1984). reported that fly ash increased crop yield of alfalfa (*Medicago sativa)*, barley (*Hordeum vulgare*), Bermuda grass (*Cynodon dactylon*) and white clover (*Trifolium repens*). Addition of unweathered

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western US fly ash up to 8% (w/w) to either calcareous or acidic soils resulted in higher yield of several agronomic crops (Page *et al*., 1979) mainly due to increased availability of S to plants. Furr *et al*. (1977) demonstrated that alfalfa, sorghum (*Sorghum bicolor*), field corn (*Zea mays*), millet (*Echinochloa crusgalli*), carrots (*Daucas carota*), onion (*Allium cepa*), beans (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), potatoes (*Solanum tuberosum*) and tomatoes (*Lycopersicon esculentum*) could be grown on a slightly acidic soil (pH 6.0) treated with 125 mt ha^{-1} of unweathered fly ash. These plants exhibited higher contents of As, B, Mg and Se. Also winter wheat (*Triticum aestivum*) grown on a deep bed of fly ash produced grains containing higher. Greenhouse experiments conducted by showed that application of 2-4% fly ash significantly increased N, S, Ca, Na and Fe content of rice (*Oryza sativa*) plants. The foliar application of fly ash also enhances growth and metabolic rates, as well as increasing the photosynthetic pigments of crops like maize and soybean (Mishra and Shukla, 1986).

1.1 Objective

Objective of this experiment to understand growth response and yield of *Crotalaria juncea* L., parameters like per cent colonization, and spore number were determined at 60, 90 and 120 days. The mycorrhizal root colonization was found to be varied in each experimental plant.

2. Materials and Method

The used soil for the experiments was sandy loam having pH 7.0, organic carbon 0.84%, Nitrogen 1.41 mg/kg, Potassium 2.41 mg/kg, phosphorus, 0.18 mg/kg, zinc, 202 mg/kg, copper, 1.04 mg/kg, magnesium 1.42 mg/kg and E.C 10.17m mho/cm. The soil was steam sterilized for one hour on two consecutive days. The physicochemical property of the soil was determined as per Jackson (1973). Per cent of organic matter was determined according to Piper (1950). Electric conductivity was measured using Bridge meter and pH by 1:1 (w/v) soil to water ratio. The fly ash was collected from poly fiber industry Harihar in Davangeri district of Karnataka (India).

3. Experimental design

The experimental pots were filled with growth media (Soil: Sand in 3:1.) each experimental potting mixture was amended with three different levels of fly ash (1%, 3% and 5%) with provided with 10 g of AM fungal inoculum. (*Glomus fasciculatum*). The control treatment was maintained with out AM fungal inoculum and fly ash. The treatments maintained for each plants species were as follows.

1) *Control (Uninioculated)*
2) *Mycorrhiza (Glomus fasciculatum) inoculated*
3) *Mycorrhiza (GF) + 1% fly ash (100g/10kg soil)*
4) *Mycorrhiza (GF) + 3% fly ash (300g/10kg soil)*
5) *Mycorrhiza (GF) + 5% fly ash (500g/10kg soil)*

All the experimental pots were arranged incompletely randomized block design with triplicate per treatment. The experimental pots were kept free of weeds, insects, pets, rodents etc. the pots were watered every alternate day and 10 ml of Hoagland solution without P was given to each seedling at the interval of 15 days.

3.1 Analysis of growth parameters:

Plants were harvested after 60, 90 and 120 days after sowing. The plants parameters like shoot and root length, fresh weight of shoot and root, shoot and root dry weight, stem diameter and number of leaves, the per cent root colonization, spore number per 50 g soil, and phosphorus uptake in shoot were recorded. After the harvest, experimental plants shoot and root was oven dried at 70^0C until a constant weight was obtained to determine the dry weight.

3.2 Root colonization

The per cent root colonization was evaluated microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described by Phillips and Hayman (1970). The following formula was used to calculate the root colonization (Giovannetti and Mosse, 1980).

$$\text{Percent mycorrhizal colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

3.3 Determination of AM fungal spores

Spores were separated from the soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). 50 g of soil was mixed with water. The mixture was pour through different sieve size (250, 106, 45µm). After several times of sieve washing, the supernatant was collected in petridish and spores counted under binocular-microscope.

3.4 Determination of Fiber content

Smallholder plots are usually harvested by hand. The plants are cut at 2 to 3 cm above the soil and left on the ground to dry. The cut *Crotalaria juncea* L., is laid in swathes to dry for up to four days. This was followed by water retting (the bundled hemp floats in water) and the fiber yield in *Gossypium hirsutum* L., was measured by taking dry weight of fruits.

3.5 Phosphorus content:

The phosphorus content in the shoots was determined by the vanado-molybdate phosphoric acid yellow color method outlined by Jackson (1973).

3.6 Statistical Analysis

Analysis of variance (ANOVA) was performed on all data and the means were separated using Duncan's multiple Range Test (DMRT), by the help of SPSS student version-9 software.

4. Results

The different dosages of fly ash with AM fungus *Glomus fasciculatum* inoculated to test the effect of fly ash on mycorrhizal and four fiber yielding plants. All the plant species inoculated with 3% fly ash with AM fungus have shown increased growth parameters over the other treatments and control plants. The experimental results revealed that, not only the growth parameters of four experimental plants were increased but also the nutrient uptake and mycorrhizal status was significant compared to plants provide with high levels of fly ash. The growth parameters of all the experimental plants were determined at 60, 90 and 120 days after sowing. The greater values for growth parameters such as shoot length, fresh weight of shoot, dry weight of shoot, root length, fresh weight of root, dry weight of root, stem diameter, number of flowers, numbers of fruits and increased "P" uptake were recorded in experimental plants with 3 % fly ash and AM fungus *Glomus fasciculatum* over the reaming treatments.

Where as, plants provided with 5% fly ash have shown to be less significant . The intermediate growth rate had been recorded in plants provided with 1% fly ash in presence of AM fungus. Mycorrhizal parameters like per cent colonization, and spore number were determined at 60, 90 and 120 days. The mycorrhizal root colonization was found to be varied in each experimental plant. It was less in beginning (at 45-60 days) but steadily increased after 90 days. It was observed that at 120 there was maximum colonization in the experimental plants roots grown with 3% fly ash over control plants.

Table: 1: Effect of *Glomus fasciculatum* and fly ash different levels treatments on the Fiber yield of *Crotalaria juncea* L, at different interval plants at different interval (60, 90 and 120).

Treatments	*Crotalaria juncea* L.
CN	3.200±0.057e
GF	7.366±0.088d
GF+1%Fly ash	8.566±0.202b
GF+3% Fly ash	10.93±0.202a
GF+5 % Fly ash	8.066±0.088c

Values represent the mean ± SD. Means followed by the same letter within a column are not significantly P= 0.05 according to DMRT.

The maximum per cent mycorrhizal colonization (PMC) was recorded in *Crotalaria juncea* L., AM fungal spore number was recorded in all experimental plants. It was found to be highest at 120 days and least was noticed at 60 days, with increase in duration the spore number was increased. Maximum AM fungal spore number was observed in the rhizosphere soils of the experimental plants inoculated with *Glomus fasciculatum* and 3% fly ash. It was least in plants inoculated with *Glomus fasciculatum* and 5% fly ash whereas,, moderate spore number was noticed in plants provided with 1% fly ash. The plants were also analyzed for its nutrient continent in shoot, particularly phosphorus. Maximum increased P uptake was observed in plants inoculated with *Glomus fasciculatum* in presence of 3% fly ash. The moderately increased P Uptake in shoots was estimated in plants grown with 1% fly ash and it was least in plants with 5% fly ash in the potting mixture. The fiber content in all the experimental plants was measured. The fiber content in the mycorrhizal plant with 3% fly ash was more when compared to control plants and mycorrhizal plants with 5% fly ash. It can be evident from the above results that, AM fungus *Glomus fasciculatum* with 3% fly ash was found to be the more efficient treatment of the enhancement of plant growth and fiber yield in four experimental plants.

5. Discussion

In the present study the best effects on biomass production were noticed in AMF inoculated plants grown with 3% fly ash. The experimental results revealed that, AM inoculation with fly ash was successful in enhancing the plant growth parameters due to improved supply of nutrients, especially phosphorus and minerals such as Zn, Cu, K and Ca (Copper and Tinker, 1978). In the present study increased growth and higher values for mycorrhizal colonization and spore number have been reported with AMF and 3% fly ash. The AMF helps in binding the fine particles of fly ash and arrest the movement heavy metals and also helps in uptake of micronutrients and phosphorus solubilization (Adholeya, 2000). Fly ash contains many essential plant nutrients and their availability to the plant may be problematic as reported by Pandey *et al*., (1994) and Singh *et al*., (1997). In the present study, inoculation of AM fungus along with 3% fly ash significantly increased biomass of all the experimental plants. The uptake of nutrients such as P significantly increased in the shoot tissues compared to other treatments. The AM fungi may enhance plant P nutrition and increase the plant growth by

diluting metal effect in host plant or by binding of the metal to the fungal mycelium through chitin or glomalin and immobilize them in rhizosphere or roots (Chen et al., 2001 and Gonza´lez-Cha´vez *et al*., 2004). It has also been found that AM fungi alleviate metal toxicity of fly ash and enhance plant growth (Ning, 2000).

6. Conclusion

The experimental plants grown in the earthen pots containing 10 kg of growth media (soil:sand=3:1) amended with 1%, 3% and 5% of fly ash with AM fungal inoculation. In general significantly increased growth was observed in plant treated with 3 % fly ash and AM fungus *Glomus fasciculatum* in all the experimental plants. There was a greater mycorrhizal colonization and viable AM fungal spore numbers were recorded in presence of 3 % fly ash in the growth media. More fiber yield was measured in plants treated with same concentration of fly ash. It can be concluded that, 3% of fly ash along with AM fungus *Glomus fasciculatum* was the best treatment to increase the growth, nutrient content and fiber yield of the four experimental plants over the remaining treatments.

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Table:2: Effect of *Glomus fasciculatum* and fly ash different levels treatments on the growth of *Crotalaria juncea* L., plants at different interval (60, 90 and 120).
Note: SL: Shoot length, FWS: Fresh weight of Shoot, DWS: Dry weight of Shoot, RL: Root Length, FWR: Fresh weight of Root, DWR: Dry weight of Root, STD: Stem Diameter, PC: per cent of mycorrhizal colonization, NFL: Number of Flowers, NFR: Number of fruits, SP: Spore Number, P: Phosphorous. Values represent the mean ± SD. Means followed by the same letter within a column are not significantly P= 0.05 according to DMRT.

Treatments	SL	FWS	DWS	RL	FWR	DWR	STD	PC	NFL	NFR	SP	P-uptake
60 DAYS												
CN	24.66 ±0.088e	10.60 ±0.115e	4.433 ±0.120e	10.73 ±0.088e	2.666 ±0.120e	1.233 ±0.120e	2.993 ±1.006e	0.000 ±0.000e	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000e	0.050 ±0.000e
GF	39.63 ±0.088d	15.70 ±0.057d	9.700 ±0.057d	18.40 ±0.057d	4.766 ±0.088d	2.033 ±0.033d	1.500 ±0.208d	52.00 ±1.154d	0.000 ±0.000	0.000 ±0.000	107.3 ±1.763d	0.100 ±0.000d
Gf+1%FA	45.76 ±0.088b	21.46 ±0.120b	13.76 ±0.088b	20.50 ±0.057b	5.800 ±0.057b	2.600 ±0.115b	1.466 ±0.066b	60.66 ±0.666b	0.000 ±0.000	0.000 ±0.000	116.6 ±0.881b	0.140 ±0.000b
GF+3%FA	57.10 ±0.152a	25.76 ±0.088a	16.70 ±0.152a	24.63 ±0.088a	6.466 ±0.033a	3.100 ±0.057a	1.733 ±0.088a	64.00 ±1.154a	0.000 ±0.000	0.000 ±0.000	130.3 ±2.728a	0.170 ±0.000a
GF+5% FA	40.70 ±0.152c	18.43 ±0.088c	10.96 ±0.185c	20.13 ±0.033c	5.666 ±0.066c	2.033 ±0.033c	1.233 ±0.120c	57.66 ±0.881c	0.000 ±0.000	0.000 ±0.000	114.6 ±2.603c	0.120 ±0.000c
90 DAYS												
CN	36.80 ±0.057e	16.10 ±0.100e	10.30 ±0.152e	15.80 ±0.057e	3.733 ±0.166e	1.966 ±0.088e	1.433 ±0.033e	0.000 ±0.000e	0.333 ±0.333e	0.000 ±0.000e	0.000 ±0.000e	0.070 ±0.000e
GF	55.13 ±1.334d	23.63 ±0.120d	14.26 ±0.120d	28.60 ±0.057d	9.033 ±0.066d	4.533 ±0.120d	1.633 ±0.033d	61.00 ±0.577d	1.333 ±0.333d	1.000 ±0.577d	147.6 ±2.962d	0.150 ±0.000d
Gf+1%FA	64.80 ±0.057b	32.73 ±0.088b	20.23 ±0.202b	32.66 ±0.120b	12.60 ±0.115b	6.566 ±0.088b	1.766 ±0.033b	65.33 ±0.666b	1.666 ±0.333b	1.000 ±0.577b	158.0 ±2.309b	0.180 ±0.000b
GF+3%FA	72.76 ±0.088a	39.40 ±0.057a	24.73 ±0.088a	36.83 ±0.033a	15.66 ±0.120a	8.600 ±0.115a	2.033 ±0.033a	72.00 ±1.154a	3.666 ±0.333a	2.000 ±0.577a	178.6 ±2.027a	0.210 ±0.000a
GF+5% FA	61.66 ±0.120c	29.70 ±0.100c	17.93 ±0.145c	30.03 ±0.272c	10.43 ±0.033c	5.866 ±0.145c	1.666 ±0.033c	63.66 ±0.333c	1.666 ±0.333c	1.333 ±0.333c	155.0 ±2.081c	0.170 ±0.000c
120 DAYS												
CN	49.53 ±0.033e	28.73 ±0.088e	13.06 ±0.033e	27.43 ±0.120e	10.90 ±0.173e	5.700 ±0.100e	1.666 ±0.033e	0.000 ±0.000e	2.333 ±0.881e	1.666 ±0.333e	0.000 ±0.000e	0.080 ±0.000e
GF	69.76 ±0.088d	38.76 ±0.133d	25.10 ±0.057d	38.76 ±0.088d	16.70 ±0.115d	10.43 ±0.033d	2.133 ±0.033d	77.33 ±0.666d	5.666 ±0.333d	2.333 ±0.333d	174.0 ±1.000d	0.160 ±0.000d
Gf+1%FA	81.20 ±0.230b	46.66 ±0.120b	30.43 ±0.088b	42.60 ±0.173b	18.73 ±0.088b	11.96 ±0.088b	2.366 ±0.033b	83.33 ±0.666b	7.333 ±0.333b	5.333 ±0.666b	196.0 ±3.055b	0.190 ±0.000b
GF+3%FA	96.83 ±0.033a	55.86 ±0.033a	36.83 ±0.033a	49.76 ±0.088a	20.63 ±0.145a	13.90 ±0.208a	2.766 ±0.088a	95.33 ±0.666a	9.666 ±0.333a	7.000 ±0.577a	228.6 ±2.027a	0.230 ±0.000a
GF+5% FA	76.33 ±0.240c	42.56 ±0.202c	27.60 ±0.057c	39.60 ±0.264c	17.10 ±0.200c	10.66 ±0.284c	2.233 ±0.033c	79.66 ±0.333c	6.666 ±0.333c	5.000 ±0.577c	185.6 ±2.027c	0.180 ±0.000c