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Impact of Fly Ash on Germination and Initial Seedling Growth Performance of *Acacia auriculiformis* A. Cunn. Ex Benth

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Impact of fly ash (FA) on germination and initial seedling growth performance of *Acacia auriculiformis* was studied. Growing media was prepared by mixing FA to forest soil (S) at five concentrations 20%, 40%, 60%, 80% and 100% (w/w). The experimental design was CRD with six treatments and three replications. Freshly collected seeds were treated with warm water followed by cold water and sown at 1.0-2.0 cm depth in germination trays filled with media of different treatments. Significant ($P<0.05$) variation in germination (G) period, G. rate, G. capacity and G. Index with respect to FA concentration in media was observed (n=100). Maximum G. rate (84.49%) and G. index (25.94) were found in media having 20% FA (T_1) after 30 days of sowing. After 90 days of transplanting in poly pots containing the mentioned substrates, significant difference ($P>0.05$) in seedling survival rate, plant height, diameter growth, leaf number, nodules per plant and seedling quality index were observed. The survival rate (83.24%), plant height (68.87 cm), collar diameter (0.83 cm), root length (39.00cm), nodule number per plant (24.16) and seedling quality index (0.88) were at maximum in growing media having 40% FA (T_2). It is concluded form the present investigation that FA can be admixed @ 20% (w/w) in forest nurseries for improving germination and @40% (w/w) for promoting seedling growth and quality improvement of *Acacia auriculiformis.*

Introduction

Fly ash (FA) is one of the major solid industrial wastes of concern in this twenty first century. It is being generated from coal fired thermal power plants which are the backbone of electricity supply in the world. In India lignite grade is primarily used and it generates about 30-45% ash as compared to imported high quality coal which has low ash content in the order of 10-15%. As a result huge quantities of FA are being produced at thermal power stations requiring large area of precious land for proper disposal. India ranks fourth in the world in the production of coal ash as by-product waste after USSR, USA and China, in that order (Senapati, 2011). 196.44million tons of Fly ash is being generated from 167 thermal power stations, during the year 2017-18. Tough there is a stringent government regulation for cent percent utilization of FA, only 67.13% have been utilized (CEA 2018).

FA contains a number of toxic metals such as arsenic (As), barium (Ba), mercury (Hg), cadmium (Cd), selenium (Se), chromium (Cr), nickel (Ni), vanadium (V), lead (Pb) and zinc (Zn) depending upon the source of coal (Dwivedi and Jain, 2014). Proper disposal and management of such a huge quantity of FA possessing potential threats of air and water soil pollution is a great challenge (Rawat *et al.,* 2018). Utilization of FA for a particular purpose depends up on its elemental content which is primarily controlled by type of coal and its source. FA is being used in manufacturing cement, concrete, bricks, wood substitute products, in road construction, wasteland reclamation; filling of underground mine spoils (Kaur and Goyal, 2015). In India major sectors include construction of roads and embankments, production of cement, mine-filling, reclamation of low-lying areas, making bricks and tiles (Environment Annual Reports, 2014-15).

FA contains almost all the plant nutrients except nitrogen, phosphorous and humus, which can be supplemented by organic matter (Sharma and Karla 2006). Hence there is a scope for utilization in agriculture and forestry sector. Many research findings infers to the positive growth and nutritional efficiency of FA. Crop plants of the families *Brassicaceae*, *Chenopodiaceae*, *Fabiaceae*, *Leguminoceae* and *Poaceae* are most tolerant to FA toxicity (Cheung *et al.,* 2000). Low bulk density, high water holding capacity and porosity, rich silt-sized particles, alkaline nature, negligible solubility of Indian FA makes it a better choice for reclamation material for wasteland and mine overburden soils. Still then a large quantity of FA is being dumped up in ash ponds and lagoons. Some of the FA contains deadliest toxic metals like As, Hg, Cd, Cr and Se. These toxic metals along with other toxicants can cause cancer and neurological damage in human. They can also harm and kill wildlife, especially fish and other water-dwelling species (Ahmad *et al.,* 2014). The current status of utilization of FA in India is only 60-70% (CEA, 2018), providing a wide scope for searching new avenues.

One of the most potential areas of utilization is in forestry sector where it can be consumed either in nursery or for tree plantation activities. This will help in locking the toxic heavy metals in the wood biomass for longer period of time. FA as planting material in forest nursery is not a new concept. Goyal *et al.,* (2002) reported its use in nursery as growing media but commercial use is scanty or absent. Hence attempt is made to know its impact on seed germination and growth of seedlings at early stages.

Acacia auriculiformis (Fabaceae) commonly known as earleaf acacia was selected because of its nitrogen fixing ability, rapid growth potential and diverse ecological significance. It is an important species for social and agroforestry plantation. Tolerance in infertile, acid, alkaline or seasonal waterlogged soil, mine overburden soil makes it very useful species for reclamation purpose.

Materials and Methods

The experiment was conducted in College of Forestry, Odisha University of Agriculture and Technology, Bhubaneswar situated at 20° 15' N latitude and 85° 52' E longitude with altitude 25.9 m amsl. FA was collected from one of the silages of Indian Metals and Ferro Alloys (IMFA) Limited, Choudwar, Cuttack (Odisha). The mean monthly temperature, relative humidity and rainfall of the experimental site is given in Fig.1.

Growing media preparation and analysis for physicochemical properties

Growing media was prepared by mixing FA to forest soil (S) at concentrations 20%, 40%, 60%, 80% and 100% W/W. There were six treatments (T_1-20% FA+S, T_2-40% FA+S, T_3-60% FA+S, T_4-80% FA+S, T_5-100% FA) including control (T_6-S). The growing media was analysed for physical and chemical properties. Bulk density (BD) and water holding capacity (WHC) was determined by using the protocol given by Piper (1966). pH and electrical conductivity (EC) were measured following protocol given by Jackson (1967), organic carbon (OC) was estimated as per Walkley and Black (1934). Available nitrogen, phosphorus and ammonium acetate extractable potassium were estimated as per the procedure given by Subbiah and Asija (1956), Olsen *et al.,* (1954) and Merwin and Peech (1951) respectively. The physical and chemical properties of growing media are given in table-1.

Seed treatment and sowing

Freshly collected seeds were given hot water treatment prior to sowing. Seeds were soaked in warm water at 80^0 C for 10 minutes followed by cold water treatment for 24 hours (Azad *et al.,* 2011). Eighteen germination trays having dimension 90 cm (L) × 45 cm (B) × 15 cm (H) were filled with above six mentioned growing media to the brim leaving 3.0 cm. Hundred seeds per replication (totalling 300 seeds per treatment) were sown at 1.0-2.0 cm depth, covered with paddy straw and kept at open nursery condition. Regular watering was made during morning hours as per the requirement. Observations pertaining to germination parameters were recorded daily up to 30 days after sowing. Germination period was determined by observing the day taken for first germination (DTFG) to 30^{th} day when about 80-85% seeds have germinated. Based on the number of seeds germinated the following parameters were calculated as per the standards given by Czabatore (1962) and AOSA (1983).

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Germination capacity} = \frac{\text{Total seeds germinated} + \text{viable seeds}}{\text{Total No of seeds sown in all replications}} \text{ X } 100$$

$$\text{Germination Value} = \text{PV} \times \text{MDG}$$

Where, PV = Peak value of Germination
MDG = Mean daily Germination

$$\text{Germination Index} = \frac{\text{No. of Germinated seed}}{\text{Days of first count}} + \cdots\cdots + \frac{\text{No. Germinated seed}}{\text{Days of Final Count}}$$

Seedling growth and quality

After completion of germination study, seedlings were transplanted into poly pots (22.86 × 12.7 cm) containing growing media of above mentioned treatment combinations. Growth parameters such as shoot length, collar diameter and number of leaves were assessed monthly after 30 days of transplanting for 3 months. Total shoot length was measured by using ruler (taken from the apical bud of the plant to the base of the shoot) and stem diameter by using electronic digital calliper (6"/150 mm, accuracy ± 0.01 mm, Mitutoyo- CD-6″ASX:500-196-30).

For recording the quantitative parameters pertaining to root growth, the entire seedling was dipped in a bucket of water at 90 days to remove adhering soil from it. It was then carefully washed so that no damage was made to root system. Length of roots (starting from collar region to the end point) and number of root nodules were recorded. Thoroughly washed seedlings (without damage to root and shoot) were dried under sun for 30 minutes.

The shoot was cut from the collar portion and weighed. Then the root and shoot sample were put in paper bags separately and were oven dried at 80°C until constant weight observed. Growth observation was based on 45 numbers of randomly selected plants from each treatment. The seedling quality index (SQI) was calculated by using the formula as described by Dickson *et al.,* (1960).

$$SQI = \text{Seedling dry wt. (gm)} / [\frac{\text{Height(cm)}}{\text{Diameter(mm)}} + \frac{\text{Shoot dry Wt. (gm)}}{\text{Root dry Wt. (gm)}}]$$

The experiment was completely randomized design (CRD) with six treatments and three replications. The collected data were analysed by using SPSS software version 20 for windows operating system. Analysis of variance (ANOVA) was carried out to determine the treatments effect on seed germination and early seedling growth. Means were analysed according to the Duncan Multiple Range Test (DMRT) at $P < 0.05$ (Duncan 1955).

Results and Discussion

Fly ash is a noxious solid waste seeking proper disposal and management. It has some multifarious utility. Still ample amount left unutilised at disposal sites of thermal power plants polluting air and water. There exists a vast scope for utility in forestry sector as potting mixture ingredient and soil improvement material at difficult sites prior to plantation. The matrix of application depends upon the elemental composition of FA to be used, tolerance limit of plant species selected and physiochemical property of plantation site soil or growing media in which FA need to be added. Fertility status of poor degraded waste lands and problematic soils are successfully improved by FA addition to varying degrees in different agro-climatic situation. Enhancement in crop yield and vegetative growth tree species have been reported by many workers when applied judiciously (Kumar *et al.,* 2002, Sinha *et al.,* 2005, Ramesh *et al.*, 2008, Chaudhary *et al.*, 2009, and Krzaklewski *et al.,* 2012, Behera *et al.*, 2018.)

Effect of substrate on seed germination

A success of plantation programs needs uninterrupted supply of quality seedlings. Growth media have a profound impact on germination and subsequent growth of embryo. Seedlings raised on good media ensure better establishment and growth when planted to the main field. The ultimate advantage of a suitable substrate is good drainage, water holding capacity and adequate supply of nutrients thereby, producing excellent disease-free seedlings (Noble 1993). Substrate property especially pH and water retention capacity have a marked impact on germination. pH affects germination either by increasing the osmotic pressure of the media to a plant that will retard or prevent the intake of water or by causing toxicity to the embryo (Rashid 2004).

The present study indicates that, FA have a significant ($P< 0.05$) impact on seed germination parameters like g. period, g. rate, g. capacity and g. index, however did not have any impact on the number of days taken for first germination (NDFG) and germination value (Table 2). Addition of FA to growing media reduced germination period in a dose-depended manner due to an increase in pH towards alkalinity. Similar type of observations was reported by Behera *et al.,* (2020) in *Leucaena leucocephala.*

The highest seed germination rate (84.49%) was observed in substrate having 20% fly ash (T_1) and statistically at par with ($P>0.05$) treatment T_2 (80.02%). The germination rate of 77.40% in T_3 and 75.00% in control (T_6) are statistically at par ($P>0.05$) with each other. Minimum germination rate (64.65%) was observed in substrate having 100% FA (T_5, Table-2).The increased germination rate (84.49%) in 20% FA admixed growing media was attributed to the improvement in the physicochemical condition of germinating media over control (75.00 %, Table-2). Azad *et al.,* (2011) reported maximum 83.75±1.25% germination for hot water treatment (Immersion in hot water at 80°C for 10 min) of seeds of *A. auriculiformis*. The reduction in germination rate beyond 20% FA addition (w/w) in media was due to enhanced pH and elemental toxicity. Higher pH and metals like Cu^{2+}, Zn^{2+} at higher EC are reported toxic to embryo and reduces biological activity during germination process (Gupta *et al.*, 2000). There existed negative relationship between FA rate with germination percentage, germination capacity but it was positive with DTFG, germination period and germinative index (Table-2).

Effect of substrate on seedling growth

After 90 days of transplanting significant difference (P>0.05) in survival rate, plant height, diameter growth, number of leaves, mean root length, nodules per plant and seedling quality index of *A. auriculiformis* was observed (Table-3).

Highest seedling survival rate (83.24%) was found in substrate containing 40% FA (T_2) which was statistically (P>0.05) indifferent from treatment T_1 (81.83 %), T_3 (79.80 %) and control (80.93%). The maximum survival rate (83.24%) of seedlings in substrate containing 40% FA (T_2) was due the improved aeration, water retention capacity and favourable pH of substrate (Table-1). The survival rate decreased linearly with increased concentration of FA up to minimum 46.57% in growth media having 100% FA (Table-3).

Table.1 Physicochemical properties of fly ash, forest soil and fly ash substratum

Property	Forest Soil (S)	Fly ash (FA)	FA substrate (Forest soil + FA % (w/w))			
			S+ 20% FA	S + 40% FA	S + 60% FA	S+ 80% FA
pH	6.97	7.67	7.06	7.26	7.32	7.45
EC(dS m^{-1})	0.684	0.212	0.230	0.265	0.311	0.476
N (kg ha^{-1})	0.002	125.50	106.15	98.67	82.50	37.50
P (kg ha^{-1})	6.70	56.13	48.23	36.92	20.58	14.69
K (kg ha^{-1})	146.43	474.36	496.41	509.92	546.2	613.15
OC (%)	0.005	0.570	0.519	0.464	0.382	0.261
BD(g cm^{-3})	0.69	1.48	1.41	1.24	0.95	0.76
Pore space (%)	49.52	32.8	43.2	46.4	48.25	49.35
WHC (%)	58.2	41.0	43.25	45.84	48.72	54.33

Values are Mean (N=Arithmetic mean); FA- fly ash; S- Forest Soil, EC- Electrical conductivity, NPK-Available Nitrogen, Phosphorous and Potash, OC-Organic carbon, BD-Bulk density, WHC-Water holding capacity.

Table.2 Effects of substrates on germination of *A. auriculiformis* seeds at 30 days after sowing

Parameters→ Treatments↓	DTFG	G. Period	GP (%)	GC	GV	GI
T_1	9.0_{b}	11.33_{ab}	84.49_{d}	87.98_{d}	69.67_{c}	25.94_{c}
T_2	8.67_{b}	11.0_{ab}	80.02_{cd}	84.20_{cd}	60.39_{bc}	23.67_{bc}
T_3	7.67_{ab}	10.0_{a}	77.40_{bc}	80.54_{bc}	56.80_{abc}	20.98_{ab}
T_4	7.33_{ab}	9.0_{a}	72.38_{b}	74.61_{b}	50.29_{ab}	20.78_{ab}
T_5	5.67_{b}	8.0_{a}	64.65_{a}	66.52_{a}	42.67_{a}	21.43_{ab}
T_6	12.33_{c}	14.0_{b}	75.00_{bc}	78.83_{bc}	48.07_{ab}	18.45_{a}
Statistical analysis						
P (0.05)	0.005	0.12	0.001	0.008	0.14	0.004
SE	0.56	0.58	1.69	1.83	1.64	0.67
F	0.67	4.30	9.72	11.66	4.67	6.5
Linear Regression analysis (y = concerned parameter, x = FA rate)						
y=	12.14-.82x	13.27-	80.12-.44x	84.18-.49x	57.31-.16x	20.70-.28x

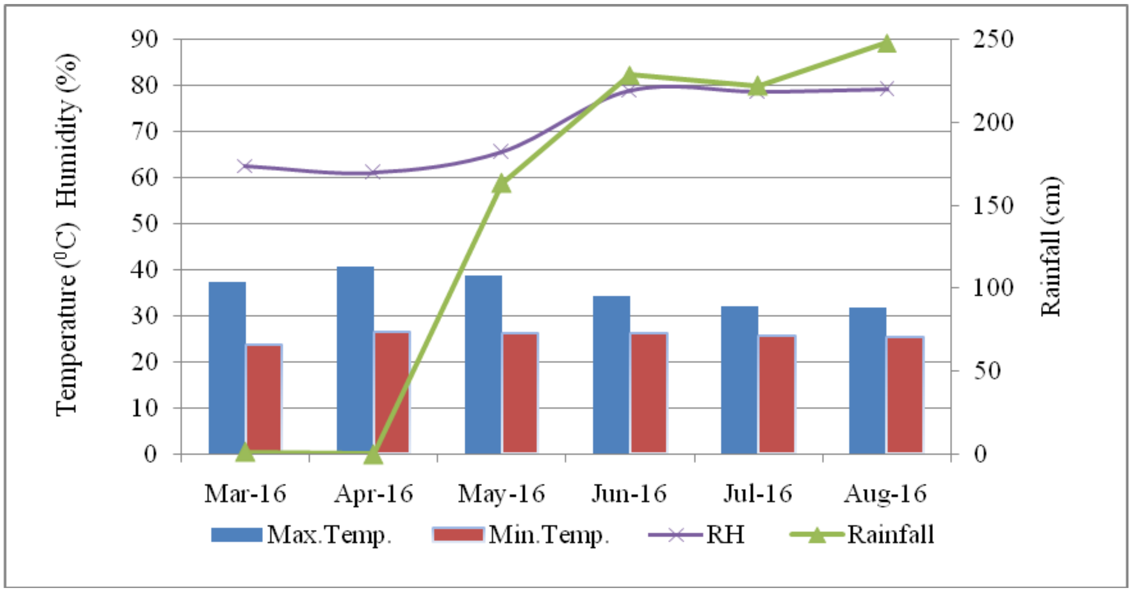
Treatments T_1=(20% FA+S), T_2=(40% FA+S), T_3=(60% FA+S), T_4=(80% FA+S), T_5=(100% FA), T_6=(Soil/Control), FA- fly ash, S- Forest Soil, DTFG- Days taken for first germination, GP- Germination Percentage, GC- Germination Capacity, GV- Germination Value, GI- Germination index. Mean values followed by same letter are statistically indifferent.

Table.3 Effects of substrate on growth and quality of *Acacia auriculiformis* seedlings at 90 DAT

Parameters→ Treatments↓	Survival (%)	Plant height (cm)	Collar diameter (cm)	Number of leaves	Mean root length (cm)	Nodules/ Plant (No.)	SQI
T_1	81.83_{b}	53.43_{b}	0.47_{a}	24.27_{b}	26.24_{b}	12.60_{a}	0.26_{ab}
T_2	83.24_{b}	68.87_{c}	0.83_{b}	42.51_{d}	39.00_{c}	24.16_{c}	0.88_{c}
T_3	79.80_{b}	63.70_{c}	0.78_{b}	35.32_{c}	21.77_{ab}	14.30_{b}	0.77_{c}
T_4	56.97_{a}	45.77_{b}	0.53_{a}	18.79_{ab}	17.87_{ab}	8.30_{a}	0.51_{b}
T_5	46.57_{a}	36.51_{a}	0.50_{a}	14.79_{a}	13.76_{a}	2.30_{a}	0.22_{a}
T_6	80.93_{b}	52.10_{b}	0.44_{a}	21.28_{ab}	26.00_{b}	10.30_{ab}	0.24_{ab}
Statistical analysis							
P (0.05)	0.002	0.004	0.006	0.01	0.006	0.001	0.007
SE	3.76	2.7	0.04	2.4	2.29	2.16	0.06
F	14.15	19.69	1.8	16.73	5.8	8.9	2.3
Linear Regression analysis (y = concerned parameter, x = FA rate)							
y =	89.40-.79x	60.43-.42x	0.56+.11x	29.56-023x	26.8-0.2x	10.3-.13x	0.45+.08x

Treatments T_1=(20% FA+S), T_2=(40% FA+S), T_3=(60% FA+S), T_4=(80% FA+S), T_5=(100% FA), T_6=(S/Control), FA- fly ash, S- Forest Soil, SQI-Seedling Quality Index, Mean values followed by same letter are statistically indifferent.

Fig.1 Climatic parameters of the experimental site



The maximum height (68.87 cm) was found in treatment having 40% FA (T_2) and it was statistically at par with Treatment T_3 (63.70 cm). The diameter growth was maximum in treatment T_2 (0.83 cm) and statistically at par with T_3 (0.78 cm). The number of leaves (42.51**),** mean root length (39.00 cm) and nodules per plant (24.16**)** was significantly higher in treatment T_2. The seedling quality index in treatment T_2 (0.88) and treatment T_3 (0.77) were statistically at par with each other. A similar trend in growth of seedlings with respect to FA concentration was reported by Gupta *et al.*, (2000) and Pandey *et al.*, (1996).

The vigour in seedling height, diameter and root growth of this species at 40% FA was due to optimum pH, improvement in availability of nutrients in ionic form at rhizosphere solum, improved nitrogen fixation rate (Table -2) and reduced or no attack of nursery insect and pest. Goyal *et al.*, (2002) observed 10% increase in the growth of *Eucalyptus tereticornis*, *Acacia auriculiformis* and *Casuarina equisetifolia* during early 6 months, grown in FA amended soils (ESP FA@18–24% (v/v)). Good root nodulation per plants (24.16) in substrates having 40 % FA could be attributed due to uptake of optimum amount of metals by the roots. However, the nodulation rate decreased after 40% FA linearly up to 100% FA which is due to the reduced ability of nitrogen fixing bacteria with increasing stress level (Faizan and Kaushar 2010). The depressive nodulation effect was substantiated by reduced plant height, collar diameter growth and SQI. Further the plants grown in 40% FA were observed to be very healthy. Better seedling quality index in T_2 (0.88) was obviously due to the improved availability of micronutrients that supported higher biomass production and shoot: root ratio (Gupta *et al.*, 2000). Ariful Islam *et al.*, (2019) observed seedling quality index in the range of 0.61-1.81 for *Acacia auriculiformis* after eight months grown on different substrates. The present SQI after three months of transplanting is within that range.

In conclusion, *A. auriculiformis* is a rhizobial fast growing multipurpose legume tree species. It is a much common species in social forestry, agroforestry, multipurpose wood lots and energy plantations of India. Robust quality seedlings not only ensure successful planting action but also reduce beating up cost. The results of present investigation recommends FA should be admixed at 20% (w/w) level in nursery beds for early sprouting and improving germination percentage. However it should be admixed at 40% in potting mixture for production of healthy and quality planting material. Further study is necessary to quantify the economic benefit or net profit gain from utilizing FA in forest nursery.

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