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Growth and Physiological Activities of Selected Leguminous Crops Grown in Carbonated Fly Ash Amended Soil

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ARTICLE INFO	ABSTRACT
Article No.: 021114104 DOI: 10.15580/GJAS.2014.3.021114104 Submitted: 11/02/2014 Accepted: 26/03/2014 Published: 03/04/2014 *Corresponding Author Muduli S. D. E-mail: surabhidipali@gmail.com	Present study was conducted to analyze the impact of varying levels of fly ash on the soil quality and plant growth. Pot experiments in random block design were conducted with different proportions of fly ash added to the soil to assess the effect of fly ash and carbonated fly ash on various growth parameters, biochemical content and antioxidant activities of <i>Vigna radiata</i> and <i>Vigna mungo</i>. Observations suggested normal seed germination in fly ash treated plants. No visible symptoms of either nutrient deficiency or phytotoxicity were observed in any case. Significant positive ($p \leq 0.01$) correlations between heavy metals concentration in the soil and physical growth as well as photosynthetic pigments indicates the stimulating effect of fly ash on various growth parameters like length, fresh and dry weight of roots and shoots, germination rate and chlorophyll content. The increase in the activity of antioxidative enzymes and antioxidants synthesis in plants with higher doses of carbonated fly ash is indicative of plant's ability to tolerate heavy metal toxicity. Thus, soil application of carbonated fly ash has the potential not only for improving their production but also for solving their disposal problem.
Keywords: Carbonated fly ash, antioxidants, germination, growth, heavy metals	

INTRODUCTION

Fly ash is a coal combustion residue generated from thermal power plant during burning of coal. Chemically, fly ash contains oxides, hydroxides, carbonates, silicates, and sulfates of calcium, iron, aluminum, and other metals in trace amount (Adriano et al., 1980). The mineralogical, physical and chemical properties of fly ash depend on the nature of the parent coal, conditions of combustion, type of emission control devices and storage and handling methods. In India, coal is the most extensively used and most important source of energy, and will probably continue to be so. Formation of fly ash depends on the ash content of coal and Indian coal used in power plants generally has very high ash content (35–45%) and is of lower quality (Mathur et al., 2003). As a consequence, a large amount of fly ash is generated in thermal power plants, and is disposed off in unmanaged landfills, lagoons and ponds. Disposal of fly ash in an unscientific way affects the local ecosystems due to the heavy metal pollution through erosion and leachate generation.

Phytomanagement employs flora and soil amendments to decrease the environmental risk posed by contaminated areas. Phytoremediation is a plant-based technology which aimed to use metal accumulating plants to remove, transfer or stabilize these contaminants from fly ash. Application of fly ash in agricultural sector helps in the saving of chemical fertilizer (Mittra et al., 2005). Crop plants of the families Brassicaceae, Chenopodiaceae, Fabiaceae, Leguminosae and Poaceae are most tolerant to fly ash (Cheung et al., 2000). In addition, legume plants and symbiotic nitrogen-fixing bacteria can improve the nitrogen content of infertile soils (Vajpayee et al., 2000). Leguminous plants could grow well on fly ash amended soils without manifestation of any injury symptoms. The micro- and macro- nutrients present in fly ash modify the physical properties of soils and works as a soil conditioner enhancing the yield of the crops. Fly ash is deficient in nitrogen and phosphorous. So we may overcome this deficiency by adding various organic substrates and by growing of leguminous crops. The focus of the present research work is that the by chemical activation through sulfatization reaction fly ash is being carbonated and can be a good source for sequestration of atmospheric CO₂ (Muduli et al. 2013). Here weathering of fly ash

has been done in atmospheric condition without applying any elevated temperature and pressure. After being carbonated, it is more suitable for use in agriculture. As the carbonation reaction happens in alkaline condition, it can be a good additive for neutralizing the acidic soil and will also help as a fertilizer. This study is a primary observation between the use of fly ash and carbonated fly ash in the growth and physiological behaviour of some selected plants. By which the fly ash can be used in management of degraded waste land like filling of mines and their reuse as agricultural land. The basic purpose of this study deals with the effect of fly ash in growth and physiological activities of certain leguminous crops like *Vigna radiata* (family Leguminosae) and *Vigna mungo* (family Fabaceae).

MATERIALS AND METHODS

The experiment was conducted on fly ash and carbonated fly ash amended soils, set up at medicinal plant garden of CSIR-Institute of Minerals and Materials Technology Bhubaneswar. During the experimental study fly ash was collected from one of the thermal power plant of Eastern India. The chemical composition of fly ash has been carried out by XRF study and given in Table-1. Different types of substrate were chosen for the experiment and conducted in pots with different weight proportion of fly ash, carbonated ash (Muduli et al., 2014) and soil. The carbonated ash used in the research work is conditioned fly ash. The fly ash has been undergone for sulfatization reaction in presence of some chemical activator, which makes the fly ash porous (Muduli et al 2013). The weight proportion and mix design of substrates has been given in Table -2. The seeds of selected leguminous crops *Vigna radiata* (Mung Bean) and *Vigna mungo* (Black Gram) were collected from Orissa University of Agriculture and Technology (OUAT) and soaked in water for 2 hour and then sown in substrate. Seeds were sown in all the pots at a depth of 1-2 cm for both the crops. Pots were placed in normal atmospheric condition and watered at regular intervals to keep the substrate saturated. After seven days from sowing the plants were uprooted to study the changes in various growth and physiological activities of the plant.

Table 1: Chemical analysis of fly ash

Constituent %	Na ₂ O	MgO	PO ₂ O ₅	SO ₃	CaO	Al ₂ O ₃	SiO ₂	TiO ₂	Cr ₂ O ₃	Fe ₂ O ₃
Fly ash	0.09	0.285	0.51	0.14	0.47	35.5	59.5	0.94	0.01	2.01

Table 2: Mix design of substrate

Sample ID	Soil (Wt %)	Fly ash (Wt %)	Carbonated ash (Wt %)
T0	100		
T1	90	10	
T2	80	20	
T3	70	30	
T4	60	40	
T5	50	50	
T6	90		10
T7	80		20
T8	70		30
T9	60		40
T10	50		50

Percentage of germination:

The number of emergent seedlings were counted at regular intervals of 24, 48 and 72 hours after sowing and germination was considered to be completed on any given pot once there was no further increase in the number of seedlings. For all species germination was completed by 12 DAS (Day After Sowing), when no new plants emerged.

Growth and biochemical parameters:

The growth parameters such as length, fresh and dry weight for roots and shoots were recorded after seven days of observation. The plants were uprooted and washed thoroughly with tap and double distilled water to remove the particles of Fly ash as well as soil from the plat surface. The plants were then divided into root and shoot followed by the measurement of various growth parameters. The chlorophyll (Chl-a, Chl-b and total Chl) and carotenoids were measured spectrophotometrically following the methods of Porra et al. (1989) and Lichtenthaler (1987) respectively. Catalase (CAT) was measured following the method of Chance and Mahly (1995). Total carotenoids were determined by using acetone and petroleum ether as extracting solvents and measuring the absorbance at 450 nm (Singh and Pandey, 2013). The quantitative estimation of ascorbate and glutathione was done

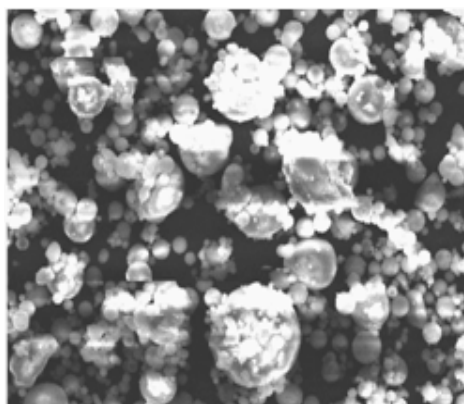
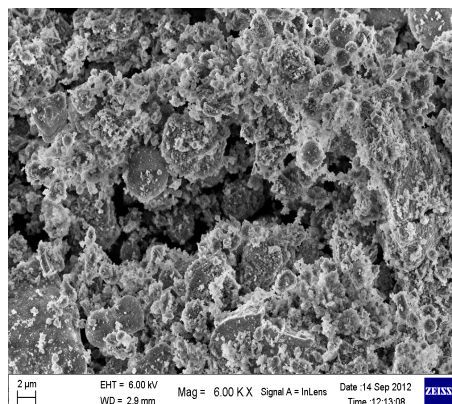
according to Law et.al. (1983) and De Vos, et al., (1992).

Statistical analysis

One-way ANOVA at 95 percent probability level was applied to examine the impact of carbonated fly ash on as heavy metal concentration of soil, growth, photosynthetic pigments and antioxidants activity of the selected leguminous crops. Pearson's correlation analysis was used to assess the significance of the interrelationships between the soil heavy metals and various growth as well as biochemical activities across different treatments. All statistical analyses were conducted using SPSS software (Version-13).

RESULTS AND DISCUSSIONS

The chemical analysis of the fly ash given in Table -1 indicates that it contains SiO_2 and Al_2O_3 , as the major constituent and oxides of Fe, Ti, Cr, Ca, Mg, P and S in trace amount. The fly ash used in this research is classified as class F fly ash according to the requirement of American Society for Testing and Materials (ASTM- C618-08, 2008). The scanning Electron Image of both fly ash and carbonated ash has been presented in Figure (1) to show the surface morphology of the sample.

**Fly ash****Carbonated ash****Figure 1: Scanning Electron Micrograph of fly ash and carbonated ash.**

Impact of carbonated fly ash on germination

The seed germination is an important event in plant growth and development. The comparison of seed germination rate of both the crops is depicted in Fig 2 and 3. From the results, it is evident that the germination of seedlings was enhanced by the application of carbonated fly ash. Significant positive ($p \leq 0.01$) correlations between germination percentage and concentration of various metals proves the positive effect of carbonated fly ash on seed germination. Application of carbonated fly ash in different proportions showed significant positive effect on germination percentage on both the plant species. Addition of carbonated fly ash at the rate of 50%

resulted in higher seed germination rate in *Lactuca sativa* (Kishor, 2010). Maximum germination was recorded in T₁₀ in comparison to T₀. So it is observed that the use of 50% carbonated ash with 50% soil shows better growth and germination capacity in comparison to the soil and the substrate mixed with 50% fly ash. Carbonated fly ash amendment enhances water-holding capacity and aeration, which results in an increase in germination percentage of seeds. Presence of macro- and micro- nutrients in the carbonated fly ash has high impact on plants, it not only enhances the seed germination but also stimulates the seedling growth at subsequent stage (Singh et al., 1997).

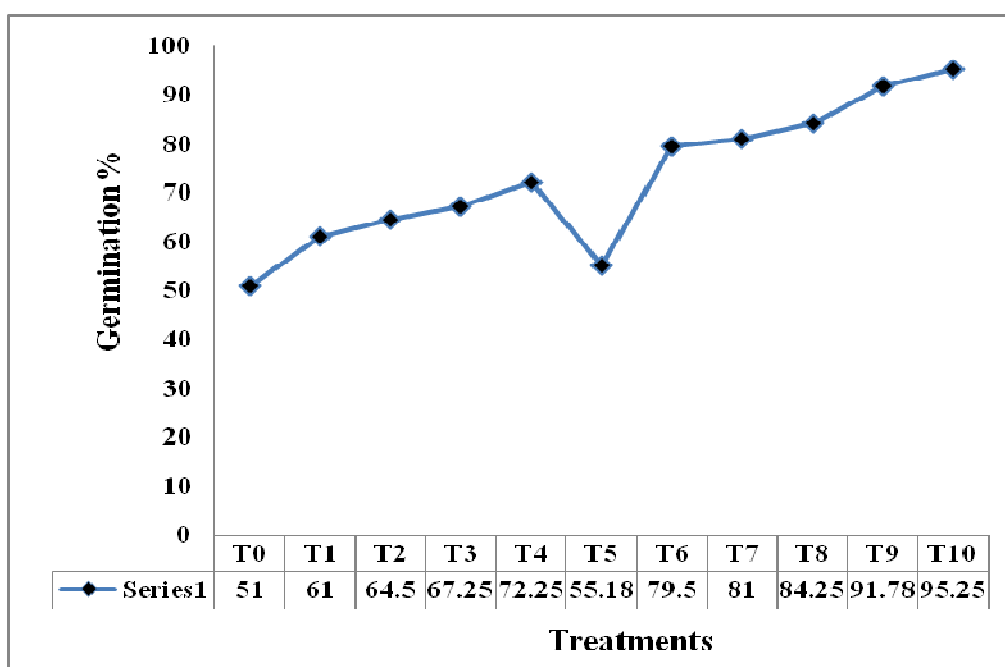


Figure 2: Effect of fly ash and carbonated ash in germination of *V. moongo*.

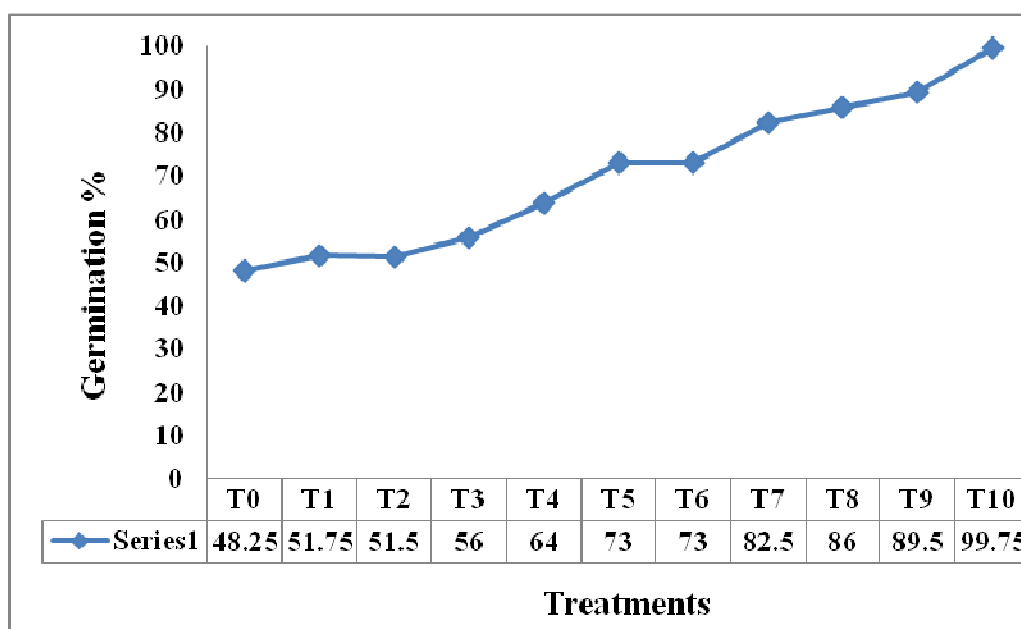


Figure 3: Effect of fly ash and carbonated ash in germination of *V. radiata*.

Impact of carbonated fly ash on growth

Carbonated fly ash was found to have stimulatory effect on the physiological parameters of both the crops depending on the increasing concentrations. A remarkable increase of length of roots and shoots of all the plants as per their proportions of carbonated fly ash application and fresh weight and dry weight of roots and shoots was observed in increasing order from T₀ to T₁₀ (Table.3). Pearson's correlation analysis indicates significant correlation with various growth parameters in both plants which further indicates the positive effect of carbonated fly ash on plant growth. Pandey et al. (2009) observed that addition of Fly ash shows positive results in most of the studied parameters of growth and yield. Similar increase in growth and yield of numerous crops and vegetables like *Medicago sativa*, *Hordeum Vulgare*, *Zea mays*, *Sorghum bicolor*, *Echinochola crusgalli*, *Daucus carota*, *Allium cepa*, *Phaseolus vulgaris*, *Brassica oleracea*, *Solanum tuberosum*, *Lycopersicon esculentum* and *Triticum aestivum* has been observed by various researchers also (Aggarwal et al., 2009; Siddiqui and Singh, 2005). The presence of essential plant nutrients such as K, Mg, S and micronutrients make fly ash a source of important plant nutrients which influences the plant growth. Fresh and dry weights of carbonated fly ash treated plants were significantly different from those of control. The increase in fresh and dry weight of roots and shoots, depending upon the increasing concentrations of carbonated fly ash might be due to the intake of essential nutrients from the fly ash to the crops. Yunusa et al. (2009) also reported significant increases in dry weight of Barley, canola, and radish at carbonated fly ash doses of 2.5 and 5.0 Mg ha⁻¹.

Photosynthetic pigments

The chlorophyll a, b and total contents in the leaves of both the leguminous crops plants were significantly higher at T₁₀ (50% soil + 50% carbonated ash). An increase in chlorophyll content of all the treated plants in both the species was observed as compared to control (Table 4). Like chlorophyll carotenoid production was also greater in treatments compared to control in both *V. mungo* and *V. radiata*. Increases in carotenoid levels are a common response to elemental stress because it is a precursor of abscisic acid (ABA) synthesis and also assists in quenching excess harmful excitation energy absorbed by the chlorophylls. Mishra et al. (2007) observed increases in the concentrations of chlorophyll b with ash addition but found no significant change in the concentrations of carotenoid and chlorophyll in the leaves of rice when supplied with up to 15 mg ha⁻¹ of a mildly alkaline fly ash. However, Yunusa et al. (2008) found significant reductions in relative chlorophyll concentrations in canola (*Brassica napus*) only when an alkaline fly ash was added to soil at rates exceeding 125 Mg/ ha.

Impact of fly ash antioxidants and antioxidative enzyme activity

Results indicated that the antioxidants levels (ascorbate, glutathione and carotenoids) and Catalase activity (Figure 4) was increased in leaves of the plants with increase in carbonated fly ash concentrations. A significant difference in the levels of antioxidants and Catalase activity in leaves was noted due to different carbonated fly ash amendments. Among the two plants concentration of various antioxidants in *V. radiata* was significantly higher than the *V. mungo* (Table 3). Pandey and Singh (2010) also suggested that antioxidants in plant samples of chickpea increase with increasing carbonated fly ash doses to combat stresses due to fly ash heavy metals. Increase in the activity of antioxidative enzymes in plants may result in

the antioxidants synthesis and ultimately confers the tolerance of plants against heavy metal toxicity. The higher oxidative enzymes activity under fly ash stress is possibly the result of gradual shift of reductive metabolism to oxidative metabolism. Significant

positive correlations (Table 4) between various antioxidants and metal concentration suggest induction of oxidative stress in the selected plants which in turn enhances the activity of antioxidative enzymes.

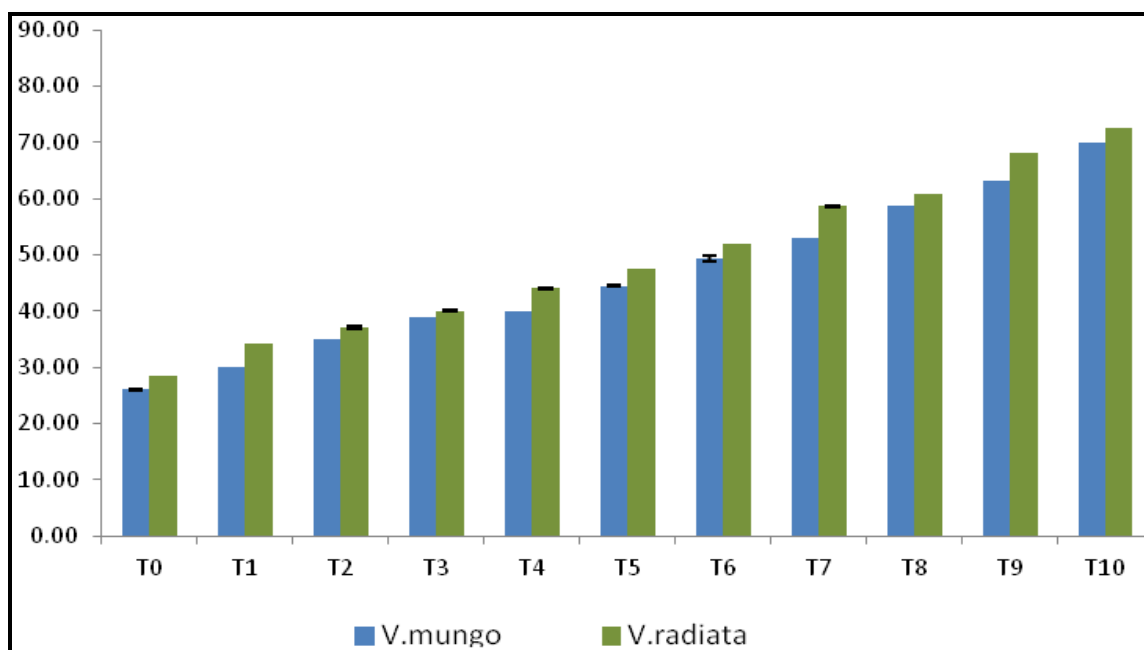


Figure 4: Effect of Fly ash and carbonated ash on germination of *V. mungo* and *V. Radiata*.

Table 3: Effect of Fly ash and carbonated ash on various growth parameters of *V. mungo* and *V. radiata*

Treatments	Root length		Root Fresh weight		Root Dry weight		Shoot length		Shoot Fresh Weight		Shoots Dry Weight	
	<i>V. mungo</i>	<i>V. radiata</i>	<i>V. mungo</i>	<i>V. radiata</i>	<i>V. mungo</i>	<i>V. radiata</i>	<i>V. mungo</i>	<i>V. radiata</i>	<i>V. mungo</i>	<i>V. radiata</i>	<i>V. mungo</i>	<i>V. radiata</i>
T0	2.72±0.15	3.22±0.095	0.097±0.134	0.022±0.001	0.029±0.001	0.017±0.001	12.7±0.081	13.15±0.129	4.315±0.012	3.02 ±0.012	2.81±0.012	1.25±0.008
T1	3.27±0.17*	3.55±0.129*	0.033±0.0001*	0.022±0.001*	0.032±0.002*	0.018±0.112*	13.15±0.129*	13.63±0.095*	5.828±0.009*	4.38±0.009*	3.47±0.017*	2.31±0.009*
T2	3.47±0.095*	4.12±0.125*	4.00 ±0.0001*	0.023±0.011*	0.033±0.001*	0.020±0.002*	13.525±0.095*	14.08±0.095*	6.23±0.008*	5.01±0.012*	4.56±0.009*	4.06±0.012*
T3	3.52±0.095*	4.72±0.0*	0.038±0.0001*	0.024±0.001*	0.034±0.003*	0.021±0.019*	14.00±0.081*	15.00±0.081*	6.285±0.012*	6.02±0.008*	4.77±0.009*	4.51±0.017*
T4	3.62±0.095*	5.07±0.09*5	0.039±0.0001*	0.034±0.013*	0.035±0.001*	0.024±0.001*	14.55±0.129*	15.25±0.129*	6.655±0.012*	6.36±0.012*	4.79±0.017*	4.72±0.017*
T5	4.25±0.129*	5.22±0.095*	0.041±0.0012*	0.036±0.001*	0.039±0.015*	0.025±0.011*	15.07±0.095*	16.15±0.129*	6.8275±0.009*	6.46±0.009*	4.90±0.012*	4.82±0.008*
T6	4.57±0.095*	5.65±0.057*	0.044±0.0009*	0.038±0.015*	0.043±0.021*	0.030±0.019*	15.40±0.081*	16.6±0.081*	7.02±0.018*	6.56±0.012*	5.07±0.009*	4.86±0.008*
T7	5.1±0.173*	6.22±0.17*	0.046±0.000*	0.040±0.001*	0.046±0.011*	0.036±0.001*	16.07±0.095*	16.98±0.095*	7.46±0.008*	7.17±0.008*	5.41±0.015*	5.43±0.008*
T8	6.67±0.15*	7.07±0.095*	0.048±0.0001*	0.045±0.081*	0.047±0.019*	0.038±0.051*	16.35±0.095*	17.25±0.129*	7.538±0.009*	7.35±0.012*	5.57±0.009*	5.59±0.012*
T9	7.15±0.129*	7.37±0.095*	0.049±0.0001*	0.048±0.010*	0.048±0.001*	0.040±0.013*	16.85±0.129*	17.9±0.081*	7.595±0.012*	7.46±0.009*	5.75±0.009*	5.76±0.009*
T10	7.47±0.095*	8.07±0.15*	0.050±0.0001*	0.050±0.013*	0.049±0.017*	0.049±0.001*	17.20±0.141*	18.25±0.129*	7.705±0.012*	7.97±0.008*	5.90±0.012*	5.91±0.012*

*** The given values are means of three replicates± SE.

Table 4: Effect of fly ash and carbonated ash on Chlorophyll & antioxidant activities of *V. mungo* and *V. radiata*

	<i>V. mungo</i>			<i>V. radiata</i>			<i>V. mungo</i>			<i>V. radiata</i>		
	Chla	Chlb	Chlt	Chla	Chlb	Chlt	Ascorbate	Glutathione	Carotenoids	Ascorbate	Glutathione	Carotenoids
T0	20.05 ±0.058	30.06 ± 0.07	49.12 ± 0.539	25 ±0.082	34.46 ± 0.03	54.12 ± 0.009	3.19±0.010	21.92±0.500	0.458±0.01	4.23±0.015	39.03±0.055	0.94±0.010
T1	23.33 ±0.082*	35.06 ± 0.001*	55.73 ±0.096*	33.11 ±0.022*	43 ± 0.005*	61.34 ±0.096*	5.27±0.032*	29.05±0.061*	0.567±0.011*	5.08±0.020*	44.78±0.005*	0.94±0.012*
T2	29.3 ±0.163*	39.73±0.096*	74.07 ± 0.024	38.99 ±0.131*	50.73±0.096*	77 ± 0.024*	7.79±0.017*	36.22±0.010*	0.666±0.009*	6.95±0.014*	51.85±0.173*	0.94±0.013*
T3	33.98 ±0.001*	46.38 ± 0.096*	80.08 ± 0.096*	45.31 ±0.001*	59.98 ± 0.016*	83.03 ± 0.009*	9.91±0.014*	41.23±0.015*	0.793±0.005*	7.72±0.025*	59.98±0.013*	0.94±0.005*
T4	42.09 ±0.005*	54.43 ± 0.001*	99.97 ± 0.010*	52.17 ±0.003*	63 ± 0.015*	92 ± 0.011*	10.78±0.000*	48.18±0.010*	0.880±0.014*	8.08±0.014*	65.08±0.015*	0.94±0.024*
T5	44.33 ± 0.05*	61.23 ± 0.001*	103.08 ± 0.096*	60.33 ± 0.002	69 ± 0.001*	103.03 ± 0.096*	12.68±0.010*	54.26±0.431*	0.975±0.010*	9.46±0.010*	70.24±0.025*	0.94±0.141*
T6	47.24 ± 0.001*	65.55 ± 0.129	112.98 ± 0.112*	69 ± 0.012*	71.55 ± 0.100	111.99 ± 0.002*	14.04±0.075*	60.14±0.021*	1.18±0.096*	11.06±0.013*	76.02±0.045*	0.94±0.010*
T7	53.78 ± 0.006*	71.08 ± 0.008*	119.39 ± 0.009*	74.00 ± 0.001*	78.12 ± 0.005*	119 ± 0.015*	17.54±0.013*	65.56±0.005*	1.98±0.100*	12.03±0.060*	81.23±0.014*	0.94±0.126*
T8	59.98 ± 0.008*	77.03 ±0.053*	129.68 ± 0.001*	85 ± 0.014*	90 ±0.053*	124.68 ± 0.001*	19.26±0.021*	69.99±0.013*	2.12±0.000*	13.88±0.010*	89.96±0.005*	0.94±0.010*
T9	60.34 ± 0.013*	83.16 ±0.105*	138 ± 0.001*	90.14 ± 0.013*	99.56 ±0.105*	138 ± 0.001*	22.45±0.013*	73.23±0.015*	2.67±0.013*	14.34±0.012*	94.07±0.010*	0.94±0.013*
T10	63.45 ± 0.163*	88.13 ±0.096*	147.68 ± 0.001*	99.75 ± 0.116*	113 ±0.006*	145.10 ± 0.015*	25.67±0.013*	81.23±0.019*	3.02±0.015*	15.98±0.015*	100.02±0.045*	0.94±0.019*

*** Given values are means of three replicates± SE.

CONCLUSIONS

The present study indicated stimulating effects of carbonated fly ash on the selected plants. An enhanced rate of germination, growth and chlorophyll production clearly indicates the positive impact of carbonated on *V. mungo* and *V. radiata*. However, an increase in antioxidants and related enzymes is suggestive of the fact that the higher doses of carbonated may cause oxidative stress in the selected plants. Thus, the overall study indicates an ample scope for utilization of carbonated fly ash as an input material for agriculture applications, and also, the use of carbonated fly ash in paddy soil in agriculture practices needs further investigations in field conditions.

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