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Improvement of seedling and clone establishment capability of *Pongamia pinnata* (L.) Pierre grown in fly ash using mixed microbial inoculums

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ABSTRACT

Among various energy plants, *Pongamia pinnata* is one of the most important species because of its multipurpose uses, high oil content and high medicinal values and need to be cultivated in large-scale for commercial use. In order to facilitate survival and growth of suitable species in marginal and wastelands and for meaningful utilization of fly ash, fly ash with different concentrations and combinations of growing media were used as substrates supplemented with microbial inoculums for better establishment of vegetatively propagated plants of *Pongamia pinnata*. Maximum growth, biomass production and better nodulation in the plant was achieved using fly ash and coir pith in the proportion of 4:1 and garden soil and coir pith in the proportion of 3:2 as compared to dilution with sand and pure fly ash.

1. Introduction

Increased demand and the progressive worldwide shortage of fossil fuels warrants for search of alternate source of energy. Biofuels in the form of bioethanol from microbial fermentation and biodiesel from vegetable oil or any non-edible oil provide good hope as alternate source of energy. Now a days many plants with high oil content are being tried as source of biodiesel. In this context it has been observed that many plants like *Pongamia pinnata (Millettia pinnata)*, *Jatropha* spp., *Azadirachta indica*, *Simarouba glauca* etc. yield oil that can be used as biodiesel and are known as biodiesel plants. Among various energy plants, *Pongamia pinnata* is one of the valuable and important plants because of its multipurpose commercial uses (Naik *et al.*, 2008) including its high medicinal value. *Pongamia pinnata* seeds contain about 40j oil, which can be converted to biodiesel by trans-esterification method (Meher *et al.*, 2006). However, the unavailability of good arable land stands a great hurdle in this direction and only hope is to develop technology for growing *P. pinnata* in marginal and waste lands with stress soil conditions.

The state of Odisha is now facing the problem of rapid wasteland formation due to contamination by flyash generated from thermal power plants using excessive coal for power generation. Every year thermal power plants in India produce more than 100 million tonnes of fly ash, which is expected to reach 175 million tonnes in the near future (Jamwal Nidhi, 2003). Flyash is a highly insoluble particulate substance generating heavy toxic effects in environment. Disposal of this huge quantity of fly ash is posing a great problem due to its limited use in the manufacturing of bricks, cements, ceiling and other civil construction materials. This would further bring changes in land-use patterns and contribute to land, water and atmospheric degradation, if proper management plans for handling ash are not undertaken (Kalra *et al*., 1996). The countries like Germany, Denmark, France, U.K., USA, and the Netherlands utilize fly ash (up to 70 %) for construction purpose, but in India it's utilization is less than 15 % (Sinha and Basu, 1998). Use of fly ash in agriculture provides a feasible alternative for its safe disposal to improve the soil environment and enhance the crop productivity. However, a

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judicious management strategy has to be developed to abate the land pollution from the heavy metals present in the fly ash. Flyash being a fossil byproduct contains many micro and macro nutrients for plants but being insoluble, are not readily available to plants. There is a great scope of utilization of flyash as plant growth substrate through microbial amelioration (Misra *et. al*., 2000).

The present research work aims at finding out a suitable dilution factor for utilization of flyash as plant growth substrate through microbial amelioration using consortia of microbes of suitable N_2 fixer, phosphate solubilizer and AM fungi using decomposed coir pith as Mixed Microbial Inoculums for Reclamation (MMIR) to minimize the toxicity and promote proper utilization of flyash for successful growth and establishment of seedlings and clonally propagated *Pongamia pinnata* plantlets. Plantlets obtained from rooting of stem cuttings in different media containing fly ash were studied for their survival, growth, biomass yield and other parameters. Methods of vegetative propagation of *P. pinnata* has been standardized by many authors (Palanisamy and Kumar 1997; Thatoi *et al.*, 2002, Thirunavoukkarasu *et al.*, 2002, but there has been no study on mass propagation and subsequent establishment of *P. pinnata* using fly ash as growing media. In view of this, the present study was undertaken to make a comparative assessment and growth of *P. pinnata* in pure fly ash and fly ash amended with sand, garden soil and decomposed coir pith in different concentrations and combinations.

2. Materials and methods

2.1. Collection of samples

Fly ash samples were collected from ash pond of NALCO, Angul and brought to the laboratory. Coir pith was collected from coir industries located at Satasankha, Puri district, Odisha. The psysico-chemical properties of fly ash were analyzed following standard method of Jackson (1973). Further the study of microbial population of flyash was studied using standard plate technique of (Alexander, 1962).

Three ingredients such as garden soil, sand and decomposed coir pith were mixed with fly ash samples in different proportions to prepare the growing media.

2.2. Microbial decomposition of coir pith

Coir pith is a highly insoluble lignocellulosic material produced in huge quantities by the coir industries and is a rich source of organic carbon. This is not easily biodegradable and dumped along roads and wastelands causing pollution. Attempt was made to facilitate early decomposition of coir pith to provide nutrients for plant growth by mixing it with a lignocellulolytic fungi *Pleurotus* sp., a phosphate solubilizer (*Bacillus* sp.), rock phosphate and sodium chloride in a cement-concrete tank and then by providing regulated watering. The process of decomposition was carried out under anaerobic condition by covering the tank with polythene sheets for a period of one month.

2.3. Pot culture experiment

Flyash was diluted with different ingredients like garden soil, sand and decomposed coir pith in the proportions of 4:1 (fly ash: sand/ soil/ coir pith:: 4:1) and 3:2 (fly ash: sand/ soil/ coir pith:: 3:2). Thirty polypots were prepared for each category of growing medium and other thirty containing pure fly ash**.** The experiment was conducted during the month of January-April, 2009. Stem cuttings with three nodes were taken from healthy tree of *P. pinnata* during the early morning hour when the leaves are turgid. The leaves from the basal node were annexed by sharp sterile knife and basal straight cut was made just below the node. The upper end of the cutting was sealed with paraffin wax to prevent infection and to minimize the loss of water from the cut ends. The lower ends of stem cuttings were dipped in .01% $HgCl_2$ solution for 2 minutes and washed several times with sterile distilled water. The cuttings were then planted in polypot containing different proportions of fly ash and then transferred to a mist chamber and arranged in different groups in randomized block design. Intermittent mist of fresh water is provided two times for a period of thirty minutes during day time with the help of sprinklers. The rooted cuttings were uprooted after 90 days of planting and data on growth parameters were recorded. Morphological features such as number of leaves, shoot length, root length, plant biomass, and number of nodules formed and biochemical parameters such as total chlorophyll and total sugar contents were estimated (Arnon, 1942).

3. Results and discussion

Analysis of physic-chemical properties revealed that the pH of fly ash is high (8.0), water holding capacity (37.2%) is very low and percentage of carbon is negligible. Due to these adverse physico-chemical properties, the fly ash is not suitable for plant growth. The findings of the study is in conformity with Sarkar and Rano (2007), who reported alkaline nature of fly ash with poor water holding capacity affecting plant growth. In view of this, vast stretches of land close to thermal power stations get contaminated due to fly ash deposits and remain hostile to plant growth.

As could be seen from the data presented in Table-1, with addition of increasing doses of sand, garden soil and decomposed coir pith to raw fly ash, pH gradually goes on decreasing making it acidic and suitable for plant growth.

Table1
Physico-chemical properties of fly ash amended with different doses of sand, garden soil and coir pith

Types of growing medium	pH	Water-holding capacity (%)	Organic carbon content (%)
GS	6.4	31.4	1.84
FA	8.0	37.2	.85
FA:CP:: 4:1	7.1	86.2	1.63
FA:GS:: 4:1	6.8	40.22	1.37
FA:S:: 4:1	6.7	39.0	0.81
FA:CP:: 3:2	6.0	168.0	1.89
FA:GS:: 3:2	6.0	46.9	1.66
FA:S:: 3:2	6.7	41.6	0.78

FA-Fly ash, CP-Coir pith, GS-Garden soil, S-Sand, C-Carbon

On the other hand, the water holding capacity and organic carbon content increases remarkably when fly ash is amended with decomposed coir pith. The flyash when mixed with coir pith in a proportion of 4:1, the water holding capacity increases from 37.2% to 86% and to 168% in the proportion of 3:2. Similarly, the organic carbon content increases from 0.85% to 1.63% when fly ash is mixed with coir pith in the proportion of 4:1 and to 1.89% in the proportion of 3:2. Assessment of microbial diversity in raw fly ash revealed that the micro-organisms like *Rhizobium*, *Nitrosomonas*, *Nitrobacter* and *Ammonifier* responsible for N_2 cycle were very low in comparison to garden soil. Therefore it can be concluded that the flyash has very poor N_2-fixation activity which is not conducive for plant growth. However, amendment of fly ash with coir pith and garden soil increases the microbial population in it as compared to its combination with sand. Mitra *et al.* (2003) recommended the application of organic amendments to the stress soil prior to microbial inoculation.

With regard to growth performance of *Pongamia pinnata* clones, it was observed that the media containing fly ash and coir pith in the proportion of 4:1 and both garden soil and coir pith mixed with fly ash in the proportion of 3:2 were responsible for significant improvement in growth parameters like number of leaves, number of nodules, plant height and biomass yield in comparison to dilution with sand and those grown on pure flyash (Table-3). The

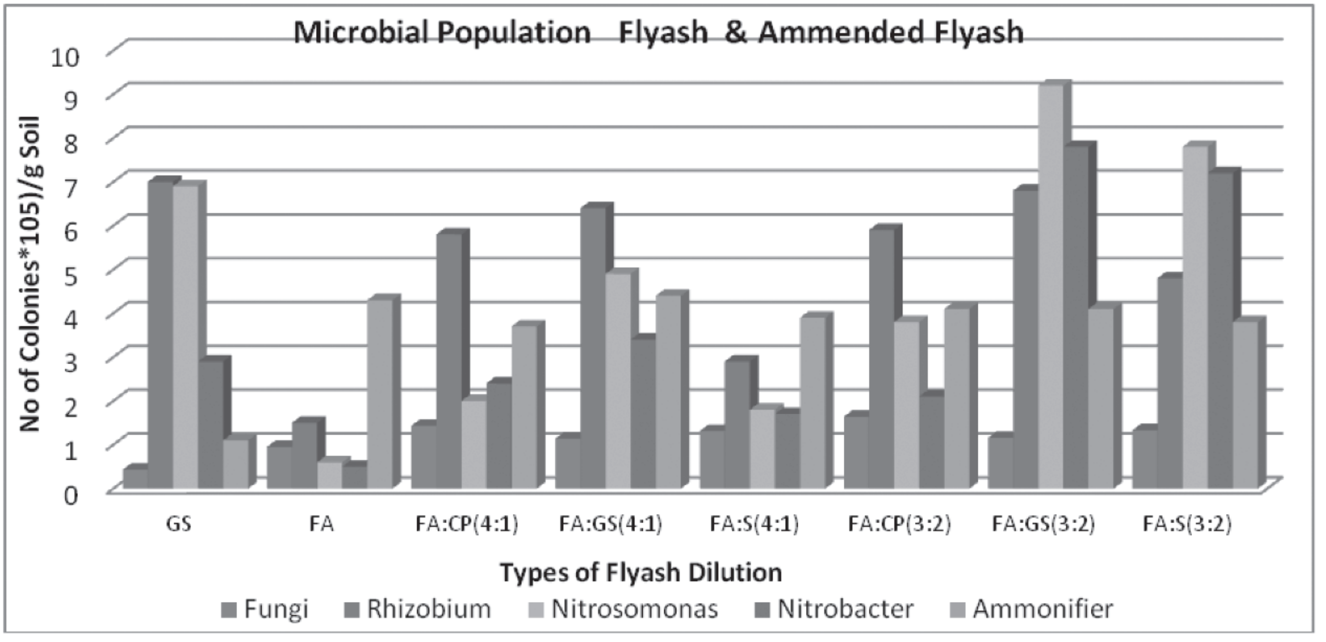

Fig 1: Microbial population in fly ash and amended fly ash (CFU isolated by Pour-plate method using Czapek's Dox media for fungi, Rhizobium by YMA media, *Nitrosomonas* by Winogradsky's medium, *Nitrobacter* by Nitrite agar and *Ammonifier* by yeast extract Mannitol agar media)

Table 3
Growth and nodulation response of *Pongamia pinnata* clones in differently diluted flyash

Parameters	Treatments							
	GS	FA	FA:CP::4:1	FA:GS::4:1	FA:S::4:1	FA:CP::3:2	FA:GS::3:2	FA:S::3:2
Leaf No.	41±16	15±8.5	38±9.5	25±5.0	26±5.0	31.8±9.0	39.4±13.0	17±3.73
Nodule No.	60±3.5	0±0	97±13	50±9.0	41±3	97±20.39	36±16	10±9.59
Shoot Length (cm)	43.3±3.8	27.9±3.0	38.2±5.06	30.6±2.93	20.14±5.58	43.3±6.64	53.62±12.37	41.56±6.36
Root Length (cm)	33.0±6.8	18.2±10.84	39.0±6.82	34.8±3.69	24.14±15.58	37.18±11.36	53.88±15.0	35.48±12.0
SDW (gm)	4.46±6.8	1.35±1.1	3.96±1.42	3.63±.16	1.59±.32	5.12±1.23	6.02±2.67	3.6±2.39
RDW (gm)	1.86±.81	0.70±0 .10	.75±.46	1.25±.45	1.25±.08	89±.09	1.89±.40	0.59±.05

SDW: Shoot Dry Weight, RDW: Root Dry Weight

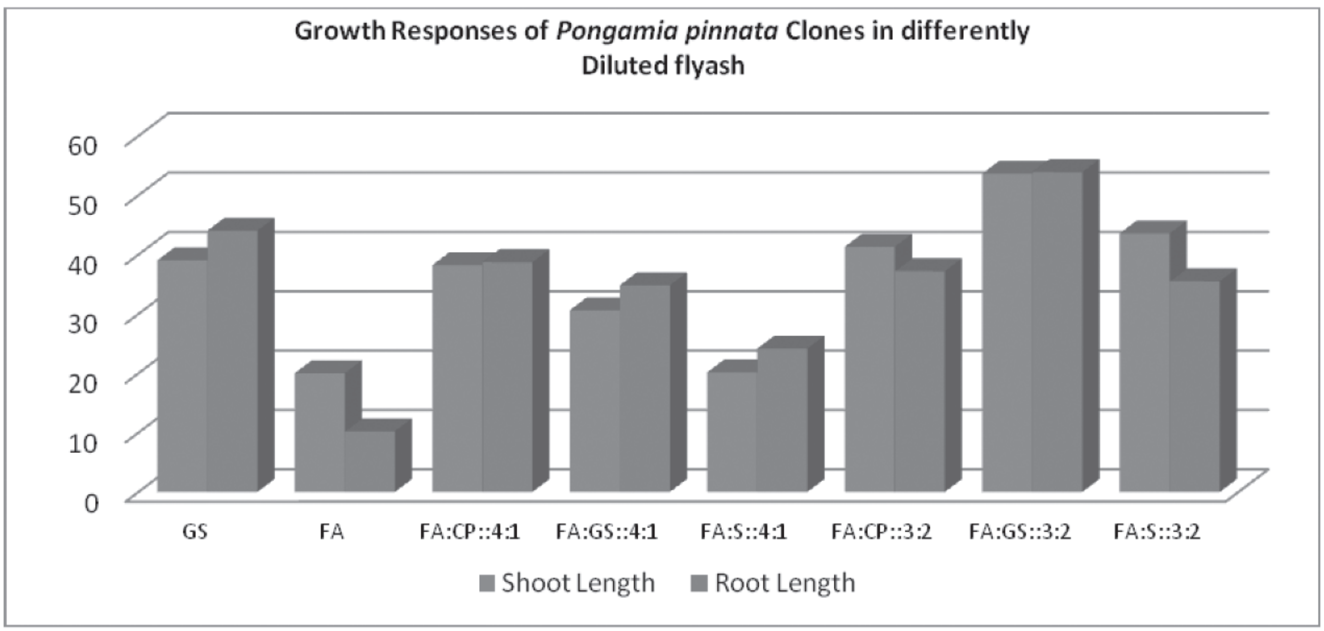

Fig 2: Growth response of *Pongamia pinnata* in differently diluted fly ash

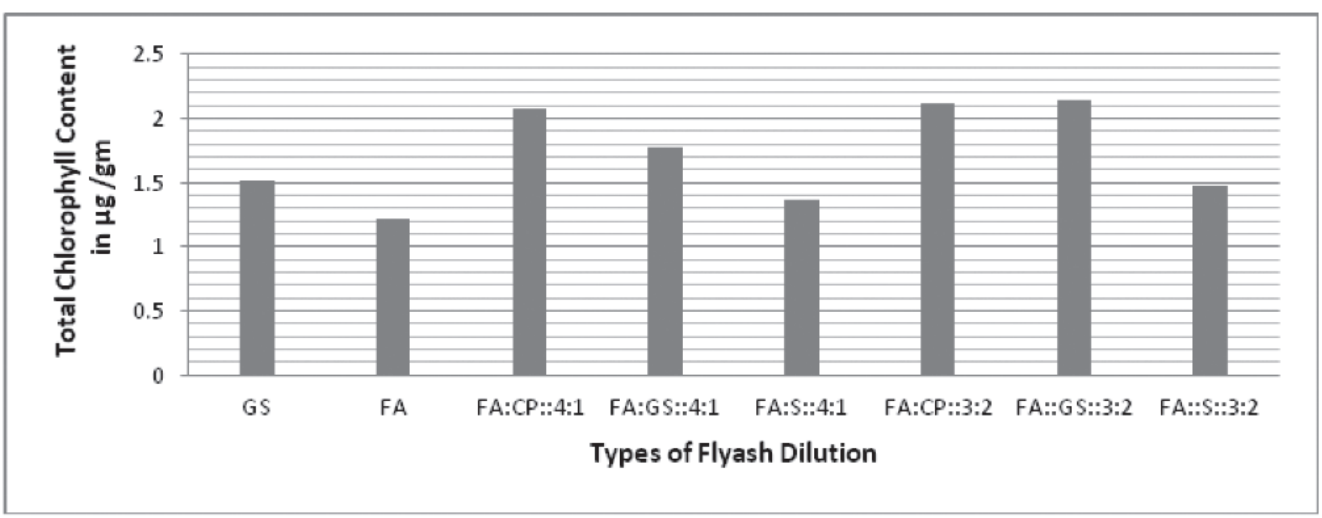

Fig 3: Changes in total chlorophyll content of *P. pinnata* grown in fly ash amended with varying doses of sand, soil and coir pith after 90 days of planting

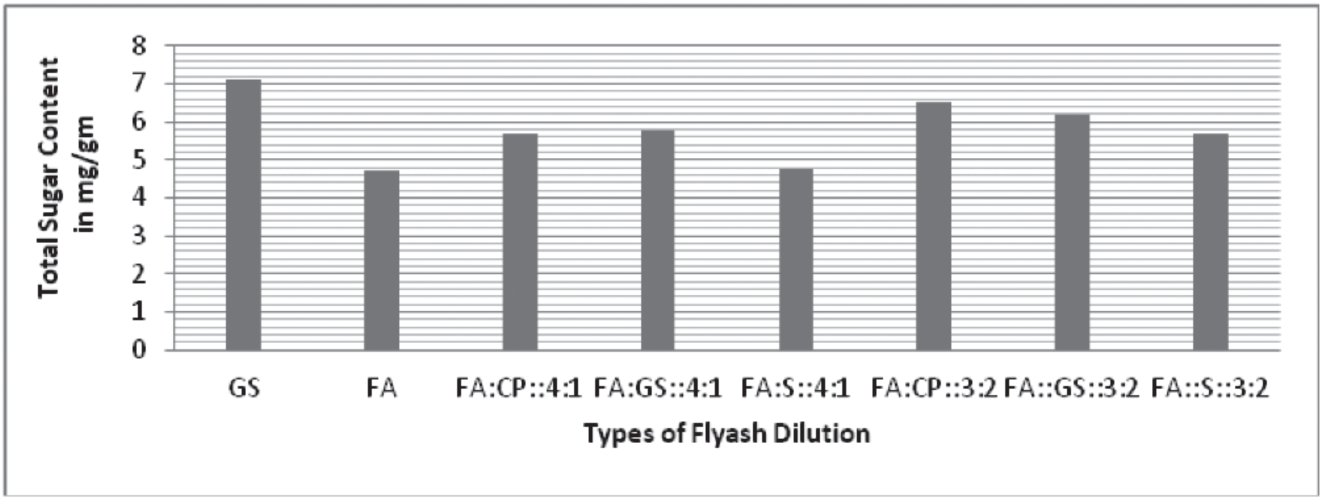

Fig. 4. Changes in total sugar content of *P. pinnata* grown in flyash and amended fly ash after 90 days of planting

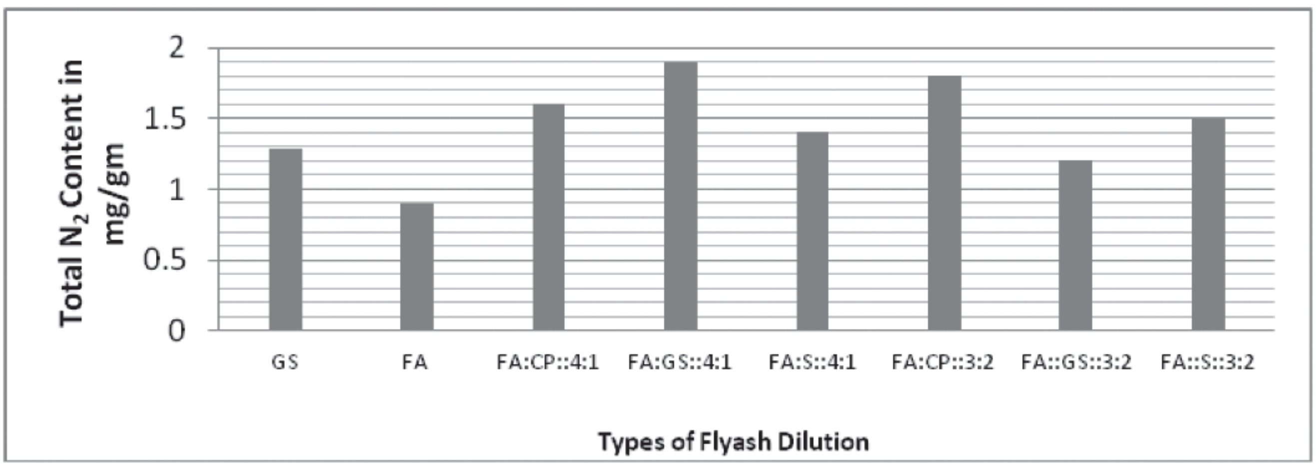

Fig. 5. Changes in N_2 content of *P. pinnata* clones grown in fly ash and amended fly ash (90 Days after planting)

increased number of nodule formation in the plant grown in a medium containing fly ash and coir pith in the proportion of 4:1 indicates improvement in *Rhizobium* establishment in the rhizosphere and enhancement of nitrogen fixing ability. Similar trend with regard to increase in chlorophyll, total sugar and protein contents was also noticed. The result is in agreement with the findings that enhancement in growth of seedlings in stress soil condition under the Mixed Microbial Inoculums (MMIR) treatment may be attributed to the release of nutrients (carbon and energy source) from decomposed coir pith to the soil favouring growth of the beneficial microorganisms and subsequent enhancement of soil enzyme activities and increase in the availability of nutrients to plants (Yunus *et al.,* 2010).

4. Conclusion

The present paper aims at standardizing suitable media and mixtures containing fly ash as a major component and methods of microbial amelioration for enhanced growth and establishment of clonally propagated plants of *Pongamia pinnata* in marginal soils and wastelands. Decomposed coir pith- a lignocellulosic waste, proved to be a very good material for amendment of flyash to promote growth and establishment of the plants under field conditions. Along with proper utilization of fly ash and coir pith, both of which are wastes and pose environmental problems, the findings of the study will help in raising large-scale plantation of *Pongamia pinnata*- a biodiesel plant species in marginal and wastelands.

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