

# Stastical Analysis and Effect of Primary Nutrient (N,P,K) in Coal Ash Admixed Wasteland Soil through Vesicular – Arbuscular Mycorrhizae Invation

## Mamta Verma

Associate Professor, Department of Civil Engineering, Bansal college of Engineering, Mandideep, Bhopal ,(M.P.), India  
E-Mail : mv.manit@gmail.com

## Kirti Verma

Associate Professor, Department of Engineering Mathematics, Lakshmi Narain College of Technology, Bhopal, [\(M.P.\)](#), India  
Email:kirtivrm3@gmail.com

## Pushpa.H

Associate Professor, Microbiology Department,  
M.S.Ramaiah College of Arts Science and Commerce, Bangalore  
Email:pushpa\_microbio@msrcasc.edu.in

## Seema Dubey

Lecturer, Govt. Higher Secondary School, Sanchi, Raisen [\(M.P.\)](#).  
Email:safadubey@gmail.com

## M. Sundararajan

Professor, Department of Mathematics & Computer Science, Mizoram University,  
AIZAWL - 796 004, MIZORAM STATE  
E-mail: dmsrajan.mzu@gmail.com

## Bilquees Jahan Khan

Associate professor, Department of life science, SAM Global University, Raisen, [\(M.P.\)](#), India  
Email: bilquees.khan76@gmail.com

**CORRESPONDING AUTHOR: Kirti Verma, Associate Professor, Department of Engineering Mathematics, Lakshmi Narain College of Technology Bhopal, M.P., India, [kirtivrm3@gmail.com](mailto:kirtivrm3@gmail.com)**

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## ABSTRACT

Low availability of primary nutrient, a very common nutritional problem in Indian soils during crop production and needs chemical fertilization. Coal ash contains nitrogen (0.0075%), phosphorus (0.0195%) and potassium (0.0265 %). The problem with the use of existing combination of soil and coal ash in agriculture is the unavailability of soluble primary nutrient. So in order to overcome the problem of unavailability of primary nutrient in indian soil and simultaneously the constructive use of coal ash in agriculture, the present work has been taken up where the effect of vesicular arbuscular mycorrhizae (VAM) isolated from the rhizospheric soil have been observed on wheat plant (*Triticum aestivum*) grown in different treatment of soil admixed with coal ash (0-25%).

Different morphological observations like plant height, leaf area index and grain yield were studied at regular interval of 20 days till their maturity phase during the entire cropping. Physico- chemical properties like pH, conductivity and primary nutrient (N, P, K) were also evaluated. The enhancement of primary nutrient nitrogen by 21.20%, phosphorus by 24.91%, and potassium by 21.70% over control have been noticed after the addition of mycorrhizae spore to their respective control (-VAM) and indicated the contribution of mycorrhizae in solubilization of unavailable nutrient. The addition of mycorrhizae spore have also improved the plant growth and grain yield.

**Keywords:** Black cotton soil, Coal ash, Vesicular arbuscular Mycorrhizae, N,P,K nutrient and Wheat plant (*Triticum aestivum*).

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## INTRODUCTION

Coal ash is the inorganic solid compound of the residue from the combustion stream, which comes out as a very fine particle from the thermal power plants. The world scenario for the total combustion of coal is 3000 million tones and around 300- 350 million tones is the total coal ash is being produced every year (TIFAC, 1992). In India annually around 90.00 million tones of coal ash is being produced by thermal power plant located at different places and is likely to across the 140 million tones barrier during 2020 (Kalra et al., 1998). Earlier works have been reported on the use of coal ash as soil amendments to field crop.

On the contrary, investigations involving the use of coal ash in agriculture show that coal ash produced undesirable effects on crop yield and on development of plant. The coal ash uses in agriculture also show salt injury, pozzolonic effects and heavy metal toxicity (Guerrero et al., 2000) in crop. The presence of toxic element in coal ash causes the negative impact on use of coal ash in agriculture. Further, disposal of coal ash in to rivers, lakes, sea etc. creates major environmental hazards (Shukla et al., 2001), keeping in view the problem of coal ash utilizations together with the use of wasteland which reveals to have primary and secondary nutrient along with heavy metals considered as important micronutrients.

Many work have been sponsored by different government agencies to promote the use of coal ash in agriculture in India. In the present paper the work is focused mainly on the availability of primary nutrients from coal ash to respective Indian soils by the use vesicular arbuscular mycorrhizae (VAM) spore. The mycorrhizae spores have been introduced in the soil to study the effect on the black cotton soil to measure the availability of primary nutrient for which coal ash is the main source in the present experiment. Based on the problem with Indian soil is the lack of phosphorus availability, phosphorus is a major plant nutrient and it occurs in the form that is not directly available to plant. In the present study the mycorrhizae spores were inoculated with soil admixed with coal ash. Since mycorrhizae symbiosis plays an important role in the mobilization, translocation of nutrients by green plant have its effect on various physical-chemical, morphological properties of soil and have been considered in the present work.

## MATERIALS AND METHODS

### Study Area

The present study was carried out in **Advanced Materials and Processes Research Institute (AMPRI)**, Bhopal.

### Sample Collection

Coal ash was collected from M.P.E.B. Sarni Betul, Thermal Power Station. In the present study the coal ash used was pond ash (i.e. mixture of coal ash and bottom ash). The soil samples used were obtained from Regional Research Laboratory, Bhopal field at a depth of 0- 30 cm.

### Sample Preparation / Soil Characteristics

The test soil and coal ash were collected randomly following the standard quarter and cone technique (Tandon 1992). They were mixed thoroughly, air dried, and passed through a 2 mm round hole sieve. Electrical conductivity and pH

were determined by potentiometry using glass colone electrode system and conductivity meter respectively, with a soil to water ratio of 1:2 (Jackson 1958). Available P was determined according to Olsen’s 1954 method. Available N in the form of NO<sub>3</sub><sup>-</sup> was detected using estimated Subbiah–Asijia 1956 method and available potassium was estimated by following Jackson method 1958.

### Experimental Design

The experiment was arranged in a randomized block design with four replication for each of six treatments. Soil sample were sieved (2 mm pore size) and sterilized then re – inoculated with a soil filtrate containing the normal micro biota without AM propagulus. Pot were filled with 5 Kg soil and soil amended coal ash of different ratio 0%, 5%, 10%, 15% 20%, 25% were taken and filled in the earthen pots which were number as T1, T2, T3, T4, T5, T6.

### Inoculum Preparation

Spore of mycorrhizae were isolated from rhizosphere soil using a wet sieving technique (Gerderman and Nicoloson 1963) and seed of maize (*Zea mays*) were sown in the sterilized soil / sand mixture containing VAM inoculum. The mycorrhizal inoculum used was Wet sieving and decantation technique (Gerderman and Nicoloson, 1963). After 2-weeks of sowing, the maize shoots were chopped off from the soil surface. The prepared inoculum was added to 2-3 cm below to each pot. A soil: sand (1:1) mixture containing extra material chlamydo spores and infected with mycorrhizae spore root segments of maize were inoculated in to soil. Seed of wheat (*Triticum aestivum*) variety WH - 147 were sown in each pot. Observation on VAM inoculation and growth of wheat plant was measured in every 20 days. After harvesting plant grain yield were recorded.

## RESULT AND DISCUSSION

### Effect of vesicular arbuscular mycorrhizae spore on soil pH of soil admixed with coal ash

Table-1 shows the pH of different treatment of soil admixed with coal ash in before and after harvesting of wheat. Initially, the pH of the coal ash and soil were 8.0 and 8.1 respectively. After the application of VAM spore in different treatments of soil with coal ash pH was found to be increased as compare to control -VAM (without inoculation of VAM spore). The maximum pH was noticed in T6. Most of the plant nutrient is released in the pH range of 6.5 to 7.5. Soil pH is an important factor in regulating spore germination. VAM fungi are the only one providing direct link between soil and root. VAM symbiosis is found sensitive to the soil pH. The availability of several essential nutrients are generally affected by soil pH. From the result as shown in the table-1 there is a drastic variation in control –VAM and +VAM (with inoculation of VAM spore) samples. The pH is found in the range of 7.8 to 7.9 in +VAM sample while in –VAM sample the pH found increasing with increasing concentration of coal ash. This trend is absent in +VAM, which is an indicative of the presence of VAM spore inoculation and its efficacy to suppress the adverse contents of coal ash. This results of pH related with VAM spores have also been reported by Hetrick, et.al., 1994) where the pH was found to be increased from acidic range to the alkalinity.

**Table–1 Effect of vesicular arbuscular mycorrhizae spore on pH of soil treated with different concentration of coal ash**

Treatment	Before sowing		After sowing	
	+ VAM	- VAM	+ VAM	- VAM
T1	7.3	7.2	7.79	7.24
T2	7.42	7.21	7.8	7.3
T3	7.5	7.4	7.8	7.58
T4	7.52	7.42	7.86	7.58
T5	7.65	7.5	7.85	7.68
T6	7.76	7.52	7.88	7.75

- ❖ (+VAM) With inoculation of vesicular arbuscular mycorrhizae spore
- ❖ (-VAM) Without inoculation of vesicular arbuscular mycorrhizae spore

**Effect of vesicular arbuscular mycorrhizae spore on soil electrical conductivity of soil admixed with coal ash**

Table-2 show the value of conductivity in  $\mu\text{s} / \text{cm}$  in different treatment in +VAM and –VAM samples. The optimum pH maintain by VAM with range of 7.8 to 7.9 the maximum conductivity was noticed in higher concentration of coal ash treated soil with +VAM while in control (–VAM), the conductivity was found to be raised from T1 to T6 treatment by less as compared to the samples with +VAM. The hyphae of mycorrhizae lead to the availability of nutrient in free ionic form. According to Khan et al., 1996, the increase in the conductivity may be due to the interaction of inorganic constituents of coal ash with the organic matter in the soil. The higher mycelia network improves the soil structure and the establishment of a nutrient cycling rather than leaching of nutrients also reported by Miller and Jastrow ,1992.

**Table–2 Effect of vesicular arbuscular mycorrhizae spore on electrical conductivity of soil treated with different concentration of coal ash**

Treatment	Before sowing		After sowing	
	+ VAM	- VAM	+ VAM	- VAM
T1	176	172	352	259
T2	187	180	352	325
T3	205	195	355	338
T4	218	207	397	365
T5	240	225	410	368
T6	242	237	412	372

- ❖ (+VAM) With inoculation of vesicular arbuscular mycorrhizae spore
- ❖ (–VAM) Without inoculation of vesicular arbuscular mycorrhizae spore
- ❖ Conductivity in  $\mu\text{s}/\text{cm}$

**Effect of vesicular arbuscular mycorrhizae spore on primary nutrients of soil admixed with coal ash**

Table-3 indicates availability of primary nutrient in percentage (%) and found maximum in T4 treatment (15% coal ash) with the inoculation of VAM spore. The primary nutrient was found to be reduced after 60-days of inoculation of cropping in +VAM sample. The result incorporates the 0 day inoculation of vesicular arbuscular mycorrhizae (VAM) spore with sowing of wheat seeds till their harvesting. The available nitrogen was measured in terms of  $\text{NO}_3^-$ . Result shows the higher availability of nitrogen in T4 treatments and measured 21.200% increased in +VAM over –VAM sample while in T1 where an increase of 11.79 % in + VAM over –VAM samples were noticed during the entire period of wheat cultivation. This increased percentage of available nitrogen in +VAM treatments could be attributed due to the increased inorganic carbon content which enhanced the biogeochemical cycling through their microbial activity. The releases of few enzymes which act upon the available complex form nitrogen source convert and released the simple form. In addition, at higher percentages of coal ash a decline in nitrogen was observed the reason is the inhibition of microbial activity due to the presence of heavy metals in coal ash.

**Table–3 Effect of vesicular arbuscular mycorrhizae spore percentage increase of N, P, and K in soil with different concentration of coal ash**

Treatment	% increase of primary nutrients														
	0 days			20 days			40 days			60 days			90 days		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
T1	5.88	10.00	9.39	8.00	9.32	10.96	9.4	15.78	12.02	11.97	17.5	12.50	4.6	7.40	7.40
T2	8.00	10.34	12.02	8.50	11.7	15.47	9.8	14.28	18.53	14.66	19.04	18.55	4.46	8.37	9.15
T3	9.20	11.76	13.93	12.00	13.5	16.86	12.5	17.07	18.53	16.45	20.00	19.13	8.0	8.00	13.33

T4	12.40	13.51	17.14	14.7	15.79	18.33	19.7	22.20	18.91	21.20	24.91	21.71	8.58	9.09	14.88
T5	5.98	8.69	8.02	6.66	10.0	8.78	6.4	13.51	9.86	9.1	15.70	10.32	4.67	8.0	6.15
T6	4.68	6.89	6.58	4.5	7.4	6.89	4.8	10.00	7.52	7.6	11.70	8.20	4.04	4.76	6.09

- ❖ (+VAM) With inoculation of vesicular arbuscular mycorrhizae spore
- ❖ (-VAM) Without inoculation of vesicular arbuscular mycorrhizae spore

A similar trend was observed in the case of phosphorous. The availability of phosphorus is very low in Indian soil, the use of coal ash as a soil modifier to such soils increases the availability of phosphorus by inoculating the VAM spores. Soil is being benefited by increased phosphorus uptake through extra radical hyphae, which has also been reported by Davis, 2002. In table-3, indicate the percentage measured phosphorus availability and which noticed maximum in the T4 treatment 24.91 % +VAM over -VAM (control). In T1 treatment of approximately 17.5 % observed availability phosphorus +VAM in over control (-VAM). The increased availability of phosphorus in inoculated VAM spore sample due to the networking of hyphae growth of endomycorrhizae fungi has converted the phosphorus in to available form for the plants (Dodd, 2002). These mycorrhizae organisms first get activated near the plant root and develop contact through a filamentous mode or thread-like structure, which creates, and extensive network, which facilitates higher amount of solutes carrying phosphorus to plants. The availability of phosphorus got reduced in control (-VAM) with increasing concentration of coal ash i.e. from T1 to T6. The presence of heavy metals in coal ash might have caused toxicity and would be nullified in +VAM samples have more amount of available phosphorus is noticed. It is reported that phosphorous interact with heavy metals at low pH, acidic medium and shows the phyto-toxicity and the main reason for such a decreased availability of phosphorous due to the high content heavy metals like Al, Fe, and Pb release by soil acidification. In the present study the percentage of availability phosphorous was low in T1 treatments with inoculation of VAM spore but did not impart toxicity to the resultant soil, as the pH was alkaline and optimum texture.

Potassium is measured in terms of K<sup>+</sup>. Potassium exists in the form of mizzonite and albite in soil and coal ash. In table-3, indicate the total potassium availability, which was noticed maximum in the T4 treatment 21.71% (+VAM) over control (-VAM) while in T1 treatment observed 12.50 % (+VAM) increased over control (-VAM). The availability of potassium to plant from the control soil was not sufficient in promoting healthy plant growth. The potassium availability has been increased on admixing coal ash with inoculation of VAM spore with the soil. The potassium present in the coal ash got activated due to the increase in cation exchanges capacity, moisture content, pH, soil structure, microbial activities which have also been reported by Saxena et al 1998.

The results indicate that root colonization by inoculation with the mycorrhizae fungi gave higher wheat yield, higher plant uptake of most of the nutrients, and may have protected the plant from excessive accumulation of Na in the shoot when grown in soil admixed with coal ash. The effect of VAM colonization on the distribution of Na between roots and shoots was very striking. Similar observation reported by other research (Bi et al., 2003). In our experiment, inoculation with the VAM fungi led to an increase in K uptake from soil, and possibly from the coal ash which is T1 treatment show the low availability of potassium is due to the low percentage of clay and cation exchange capacity.

#### Effect of vesicular arbuscular mycorrhizae spore on plant height and leaf area index of wheat plant

Table-4 (a&b) indicates the plant height and leaf area index of wheat plant noted at regular intervals of 20 days. Table-4 indicates that the plant growth was maximum T5 and T6 treatment with VAM spore. The observation is also strengthened by the physico-chemical properties of the soil mixture which were acquired by adding 25 % coal ash with inoculated VAM spore. VAM hyphal networks could increase soil aggregate and stability (Miller and Jastrow, 1992). Mycorrhizae hyphae may have contributed to improved soil aggregation by the production of glomalin, a glycoprotein which may act as a cementing agent for soil particles (Wright et al., 1996). Mycorrhizae hyphae may directly improve soil structure, which physically bind soil particles (Miller and Jastrow, 1992)

**Table–4a Effect of vesicular arbuscular mycorrhizae spore on plant height of wheat plant**

Treatment	After 20 days		After 40 days		After 60 days		After 90 days	
	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM
T1	4.21	2.2	14.64	13.5	26.37	20.12	38	36
T2	4.26	2.46	14.65	13.5	28.9	24.47	40	38
T3	4.28	2.62	15.84	13.62	29.15	25	44	43
T4	4.36	3.26	17.49	14.26	29.49	25.37	48	45
T5	4.91	3.43	178.53	14.87	30.16	25.5	49	47
T6	5.44	3.5	17.96	15.32	34.43	27.2	53	50

- ❖ (+VAM) With inoculation of vesicular arbuscular mycorrhizae spore
- ❖ (-VAM) Without inoculation of vesicular arbuscular mycorrhizae spore
- ❖ Results in centimeter (cm)

**Table–4b Effect of vesicular arbuscular mycorrhizae spore on leaf area index of wheat plant**

Treatment	After 20 days		After 40 days		After 60 days		After 90 days	
	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM
T1	5.33	4.73	12.99	9.50	20.69	19.38	35.8	32.0
T2	6.52	4.80	14.72	10.75	28.74	21.79	42.5	34.7
T3	6.68	4.88	14.85	10.81	29.07	22.6	43.5	37.0
T4	6.87	4.88	15.33	12.95	31.15	24.05	45.8	37.5
T5	6.94	4.92	18.86	15.62	31.18	24.35	45.8	38.0
T6	6.98	4.92	20.57	19.31	42.29	25.57	47.5	40.0

- ❖ (+VAM) With inoculation of vesicular arbuscular mycorrhizae spore
- ❖ (-VAM) Without inoculation of vesicular arbuscular mycorrhizae spore
- ❖ Results in centimeter (cm)

**Effect of vesicular arbuscular mycorrhizae spore on grain yield of wheat plant**

Fig. 1 show the significant increase in grain yield was observed in all the soil sample inoculated with VAM spore and found maximum in 20% and 25% concentration as compared to control. This may be due to the combined effects of supplementation of micronutrients immobilization of the toxic metals and optimum texture as also discussed by Saxena et al., (1998). Similar observation reported by other research (Subha Rao et, al. 1986) It is reported that the biomass of root and shoot could increased root system and have facilitated VAM endophyte to colonize more efficiently VAM enhanced dry matter and grain yield in plant growth due to inoculation with mycorrhizal fungi. VAM certainly enhanced availability of phosphorus in soil, which play important role in biological nitrogen fixation, reflecting that the healthy plant growth is beneficial for grain yield.

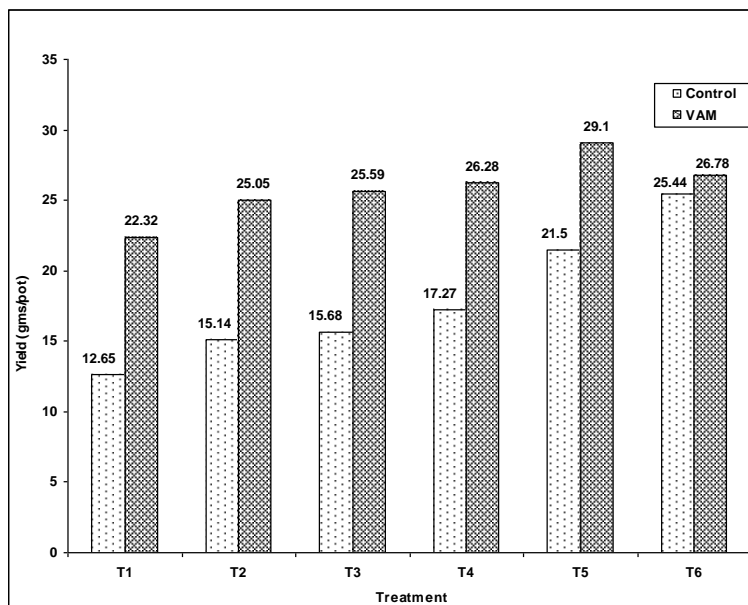


Fig1: Effect of vesicular arbuscular mycorrhizae spore on grain yield of wheat plant

**EXPERIMENTAL SET UP: EFFECT OF PRIMARY NUTRIENT (N,P,K) IN COAL ASH ADMIXED WASTELAND SOIL THROUGH VESICULAR – ARBUSCULAR MYCORRHIZAE INVATION**

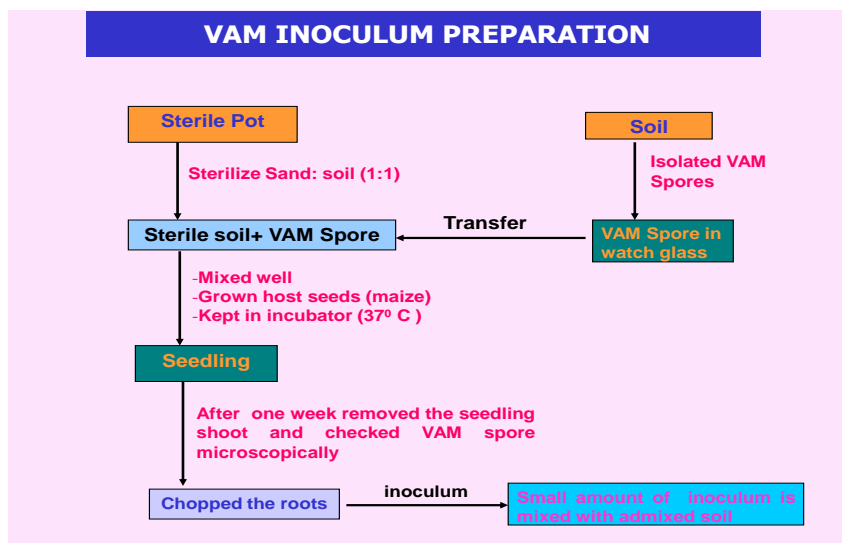
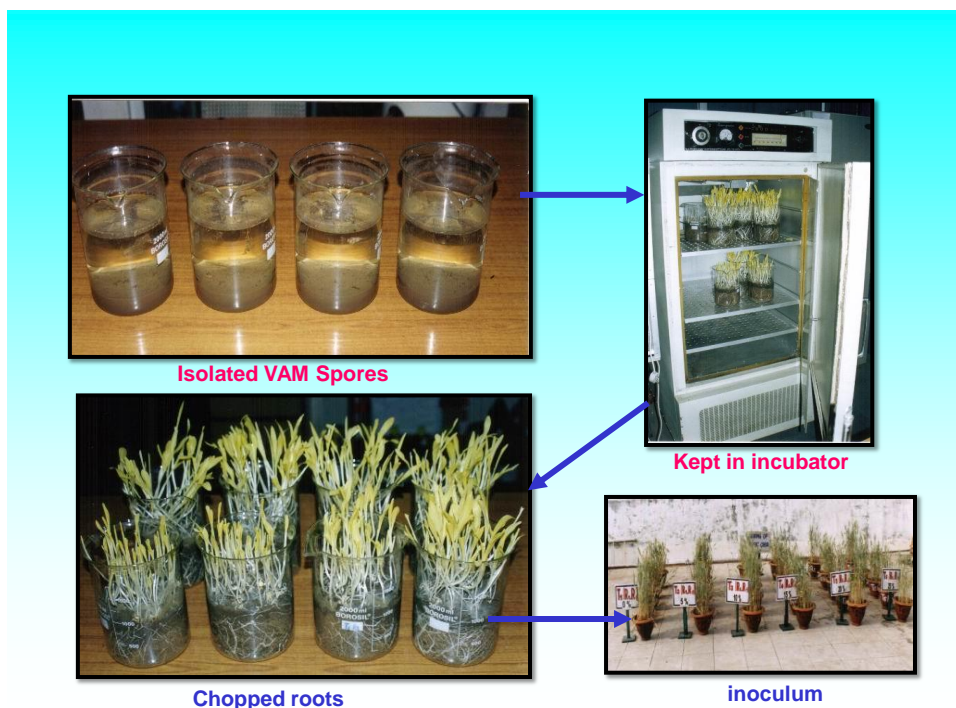
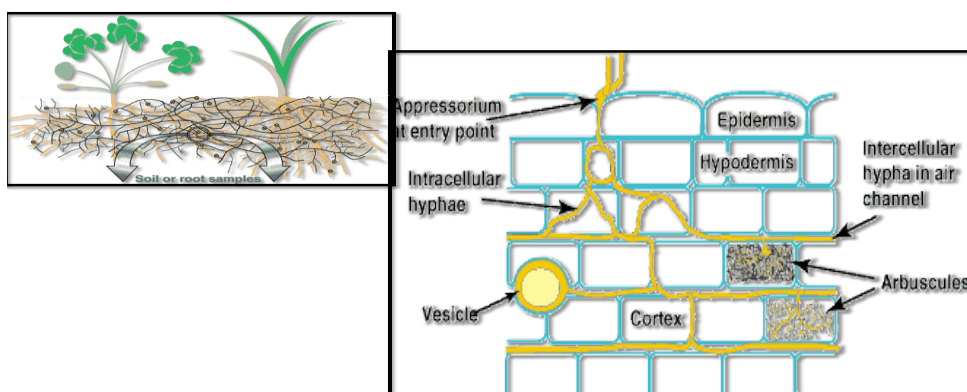


FIG 2 : VAM INOCULUM PREPARATION



**FIG 3 : VAM EXPERIMENTAL SETUP**



**FIG4 : VAM Association**

**CONCLUSION**

The present study indicates the healthy growth of wheat plant, which is possible in soil, amended with coal ash and could be improved by colonization of the plant roots by mycorrhizal fungi. Pot trials are an important step for correct management of indigenous VAM fungi. VAM fungi may also contribute to the re establishment of a general soil microflora, and of a sustainable agricultural system combined with the appropriate use of coal ash.

**REFERENCES**

1. Dodd JC. 2002. The role of arbuscular mycorrhizal fungi in agro – and natural ecosystems Outlook on Agriculture 29 (1), 55–62.
2. Davies .1993. Drought resistance of mycorrhizal papper plant- indeoendent of leaf phosphorus concentration, response in gas exchange, and water relation. Physiol. Plant 87, 45 – 53.
3. Gerderman J.W. and Nicoloson, T.H., 1963. Spore of mycorrhizal endogen, species extracted from soil Wet sieving and decanting. Trans. Br. Mycol. Soc., 46, 235 – 244.



4. Guerrero A., Hernandez M.S., S. Goni. 2000. The role of the fly ash pozzolanic activity in simulated sulphate radioactive liquid waste. *Waste management* 20. 51 – 58.
5. Hetrick, B.A.D; Wilson. G.w.t., Figge, D.A.H., 1994. The influence of mycorrhizal symbiosis and fertilizer amendment on establishment of vegetation in heavy metal mine spoil. *Environ. Pollut.* 86, 171-179.
6. Jackson M L 1958. Soil Chemical Analysis. In *chemical and microbiological properties*, Part2, edited by A L Page, 166- 179.
7. Kalra et al., 1998. Fly ash as a soil conditioner and fertilizer. *Bioresource Technology* 64, 163-167.
8. Khan S, Taria Begum, Singh J. 1996 Effect of fly ash on physico – Chemical properties and nutrient status of soil. *Journal of Environmental Health* 38(1), 41– 46.
9. Miller, R.M. AND Jastrow, J.D. 1992. The role of mycorrhizal fungi in soil conservation. In: G.J. Bethlenfalvay and R.G. Linderman (Editors). *Mycorrhizae in sustainable Agriculture*. American Society of Agronomy special Publication No. 54, Madison, WI, pp.29 – 44.
10. Saxena. M., Ashoken P., Mandal S and Chauhan A. 1998. Impact of fly ash in wasteland development. *J. Energy Conservation and Recycling Vol. (4)* 229-234.
11. S.R. Olsen. 1954. Estimation of available phosphorus in soil by extraction with sodium carbonate U.S. Dep. Agri, Madison, Wisconsin, 403 – 403.
12. Subha Rao, N.S. Tilak, K.V.B.R. & Singh, C.S. 1986. P SOIL. 95, 351-359.
13. Subbiah Ashia B.V. 1995. A rapid procedure for the determination of available nitrogen in soils. *Cur. Sci.* 25, 259-260, India.
14. Shukla S., seal. S., Akesson J., Order R., Carter. R., Rahman Z. 2001. Study of mechanism of electroless copper coating of fly ash cenosphere particles. *Applied surface Science* 181, 35 – 50.
15. Tandon H L S. 1992. Method of sampling. In *Method of Analysis of soil, water and fertilizers* New Delhi: FDCO. 156 pp 20.
16. Technology, Information, Forecasting and Assessment council TIFAC 1992. *Techno Market Survey on Industrial air pollution control technologies*, Department of Science and Technology, New Delhi (DST), Dec., pp.62 –76.
17. Wright, S.F., Franko – Snyder, M; Morton, J.B. & Upadhaya, A. 1996. Time course study and partial characterization of protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant and soil*, 181: 193.
18. Y.L. Bi, X.L. Li, P. Christie, Z.Q. Hu, M.H. Wong 2003. Growth and nutrient uptake of arbuscular mycorrhizal maize in different depths of soil overlying coal fly ash. *Chemosphere* 50, 863 – 869.