RESEARCH ARTICLE

Physiological Response of Black Gram [*Vigna mungo* (L.) Hepper] Grown on Fly Ash-Amended Soil: Growth, Photosynthesis, and Antioxidant Defense

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Abstract

The present study deals with the growth, photosynthesis, antioxidant defense along with the metal accumulation in black gram [*Vigna mungo* (L.) Hepper] grown on various combination of fly ash amended garden soil. Significant improvement of soil quality such as pH, nitrogen and organic carbon was observed in 25% of fly ash amendments. Besides, the bioaccumulation of Fe, Cu, Mn, and Zn was remarkably increased with the increase of fly ash in amended soil. Fly ash (25%) amended with garden soil led to a 62% increase in plant biomass. Photosynthetic rate, stomatal conductance, and chlorophyll content significantly increased under 25% fly ash amendments soil, though photosystem (PS) II activity remained unchanged compared to the plants grown in garden soil. Furthermore, the activity of some antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase was increased over control under different fly ash amendments. Taken together, garden soil amended with 25% of fly ash not only improved the physicochemical properties of the soil but also contributed to better growth and photosynthesis in black gram.

Keywords: Antioxidants, Black gram, Chlorophyll fluorescence, Fly ash, Photosynthesis

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Introduction

Fly ash is a waste product of thermal power plants and regarded as a problematic solid waste all over the world (Pandey and Singh, 2010). Globally, fly ash production was about 600 million tonnes per year, which may cover up to 3235 km^2 of land area by 2015 (Ram and Masto, 2014; Verma *et al.,* 2014). The management of the enormous quantity of fly ash generated every year has become a major problem ~~and~~ because of the high cost of disposal and environmental management. So the utilization of fly ash in the agricultural sector could be a viable option (Pandey and Singh, 2010). Fly ash consists of macro-nutrients such as Na, K and P and micronutrients, Fe, Co, B, Zn, Cu, and Mn. The elements, Pb, Ni, Cr, Cd and a few more, also occur abundantly and have the potential to cause toxicity (Mishra *et al.,* 2007; Varma *et al.,* 2014). Fly ash has potential benefits for use in agriculture due to its physical properties and the presence of macro and micronutrients, which are conducive for plant growth (Pandey and Singh, 2010; Verma *et al.,* 2014). Fly ash incorporation in soil modifies the physicochemical, biological and nutritional quality of the soil (Pandey and Singh, 2010). The commercialization of fly ash as a fertilizer in the agricultural sector for crop production is uncommon because of poor content of nitrogen and phosphorus (Gupta *et al.,* 2007). However, the augmented levels of non-essential and potentially toxic metals may pose a problem for plant growth (Prajapati, 2012). Therefore, fly ash with soil amendments may offer correction of these deficiencies and suitable combination to support plant growth with reduced risk of metal toxicity.

Several studies have been carried out using fly ash in combination with other organic amendments like press-mud and farmyard manure, which promotes the plant growth and the yield (Pandey and Singh, 2010; Verma *et al.,* 2014). Soil amendment with low amount of fly ash enhances both growth and yield, while adverse effects at higher levels were observed in several crops such as pigeon pea (*Cajanus cajan*), wheat (*Tritiucm aestivum*), alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*) and beans (*Phaseolus vulgaris*) (Mishra *et al.,* 2007; Verma *et al.,* 2014). In addition, several studies demonstrated that many plants belonging to the Leguminosae family have been possess high tolerance and survival in arid, infertile and metal-contaminated areas (Gupta *et al.,* 2007) and are most likely to improve the low N content of soil amended with fly ash (Cheung *et al.,* 2000; Rai *et al.,* 2004). *Vigna mungo* is an annual herbaceous and leguminous plant attaining a height of maximum 50 cm within a life span of 4-5 months and was selected for the present study.

Numerous reports suggest that the exposure of plants to toxic levels of fly ash triggers a wide range of physiological and metabolic alterations along with the effect on leaf gas exchange (Dubey, 2011; Raja *et al.,* 2014). The symptoms of fly ash toxicity are a reduction in plant growth, inhibition of antioxidative defense system with progressing senescence and plant death (Sharma and Dubey, 2007; Sethy and Ghose, 2013; Gill, 2014). The higher concentration of fly ash can inhibit photosynthesis at several levels: chlorophyll, gas exchange, structure and function of chloroplasts (Reid *et al.,* 2004; Guidi *et al.,* 2011; Gajić *et al.,* 2013). However, there is a dearth of studies regarding photosynthetic response and PS II activity of black gram plant to different levels of fly ash in soil amendments. Unlike other heavy metal stress, relatively few information is published on

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fly ash induced oxidative stress in higher plants and less information is available in pulses, such as black gram (Nadgórska-Socha *et al.,* 2013).

Keeping in view of the above, the present study aims to evaluate the effect of fly ash-amended soil on growth, photosynthesis and PSII activity as well as antioxidant response in black gram and to find out the suitable concentration of fly ash could be recommended for sustainable agriculture.

Materials and Methods

Collection and characterization of fly ash and garden soil

The fly ash used in the study were collected from the fly ash dumping site of National Aluminium Corporation Limited (NALCO), Koraput, India (18˚ 46′ 22″ N to 82˚ 53′ 23″ E) and the garden soil was collected from the campus of Central University of Orissa, Koraput (82˚ 44′54″ E to 18˚ 46′47″ N). Five different amendments of fly ash and garden soil were prepared by mixing these two indifferent ratios in dry weight, and were placed in five-Kg plastic pots (50 cm in diameter and 30 cm in height) i.e. garden soil : fly ash (100 :100), fly ash : garden soil (25: 50), fly ash : garden soil (50: 50), fly ash : garden soil (75: 25). Pots were left at the experimental site for 14 days before cultivation for physicochemical stabilization and proper conditioning of treated soil.

Analyses for different physicochemical parameters of fly ash and soil amendments were carried out in the laboratory before and after cultivation of black gram. The pH and electrical conductivity (EC) was measured using a pH meter and a conductivity meter, respectively, by diluting samples with double distilled water in a 1:2 ratio (Digimon model D1-909). Total organic carbon (TOC) was determined using a dichromate oxidation method (Walkley and Black, 1934). Available phosphorus in samples was determined by Olsen method employing sodium bicarbonate as an extracting agent (Singh *et al.,* 2007). The available nitrogen was determined using KEL PLUS nitrogen estimation system (Classic DX, Pelican Equipment).

Analysis of metal in a substrate and plant parts

For metal analysis, the fly ash, garden soil and plant samples both root and shoot tissues (1 g each) were oven-dried (80°C) and digested with a mixture of nitric, sulphuric and perchloric acid (6:1:2) at 100°C. Digested material was diluted with double distilled water and Fe, Cu, Mn and Zn contents were analyzed using an Atomic Absorption Spectrophotometer (Parkin Elmer Atomic Absorption Spectrophotometer, Analyst AA-200) (APHA, 1989) at Soil Testing Laboratory, Koraput. All the sample analyses were determined in triplicate. Blanks and internal standards were taken for quality assurance during a metal analysis of the samples.

Plant material and growth condition

The study was conducted by taking black gram [(*Vigna mungo* (L.) Hepper)] seeds collected from Regional Research Station (OUAT), Koraput, India. Five healthy seeds were selected and directly sown in the plastic pots and the experiment was carried out in three replications in a randomized complete block design. Plants were grown in the campus of Central University of Orissa, Koraput, India (82˚ 44′ 54″ E to 18˚ 46′47″ N, 880 m above the mean sea level), regularly irrigated with tap water and subjected to natural solar radiation, with daily maximum photosynthetic photon flux density (PPFD), air temperature and relative humidity being about 1320±25 µ mol m^{-2} s^{-1}, 31.6 ± 2^0C and 70-75%, respectively. All the measurements were performed three times after 90 days of sowing (at the flowering stage).

Plant growth and metal tolerance index : Plant growth was measured in terms of fresh and dry weight of 90 days-old plants of two different plants in each replication. Total plant biomass was obtained after drying at 80°C until a constant weight was recorded. Metal tolerance ability of the plant was determined through metal tolerance index (MTI) using the following formula.

$$\text{Fly ash tolerance index (FTI)} = \frac{\text{Total plant biomass under treatment}}{\text{Total plant biomass under control}} \times 100$$

Measurement of leaf gas exchange and chlorophyll content

The leaf gas exchange parameters such as photosynthetic rate (P_N) and stomatal conductance (g_s) of black gram seedlings were measured between 10-12 h on fully matured leaves of each plant using an open system photosynthetic gas analyzer (CI-304, CID, USA) under normal ambient environmental condition. The fully matured leaf from the top was selected and kept inside the chamber under natural irradiance until stable reading was recorded. The measurements were carried out at 31±2°C, 70% relative humidity, 1014±30 µmol $m^{-2}s^{-1}$ photosynthetic active radiation, 370 µmol CO_2 $m^{-2}s^{-1}$ and 21% O_2.

The leaf chlorophyll (Chl) content was measured spectrophotometrically by taking absorbance at 663.6 nm, and 646.6 nm as recently described in Bisoi *et al.* (2017). The total Chl contents were calculated using the equations of Porra (2002).

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured on the same leaves used for gas exchange measurements under different treatment using a portable Chl fluorometer (JUNIOR-PAM, WALZ, Germany). Different parameters like minimal fluorescence (Fo), maximal fluorescence (Fm), variable fluorescence (Fv = Fm - Fo) and Maximum photochemical efficiency of PS II (Fv/Fm) was measured in 20 min dark-adapted leaves (Maxwell and Johnson, 2000). In light-adapted leaves at a PPFD of 400 µmol m^{-2} s^{-1} (for 15 min) steady-state fluorescence yield (Fs), maximal fluorescence (Fm′) and minimal fluorescence (Fo′) were measured when actinic light was turned off. Further, a yield of PSII photochemistry (Y II), Quenching value due to non-photochemical dissipation of absorbed light energy (NPQ) and the coefficient for photochemical quenching (qP) was also calculated (Maxwell and Johnson, 2000).

Measurement of antioxidant enzyme activity and protein content

After measurement of photosynthesis and Chl fluorescence same leaf tissue of black gram (0.5 g) was homogenized in 10 ml of 50 mM potassium phosphate buffer (pH 7.8) as recently described by Bisoi *et al.* (2017) for measurement of antioxidative enzyme activity. An aliquot of the extract was used to determine protein content following Lowry *et al.* (1951). In brief superoxide dismutase (SOD) activity was measured by the photochemical method according to Giannopolitis and Ries (1977) with modification suggested by Choudhury and Choudhury (1985). Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm (E=2.8 mmol cm^{-1}). The activity of guaiacol peroxidase (GPX) was assayed

according to Rao *et al.* (1995) and Catalase (CAT) activity was measured according to Cakmak and Marschner (1992).

Statistical analysis

Differences between various parameters were compared by analysis of variance (ANOVA) using CROPSTAT (International Rice Research Institute, Philippines). Mean values were compared by the least significant difference (LSD, $P < 0.05$), wherever the *F*-test was significant. Correlation analysis and Duncan's multiple range tests were done by the CROPSTAT software.

Results and Discussion

Physico-chemical parameters

Fly ash is a mixture of various elements, including many that are required for the growth and development of plants. But the utilization of fly ash for vegetation is restricted because of the presence of non-essential and potentially toxic metals, which may pose a problem for plant growth. Therefore, fly ash with soil amendment may offer a suitable combination to support plant growth with reduced risk of metal toxicity (Pandey and Singh, 2010). In the present study, the nature of fly ash was alkaline pH of 8.2 with low in nitrogen (N), organic carbon (OC) and potassium (K) in comparison to garden soil (Table 1). The electrical conductivity (EC) and the available phosphorus (P) content were also higher in the fly ash compared to garden soil. Besides, fly ash contains a larger amount of Fe, Mn, and Cu in comparison to garden soil, except of Zn (Table 1). Results indicate that in garden soil the concentration of Zn and Cu were within the range of average value for soil whereas the concentrations of Mn was deficient (Kabata Pendias, 2011). However, in the fly ash, Fe and Cu were toxic and the concentration of Mn was deficient (Kabata Pendias, 2011). However, after cultivation of black gram (for 90-days) under low level of fly ash (25%) in soil amendments, significantly increases the pH, EC, N, OC compared to other treatments ($P<0.05$). The resulting high pH of amended soil may be due to the higher pH of fly ash and presence of CaO and MgO but at low level of fly ash amendments no visible adverse effect was observed as have been reported earlier (Su and Wong, 2003). Increase of EC due to fly ash application, it facilitates leaching of soluble salts from fly ash causing availability or entry of metal nutrients to growing plants, as reported earlier (Su and Wong, 2003). The increase of available P of the soil was observed with an increased application rate of fly ash (Sarangi et al., 2001). Fly ash application is therefore potentially valuable particularly in conditions where trace metals are deficient (Gupta *et al.,* 2006).

Metal accumulation in plant parts under fly ash amended soil

The metal accumulation of different plant parts (root and shoot) of black gram seedlings was presented in Table 2. The accumulation of different metal such as Fe, Cu, Mn, and Zn was remarkably increased with the increase of fly ash concentrations in amended soil compared to garden soil. The level of different metals such as Fe, Cu, Mn, and Zn, were higher in roots compared to shoot in different treatments. The metal accumulation capacity in plants species depends on pH of the substrate, type of metal in a substrate, type of plant species (Maiti and Jaiswal, 2008; Pandey, 2012). The differences in metal accumulation of the different plant tissue suggest a different cellular mechanism of bioaccumulation of metals that may regulate their translocation and partitioning in the plant systems (Sinha *et al.,* 2007). The result is consistent with the study by Gupta *et al.* (2007) who found a greater accumulation of metal in root tissue of *Cicer arietinum* under different fly ash amendments.

Plant growth and fly ash tolerance Index

The growth of black gram seedlings was remarkably inhibited under bare flay ash compared to the garden soil (Fig. 1). Application of fly ash (25%) in garden soil significantly increased the plant growth compared to other treatments (Fig. 1). The plant biomass of black gram was increased by 62% under low level of fly ash (25%) amendments compared to the control. The study suggests that fly ash in low concentration (25%) has the promoting effect on the growth of black gram seedlings but high concentrations of metal in fly ash that can inhibit plant growth as has been reported for other crops (Parveen *et al.,* 2006; Bisoi *et al.,* 2017). Fly ash has some physical and chemical properties that might be useful in a low level of soil amendment (Verma *et al.,* 2014; Ram and Masto, 2014). However, improvement of N, K and OC has taken together, the essential plant nutrients found in 25% fly ash amendments can increase the growth of black gram seedlings. But in the other hand, fly ash carries considerable amounts of metals (Table 1), many of which are toxic in elevated concentrations and this could be a contributing factor to low growth rates on a higher concentration of fly ash.

To assess the metal tolerant capacity of black gram, we used fly ash tolerance index (FTI) (Köhl and Lösch, 1999). Based on total

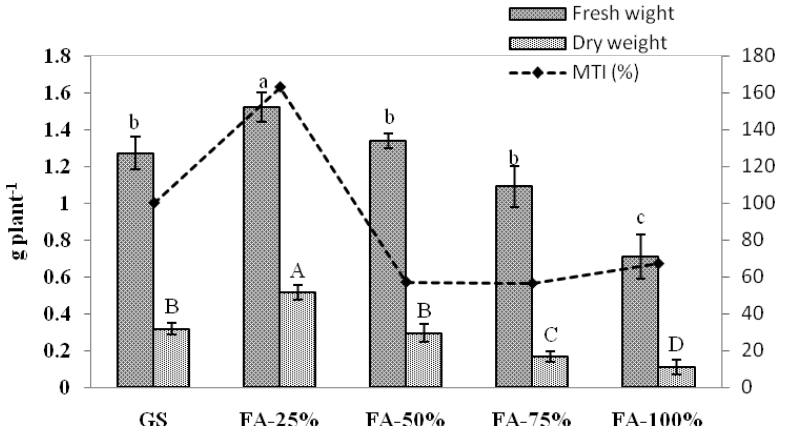

Fig. 1: Plant biomass and metal tolerance Index (MTI) of 90-days-old *Vigna mungo* L. seedlings under different levels of fly ash-amended soil. Data are the mean of three replications with vertical bar represents standard deviation. Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. GS: garden soil, FA: fly ash.

Table 1: Physicochemical parameters of garden soil and different percentage of fly ash amended soil used for the experiment. Data are the mean of three replications with standard deviation.

	Garden soil		*FA 25%*		*FA 50%*		*FA75%*		*FA 100%*		*LSD*
Parameters	*BC*	*AC*	*BC*	*AC*	*BC*	*AC*	*BC*	*AC*	*BC*	*AC*	*(P<0.05)*
pH	6.64± 0.11	6.66±0.04	6.66±0.10	6.82±0.06	7.20±0.04	7.25±0.06	7.5±0.02	7.61±0.03	8.20±0.10	8.23±0.07	0.14
EC (µMho cm^{-1})	68.5± 11.0	67.4±5.0	98.0±8.5	95.23±3.5	102.5±3.5	105.3±2.4	110.1±11.0	119.2±5.6	158±13.0	151.2±4.7	12.2
Nitrogen (µg g^{-1} dwt)	0.18± 0.02	0.22±0.04	0.16±0.03	0.27±0.02	0.14±0.04	0.15±0.02	0.12±0.02	0.13±0.03	0.08±0.02	0.08±0.01	0.04
Phosphorus (µg g^{-1} dwt)	0.02± 0.00	0.03±0.01	0.03±0.01	0.08±0.02	0.10±0.02	0.09±0.03	0.16±0.05	0.14±0.03	0.25 ±0.06	0.21±0.07	0.07
Potassium (µg g^{-1} dwt)	0.84± 0.05	0.86±0.02	0.76±0.03	0.88±0.02	0.59±0.07	0.62±0.06	0.39±0.04	0.42±0.02	0.18±0.03	0.20±0.01	0.06
Total Organic Carbon (%)	0.53± 0.01	0.56±0.03	0.43±0.02	0.51±0.04	0.36±0.06	0.32±0.01	0.25±0.03	0.27±0.04	0.26±0.02	0.28±0.04	0.06
Fe (µg g^{-1} dwt)	128±10	124.4±12	158 ± 25	150±18	1045 ± 32	985±34	1717 ± 46	1650±65	2810 ± 95	2760±105	90
Mn (µg g^{-1} dwt)	63.4± 6.6	58.2±5.4	81.3 ± 4.4	73.4±2.3	92.2 ± 5.3	82.5±6.7	116.5 ± 9.9	112.5±8.8	123.6 ± 11.2	116.5±9.5	8.5
Zn (µg g^{-1} dwt)	94.5± 3.4	90.5±4.2	83.4 ± 2.8	75.5±5.6	68.3 ± 3.3	64.3±6.5	61.3 ± 5.5	59.2±5.4	52.3 ± 9.3	48.5±4.2	8.3
Cu (µg g^{-1} dwt)	16.3 ± 2.2	13.4±2.3	28.5 ± 3.3	25.3±4.5	36.3 ± 5.2	35.6±5.2	55.2 ± 3.5	50.8±3.4	66.3 ± 4.5	64.5±4.4	7.3

BC: before cultivation; AC: after cultivation; FA: fly ash

Table 2: Bioaccumulation of different metals in *Vigna mungo* L. at garden soil (GS) and fly ash (FA) 25, 50, 75and 100%. Data are the mean of three replications ± standard deviation. Means followed by a common letter in the same column are not significantly different at the 5% level by *Fisher's* least significance difference (LSD) test.

	Fe (µg g^{-1} dwt)		*Mn (µg g^{-1} dwt)*		*Cu (µg g^{-1} dwt)*		*Zn (µg g^{-1} dwt)*	
Treatment	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*
GS	114.5±12.4^{a}	118.0±14.3^{a}	53.3±5.2^{a}	49.6±3.4^{a}	11.9±1.6^{a}	12.5±1.1^{a}	50.5±3.5^{a}	32.4±2.3^{a}
FA-25%	120.0±10.5^{a}	160.5±11.4^{a}	64.4±4.5^{a}	55.5±4.5^{ab}	16.7±2.1^{b}	13.5±2.1^{a}	57.6±4.3^{a}	39.0±1.8^{b}
FA-50%	440.5±28.4^{b}	445.0±31.2^{b}	81.8±5.1^{b}	63.5±5.2^{bc}	25.3±1.4^{c}	16.1±2.4^{ab}	63.6±2.5^{b}	44.3±2.9^{b}
FA-75%	887.0±30.3^{c}	747.5±34.5^{c}	83.7±3.8^{b}	70.8±3.8^{c}	24.3±2.1^{c}	18.4±2.8^{bc}	63.2±2.1^{b}	42.8±2.6^{b}
FA-100%	1390.0±80.5^{d}	1072.0±35.6^{d}	85.0±4.7^{b}	73.7±7.9^{c}	27.3±1.2^{c}	24.3±2.1^{c}	60.4±4.6^{b}	47.9±3.2^{b}
LSD*P<0.05	76.6	80.3	10.2	12.1	3.5	4.8	8.2	6.4
CV (%)	10.5	7.4	8.6	9.7	4.6	5.7	4.6	6.2

plant biomass of black gram under different fly ash amendments, the FTI was significantly increased in 25% of fly ash-amended soil and further decline with an increase of fly ash concentration (Fig. 1). This showed the tolerance level of black gram in low level of fly ash amended soil and capable to grow in metal-polluted soil as has been reported for other crops such as *Ciser arietinum* and *Cymbopogon citratus* (Gupta *et al.,* 2007; Gautam *et al.,* 2017).

Leaf photosynthetic parameters

The leaf photosynthetic parameters of black gram under different levels of fly ash amendments were presented in Fig. 2. The CO_2 photosynthetic rate (P_N) of black gram seedlings increased under 25% of fly ash-amended soil as compared to garden soil along with an increase of stomatal conductance (g_s) and chlorophyll (Chl) content (Fig. 2). But these parameters significantly decreased under the higher concentration of fly ash (75, 100%). The increase of leaf P_N, g_s and Chl was 18%, 9% and 10% respectively, under 25% fly ash in compared to control plants. Low level of fly ash in soil improves the leaf photosynthesis in black gram that might be due to the improvement of soil physical and chemical parameters (Pandey and Singh, 2010). The remarkable inhibition of P_N under high concentration of fly ash (75, 100%) was probably due to the structural damage suffered by the photosynthetic apparatus as evident from the fall in the values of g_s, Chl content and functional alteration of stomata as has been reported for other crops (Raja *et al.,* 2014). Overall, lower photosynthetic efficiency occurred under elevated levels of fly ash in soil system due to the synergistic activities of different factors such as degradation of Chl, alteration of PS II activity and by free radicals generated by metals present in fly ash (Mathur *et al.,* 2016; Bisoi *et al.,* 2017).

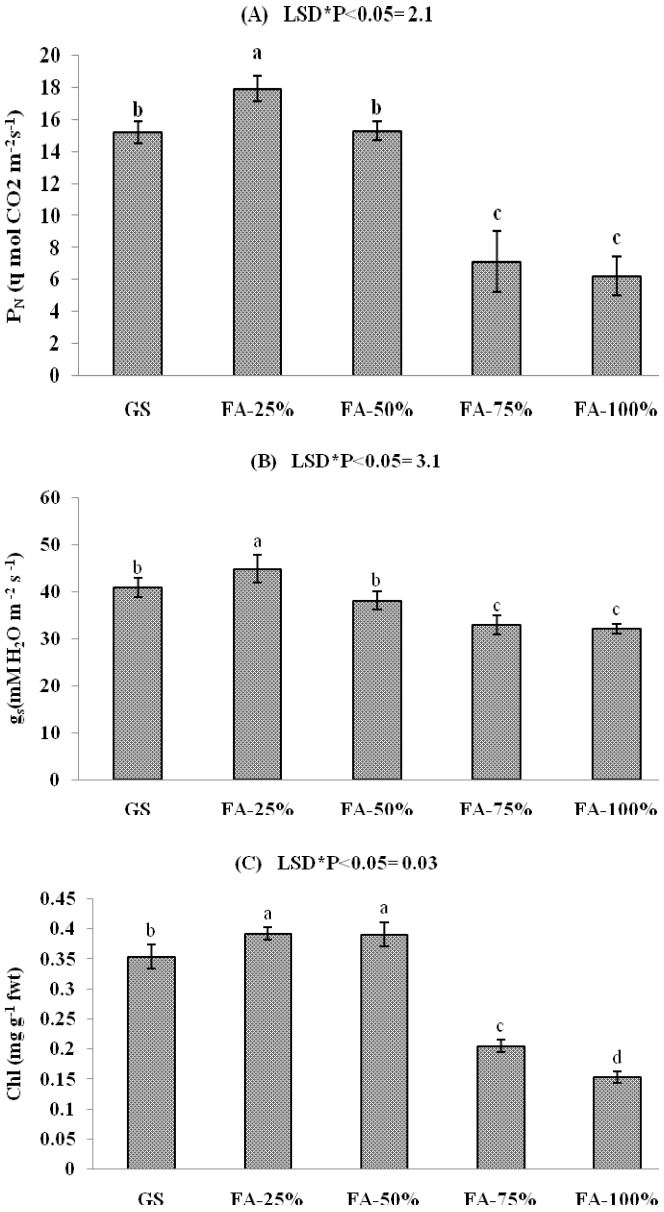

Fig. 2: Changes of CO_2 photosynthetic rate (P_N), stomatal conductance (gs) and chlorophyll (Chl) content in *Vigna mungo* L. leaves grown under different concentration of fly ash. Data are the mean of three replications with vertical bar represents standard deviation. Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test. GS: garden soil; FA: fly ash; LSD: a least significant difference.

Leaf chlorophyll fluorescence parameters

The PSII activity in black gram under different levels of fly ash-amended soil was studied by the measurement of different Chl fluorescence parameters such as Fo, Fm, Fv/Fm, Y (II), qP and NPQ (Murchie and Lawson, 2013). In the present study, these parameters were not changed in a low level of fly ash (25%) in soil amendment compared to the control seedlings (Fig. 3). But the higher concentration of fly ash (50, 75 and 100%) in amended soil inhibited the PS II activity, as reflected in the significant decline in

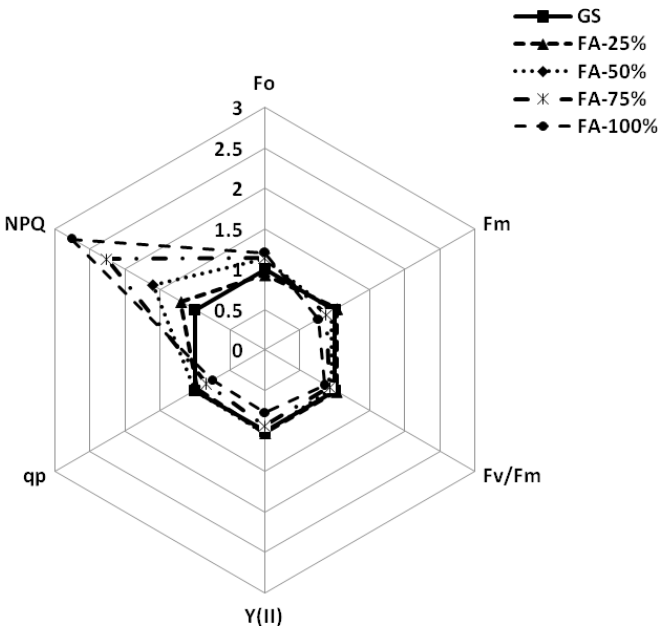

Fig. 3: Spider type visual plot showing the quantitative extent of changes in various chlorophyll fluorescence parameter in *Vigna mungo* L. seedlings grown on different concentration of fly ash. The black circle with radius 1 represents garden soil (GS) and FA-25, FA-50, FA-75, and FA-100 are a different percentage of fly ash-amendments. Data are the mean of three replications. Fo: minimum fluorescence yield obtained with dark-adapted leaf; Fm: maximum Chl fluorescence yield obtained with dark-adapted leaf; Fv/Fm: maximal photochemical efficiency of PS II; NPQ, non-photochemical quenching; qP: photochemical quenching; Y(II): yield of PSII photochemistry.

the values of Fm, Fv/Fm, Y (II) and qP and increase of Fo and NPQ (Fig. 3). The increase of Fo under high fly ash concentration was due to photoinhibition in black gram as reported in other abiotic stress such as soil salinity and drought (Calatayud and Barreno, 2001). The Fm, Fv/Fm, qP and Y (II) significantly decreased in a higher concentration of fly ash indicate the decreasing ability of PS II activity due to metal toxicity in a high concentration of fly ash (Panda *et al.,* 2006; Raja *et al.,* 2014). Besides, an increase of NPQ in black gram under high fly ash concentration suggested that there was decreasing the quantum efficiency of PSII photochemistry either by causing a decrease in the rate of primary charge separation or by an increase of heat dissipation (Maxwell and Johnson, 2000; Fu *et al.,* 2012).

Antioxidant enzyme activity and protein content

Fly ash can induce oxidative stress, causing increased production of ROS (Arora *et al.,* 2002). Plants possess several antioxidant enzymes that protect the plants against oxidative damage to control the level and effects of ROS. The activities of some antioxidant enzymes such as SOD, APX and GPX were increased in the black gram seedlings grown in different concentration of fly ash as compared to the plants grown on garden soil (Table 3). The activities of SOD, APX and GPX increased by 1.18, 1.47 and 2.02 - fold, respectively under 100% fly ash compared to the control. The rise in the activities of enzymes was considered to be the response to active oxygen activities caused by metal ion present in fly ash. Possibly, increased levels of active oxygen stimulate the cellular protective mechanism in black gram to mitigate damages (Bhaduri and Fulekar, 2012). This result agreed with the earlier reports on the other crops (Arora *et al.,* 2002). In contrast remarkably inhibition of CAT activity in black gram under 75% and 100% of fly ash, suggesting that CAT was more sensitive than other antioxidative enzymes under elevated fly ash concentration. In addition, a marked increase of protein content was observed in low level (25%) of fly ash-amended soil and decline in further treatments (Table 3). The findings suggest that 25% of fly ash amended soil was not deleterious for black gram growth and heavy metals in the fly ash induce the synthesis of stress proteins (Rastgoo and Alemzadeh, 2011).

Our experiment showed a negative correlation between antioxidant enzymes activities such as SOD, APX and GPX with growth and photosynthetic parameters such as P_N, gs, Fm, Fv/Fm, qP and Chl but a significant positive association with Fo and NPQ (Table 4). This suggests that an increase of growth and photosynthetic activity in black gram seedlings under 25% of fly ash amendment might be due to the better antioxidative protection from oxidatative damage. But the antioxidant defense in black gram seedlings lose their intrinsic balance and are unable to scavenge the generation of excess ROS under elevated levels of fly ash conditions and unable to protect the growth and photosynthesis.

Conclusion

The study showed that garden soil amended with at 25% of fly ash could be beneficial in improving the growth of black gram as well as it maintains leaf photosynthesis and PS II activity. The most suitable treatment for improving black gram growth was 25% of fly ash with soil as it not only improved the physical properties of the soil but also gave the highest plant biomass. Further, the decline in CO_2 photosynthetic rate in black gram seedlings under elevated levels of fly ash in soil system was due to the structural and functional alteration of PS II, stomatal conductance and as well

Table 3: Changes of antioxidant enzymes and protein of 30 days old *Vigna mungo* seedlings exposed to different concentration of fly ash. SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, GPX : Guaiacol peroxidase Data are the mean of 3 replications. Means followed by a common letter are not significantly different at the 5 % level by Duncan's multiple range test.

Treatment	*SOD (Units g^{-1} fwt)*	*APX (mM Asc Oxidised min^{-1} g^{-1} fwt)*	*GPX (µM Guaiacol Oxidised min^{-1} g^{-1} fwt)*	*CAT (ΔA min^{-1} g^{-1} fwt)*	*Protein (mg g^{-1} fwt)*
GS	53.4±1.6^{b}	5.2±0.1^{b}	186.3±7	3.3±0.2^{b}	44.9±0.9^{b}
FA 25%	53.8±6.6^{b}	5.7±0.01^{b}	324.5±13	3.8±0.14^{b}	50.0±1.3^{a}
FA 50%	65.4±4.4^{a}	5.8±0.1^{b}	338.5±1.9	4.4±0.14^{a}	47.6±4.2^{a}
FA 75%	62.0±0.7^{a}	7.3±0.2^{a}	338.5±12	2.4±0.07^{c}	40.3±0.4^{b}
FA 100%	60.5±3.2^{a}	6.7±0.3^{a}	376.8±9.8	1.3±0.14^{d}	36.1±2.6^{c}
LSD*P<0.05	9.7	0.61	24.3	0.43	4.9

Table 4: Correlation between growth and photosynthetic parameters with antioxidant enzymes and protein in *Vigna mungo* L. seedlings grown on different fly ash amended soil. P_N: photosynthetic rate; g_s: stomatal conductance; Chl: chlorophyll; Fo: minimum fluorescence yield obtained with dark-adapted leaf; Fm: maximum Chl fluorescence yield obtained with dark-adapted leaf; Fv/Fm: maximal photochemical efficiency of PS II; NPQ, non-photochemical quenching; qP: photochemical quenching; Y(II): yield of PSII photochemistry; SOD: superoxide dismutase; APX: ascorbate peroxidise; GPX: guaiacol peroxidise; CAT: catalase; *: P<0.05, **: P<0.01, ns: non-significant.

Parameters	*P_N*	*g_s*	*Chl*	*Fo*	*Fm*	*Fv/Fm*	*Y (II)*	*qP*	*NPQ*	*Fresh weight*	*Dry weight*
APX	-0.88**	-0.61**	-0.80**	0.68**	-0.69**	-0.70**	-0.53**	-0.78**	0.54**	-0.12^{ns}	-0.81**
SOD	-0.45**	-0.62**	-0.26^{ns}	0.13^{ns}	-0.42**	-0.26^{ns}	-0.19^{ns}	-0.14^{ns}	0.38*	-0.58*	-0.34^{ns}
GPX	-0.62**	-0.53**	-0.46*	0.32*	-0.58**	-0.49*	-0.49**	-0.57**	0.61**	-0.48*	0.60**
CAT	0.85**	0.70**	0.95**	-0.96**	0.82**	0.96**	0.91**	0.86**	-0.77**	0.90**	0.90**
Protein	0.86**	0.69**	0.92**	-0.90**	0.83**	0.93**	0.85**	0.79**	-0.72**	0.79**	0.87**

as chlorophyll damage. Further, it is also suggested that fly ash can be used as amendments for a year or two, but the continuous use of fly ash by increasing load of toxic metals can cause permanent soil contaminations.

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