



## Original article

# Coal fly ash and nitrogen application as eco-friendly approaches for modulating the growth, yield, and biochemical constituents of radish plants



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## ABSTRACT

Plants are confronting a variety of environmental hazards as a result of fast climate change, which has a detrimental influence on soil, plant growth, and nutrient status. As a result, the present study aims to evaluate the influence of various fly ash concentrations (5, 10, 15, 20, 25, 30, and 35% FA) mixed with the optimum concentrations of nitrogen in the form of urea (0.5 g pot<sup>-1</sup>) on the growth, productivity and biochemical constituents of radish plants. Energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) were used to assess soil physical–chemical properties and FA nutrient status. Results suggested that FA added many essential plant nutrients to the growth substrate and improved some important soil characteristics such as pH, electric conductivity, porosity, and water holding capacity. Also, the results revealed that the low concentrations of FA up to 20% were found to boost radish growth, yield, chlorophyll, carotenoids, and mineral content. While the highest concentrations of FA (25–35%) decreased radish growth and yield, increased oxidative stress through increased lipid peroxidation (MDA) and caused a significant boost in ascorbic acid, proline, protein, and antioxidant enzyme activities. Furthermore, SEM of radish leaf revealed an enhancement in the stomatal pore of radish leaf under different levels of FA. In conclusion, combining 15% fly ash with 0.5 g nitrogen in the form of urea significantly enhanced radish yield by enhancing antioxidant activity such as catalase, peroxidase, ascorbate peroxidase, Guaiacol peroxidase, superoxide dismutase, nitrate reductase and reducing oxidative stress, potentially reducing fly ash accumulation and environmental pollution.

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## 1. Introduction

Due to poor soil fertility, restricted farmer resource availability, and a reduction in nutrients, farmers suffer from decreased soil productivity. There is a worldwide concern over the poor soil fertility problem (Abu-Shahba et al., 2022, Mohamed, 2011). Climate change is expected to have a substantial impact on dry and semi-arid regions, resulting in lower food yields (Abu-Shahba et al., 2021). Over time, the enhancement of soil fertility for crops has

been limited to the use of inorganic fertilizers, which, if left unchecked, would result in a reduction in soil acidity, soil organic matter, and physical soil damage. Because of their exorbitant pricing, the majority of small-scale farmers are unable to purchase them. It is also critical to utilize contaminants to minimize global pollution like fly ash (FA), which is among the most crucial forms of particulate waste produced by the burning of coal combustion for the generation of energy (Ahmed et al., 2021).

Higher levels of pollutants caused by environmental stress are toxic to plants. Fly ash (FA) is an environmentally hazardous and dangerous waste generated by coal-fired thermal power stations, and its removal is one of the world's most pressing challenges, particularly in developing nations. The constant and huge manufacturing of FA necessitates a variety of dumpsites. FA is regarded as a significant environmental danger around the world due to its negative effects on the environment (Ahmed et al., 2021). Heavy metals such as Pb, Cr, Fe, Mn, Ni, Zn, or As found in significant

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quantities in FA may have a detrimental impact on plant development. Heavy metals in FA would affect plant productivity, either directly or indirectly. Some of the direct and indirect consequences include reduced photosynthetic rate, seed germination, protein content, pigment content, and so on, whereas indirect consequences include increased reactive oxygen species (ROS), activity in antioxidant enzymes, function loss and membrane lipid peroxidation (Raj and Mohan, 2018, Moustafa-Farag et al., 2020).

Because of its effectiveness in modifying soil health and plant performance, fly ash has a lot of promise in agricultural production (Tejasvi and Kumar, 2012). Electricity consumption and energy use are increasing in India. As a result, by 2035, India will have surpassed China, the United States, and Russia in terms of electric power plant capacity, accounting for 76.4% of India total capacity (Singh et al., 2018). Approximately 130 million tons of fly ash are created in India today, with an additional 140 million tons expected by the end of 2020. If FA is directly discharged into the environment from power station chimneys without proper treatment, it causes air pollution but has effects on human health (Bartonova, 2015, Ding et al., 2015).

The characteristics of FA in thermal power plants are determined by the kind of coal used and the conditions under which it is burned. Fly ash is a very fine particle with an average diameter < 20  $\mu\text{m}$  and a bulk density of 0.54–0.86  $\text{g/cm}^3$  (Lanzerstorfer, 2018). Except for N, FA contains certain important elements such as Mg, P, K, Zn, Mn, Fe, and others. By improving the availability of soil quality, FA might enhance the porosity and fertility condition of the soil, allowing plants to thrive and yield more (Schönegger et al., 2018). They also contain significant oxide constituents, such as Si, Al, Ca, Mg oxide, Na, and Ti (Bartonova, 2015). As a result, several researchers throughout the world are concerned about FA. Furthermore, the characteristics of fly ash have shown that it can reduce the cost of purchasing soil ameliorants. In terms of soil enzymatic activity, and porosity, soil microbial activities, play a variety of beneficial and functional roles. It improves its quality by reducing water-holding potential, capillary pressure, bulk density, and conductivity. It also protects soil conditions and improves crop development and production (Yunusa et al., 2012).

The crucial need to implement appropriate fertilizer management techniques in agroecosystems is linked to increased food production, the requirement for ecologically friendly agriculture, and upcoming threats related to climate change (Dimkpa et al., 2020). Nitrogen (N), which is classed as a major macronutrient, is critical for increasing crop productivity (Zhang et al. 2020). Farmers increased their use of nitrogen fertilizer on their crops year after year due to increased food availability, while taking into account the reactions of various species to N levels and forms. Since the 1990, the use of nitrogen fertilizer helps in increasing agricultural food output (Jiao et al., 2016). Nitrogen is the principal component of amino acids, nucleic acids, proteins, membranes, cell walls, rubisco, and helps the plants in the adaptation of abiotic and biotic stress conditions (Aghavani et al., 2017). Nitrogen deficiency causes a reduction in leaf area, leaf number, and photosynthetic rate, resulting in lower agricultural production (Mu et al., 2017). It also acts as a signaling molecule, influencing gene expression and regulating metabolic pathways such as nitrogen assimilation, photosynthesis, the cell cycle, and the antioxidant systems (Yao et al., 2020). Several studies have shown that the supply of nitrogen might boost plant growth and crop output. Nonetheless, the overuse and misuse of artificial nitrogen fertilizers causes the buildup of chemicals in agricultural crops. That might be harmful to individuals, as well as polluting the environment and causing economic losses (Mu and Chen, 2020).

The radish (*Raphanus sativus* L.), a member of the Brassicaceae family, is one of China's and India's most economically valuable

vegetables. Radish production is projected to be over 7 million tons per year, accounting for around 2% of all vegetables produced worldwide (Kopta and Pokluda 2013). In India, radish is grown over an area of 208,550 ha with a yield of 3061 metric tons (<https://www.agricoop.nic.in> 2018). Radish foliage and root are the edible parts. Leafy greens and sprouts, on the other hand, are becoming more popular (Manivannan et al. 2019). The root is mostly eaten raw in salads or cooked (Nishio, 2017). It also contains various minerals including iron, calcium, magnesium, potassium, and phosphorus, as well as water-soluble vitamins like B and C (Fang, 2019). Because radish leaves contain a variety of nutrients, they can be used to treat and cure a variety of illnesses (Firdous, 2019).

Considering the above-mentioned characteristics as well as the economic and therapeutic value of the radish plant. The aim of the study is to increase the growth and productivity of fly ash and to decrease the pollution that is caused due to the accumulation of fly ash waste. Also, the current study examines the physicochemical properties of soil and fly ash, as well as growth, physiological, biochemical, and yield attributes, as a result of the use of fly ash and nitrogen treatments. The novelty of this manuscript is the mixing of fly ash with the recommended dose of nitrogen in the form of urea to increase the fertility of soil and plant growth and productivity.

## 2. Materials and methods

### 2.1. Site of the experiment

The research was conducted in the greenhouse of Aligarh Muslim University Department of Botany in Aligarh. The experimental site is in a humid subtropical environment, with a latitude of 27.9135° N and a longitude of 78.0782° E. The experiment took place at a temperature of 28–38 °C (82–100 °F), with irrigation performed on alternate days. The soil type employed in this study was sandy loam.

### 2.2. Plant material and treatments

The seeds of radish var. Early minu were acquired from the Chola Beej Bhandar, in Aligarh, India. The seeds were soaked in 0.1%  $\text{HgCl}_2$  for 2 min, and then washed in de-ionized water. The soil for this experiment was scraped from the surface layer of a contamination free agricultural area up to a depth of 20 cm and autoclaved in the lab. The fly Ash was obtained from the Kasimpur Thermal Power Plant and transported to the lab in the Department of Botany. Each pot contained three kilograms of soil mixed with different levels of fly ash in earthen pots measuring 12 in. in height. The treatments were control, 5, 10, 15, 20, 25, 30, and 35 % FA. Each mixture of fly ash and soil was also mixed with 0.5 g of nitrogen in the form of urea. For a total of 40 pots, each treatment was repeated five times. The experiment was set up in such a way that it was a completely random design (CRD).

### 2.3. Determination of physicochemical characteristics of FA and soil and elemental status

Gee and Bauder (1979) used the hydrometer method to determine the texture of the soil/FA. Jackson (1973) method was used to calculate the EC and pH. The carbonates and bicarbonates of soil and FA samples were determined using Richard (1954) technique. Jackson (1973) approach was used to determine the chloride and sulphate levels in soil and FA samples. The Kjeldahl (1883) technique was used to measure the nitrogen content. After digestion by using  $\text{HClO}_4$ :  $\text{HNO}_3$ , phosphorus (P) was measured colorimetri-

cally, and potassium (K) was calculated using a flame photometer. Atomic absorption spectrometry (Perkin Elmer 3690) was used to determine the amounts of magnesium, manganese, and zinc in the diluted digests (Jackson, 1973). Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX) was used to determine the element content of dried fly ash and soil.

#### 2.4. Measurement of plant growth and yield

The experiment was terminated after 60 days from the sowing. The length of the plant was measured. A computerized weighing balance is used to calculate the fresh and dried weight of the radish. For each treatment, five average-sized leaves were collected and drawn on tracing paper to calculate the leaf area.

#### 2.5. Biochemical parameters

##### 2.5.1. Determination of photosynthetic pigments

Maclachlan and Zalik, (1963) method was used to determine the chlorophyll a, b, and carotenoid content. A spectrophotometer was used to read the supernatant at wavelengths of 663 and 645 nm against a blank of 80 percent acetone.

##### 2.5.2. Determination of leaf characteristics

SEM was used to estimate the number of stomata and their microstructural dimensions. Leaf specimens were prepared for SEM investigations according to Bertrand and Poirier, (2005).

##### 2.5.3. Determination of nonenzymatic antioxidants, osmolytes and oxidative stress

Keller and Schwanger (1977) used the 2,6-dichlorophenol-indophenol (DCPIP) dye to assess the ascorbate concentration, and the optical density was read at 520 nm. Bates et al. (1973) described the ninhydrin technique for estimating proline content using acid ninhydrin, and the absorbance of the mixture was measured at 520 nm using a spectrophotometer. Protein content was determined using the Folin–Ciocâlțeu reagent according to the Lowery et al. (1951) technique, with the optical density read at 750 nm and bovine serum as a standard. Heath and Packer (1968) method of calculating malondialdehyde content was used to determine lipid peroxidation using 0.5 % thiobarbituric acid (TBA) and the absorbance of the solution was measured at 532 nm and 600 nm. The extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  was used to calculate the absorbance coefficient of malondialdehyde.

##### 2.5.4. Determination of antioxidant enzyme activity

A half gram of leaf sample was grounded in a 5 mL solution of 50 mM sodium phosphate buffer (pH 6.5) and was centrifuged at  $15,000 \times g$  for 15 min. The supernatant was collected and used to measure the enzymatic activity. Barber (1980) approach was used to measure catalase (CAT; EC 1.11.1.6). The activity of peroxidase (POX; EC 1.11.1.7) was measured using the Kar and Mishra (1976) technique using pyrogallol. The amount of ascorbate peroxidase (APOX; EC 1.11.1.11) was measured using the Nakano and Asada (1981) method. Guaiacol peroxidase (GPX; EC 1.11.1.7) was assayed according to Mazhoudi et al. (1997) method using guaiacol. The ability of superoxide dismutase (SOD; EC 1.15.1.1) to prevent the photochemical reduction of nitroblue tetrazolium was assayed by using the Beauchamp and Fridovich (1971) method. The activity of nitrate reductase (NR; EC 1.7.1.2) was assessed using Jaworski (1971) technique.

##### 2.5.5. Determination of minerals content

Plant samples were collected, oven-dried at  $68^\circ \text{C}$  for 48 h, crushed, and passed through a 1 mm filter to measure the mineral

content of radish leaves. Total nitrogen was calculated using the Kjeldahl method. The  $\text{HNO}_3$ :  $\text{HClO}_4$  (4:1 v/v) is used to induce digestion of the leaf tissues (Jackson, 1973). Using the indophenol blue and ascorbic acid technique (Jackson, 1973) and a spectrophotometer at 660 nm and 440 nm, phosphorus in the extraction solution was determined. K, Na, Ca, Mg, Fe, Mn, Zn, and Cu elements were measured in the diluted digests by using atomic absorption spectrophotometer (Perkin Elmer 3690) (Jackson, 1973).

#### 2.6. Statistical analysis

The significance of the data was determined using SPSS (17.0 Inc.) and analysis of variance (ANOVA- one way) at a level of  $P \leq 0.05$  by using Duncan's Multiple Range Test.

### 3. Results

#### 3.1. Physicochemical properties of FA and soil

Table 1 shows the physicochemical parameters and element content found in FA (Fig. 1 A-C) and the soil (Fig. 1 D-F) chosen for amendment. SEM images revealed that fly ash is composed of microscopic spherical particles as compared to the soil. As indicated, FA that was applied to the soil has an alkaline pH (8.5), high water holding capacity (53.41%), moisture content (4.29%) and EC ( $0.205 \text{ mmhos cm}^{-1}$ ), whereas lower organic carbon (69.78%), chloride (24.19%), bicarbonate (15.32%), P (0.024%) and K (16.19%) were detected as compared to the soil without any additions. Because FA lacks the ingredient N, its content was reduced. The FA amendment boosted the water holding capacity of the soil by 53.41% above the control value (45.50%) (Table 1). In comparison to the soil, EDX profiling revealed that fly ash has the highest concentration of metals such as Si, Al, Fe, K, and Mg (Fig. 1C- F). Among these, fly ash contains the highest amount of Si and the lowest amount of Cu (Fig. 1 C). Fly ash does not contain nitrogen. The EDX profiling of soil showed that only two metals were detected Si and Al (Fig. 1 F).

#### 3.2. Interactive effect of nitrogen and fly ash on growth and yield of radish

Figs. 2-4 illustrate the effect of the combined application of optimum nitrogen (0.5 g) doses and various fly ash levels that

**Table 1**  
Physicochemical properties of experimental soil and fly ash. The given values are mean of three replicates  $\pm$  SE.

Characteristics	Soil	Fly ash
Color	Light Brown	Grey
Texture	Loam, Sandy loam	Silty
EC ( $\text{mmhos cm}^{-1}$ )	$0.050 \pm 0.11$	$0.205 \pm 0.19$
CEC ( $\text{mEq}/100 \text{ g}$ )	$2.04 \pm 0.20$	$6.5 \pm 0.58$
Porosity (%)	38.59	60.19
Water holding capacity (%)	45.50	53.41
Moisture content (%)	$2.68 \pm 0.29$	$4.29 \pm 0.21$
Particle density ( $\text{g}/\text{cm}^3$ )	$2.59 \pm 0.23$	$1.50 \pm 0.22$
pH	$6.8 \pm 0.59$	$8.5 \pm 0.70$
Sulphate (%)	$15.37 \pm 1.50$	$26.10 \pm 1.90$
Chloride (%)	$37.67 \pm 2.30$	$24.19 \pm 1.65$
Carbonate (%)	$76.23 \pm 5.44$	$69.78 \pm 7.10$
Bicarbonate (%)	$20.25 \pm 1.45$	$15.32 \pm 1.59$
N (%)	$2.65 \pm 0.38$	-
P (%)	$2.86 \pm 0.43$	$0.024 \pm 0.003$
K (%)	$18.65 \pm 1.66$	$16.19 \pm 1.28$
Zn ( $\text{mg g}^{-1}$ )	$1.21 \pm 0.29$	$2.05 \pm 0.87$
Mg ( $\text{mg g}^{-1}$ )	$40.02 \pm 3.16$	$23.13 \pm 1.90$
Mn ( $\text{mg g}^{-1}$ )	$3.82 \pm 0.95$	$9.17 \pm 1.29$



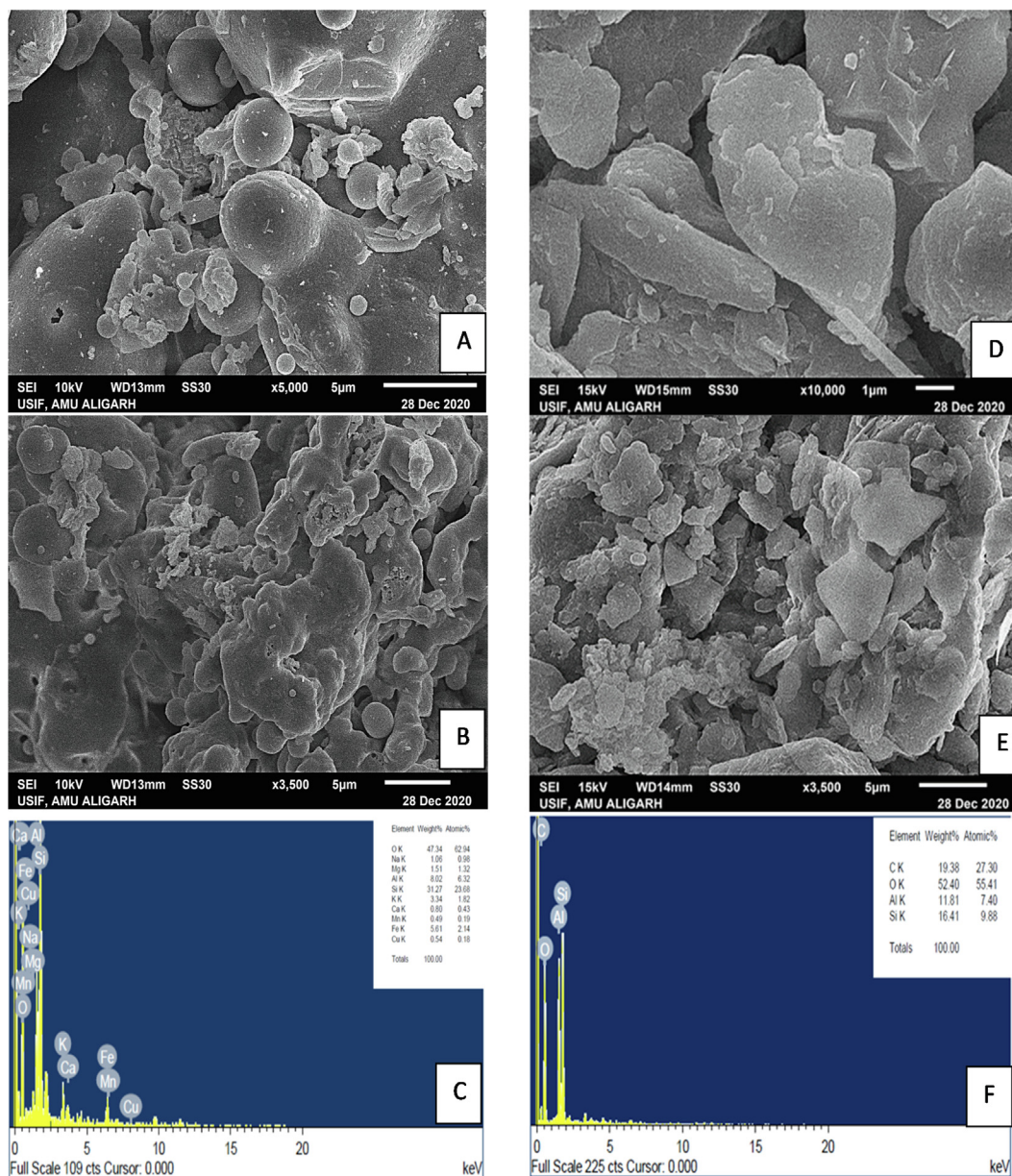


Fig. 1. Scanning electron microscopy (SEM) images and energy dispersion X-ray (EDX) profiling of fly ash (A-C) and soil (D-F).

amendments to the soil have on radish growth and yield. The low concentrations of fly ash (5, 10, 15, and 20% FA) and nitrogen substantially boost the growth and yield parameters of radish plants above the values of the control plants, while the high concentrations of FA (25, 30 and 35%) and nitrogen have a negative effect on the growth and yield performance of radish. Soil amendments with 15% FA and 0.5 g of nitrogen caused a significant boost in growth performance and recorded the maximum values of foliage length (49.8%), root length (41.9%), foliage fresh weight (45.2%), foliage dry weight (130.8%), root fresh weight (137.7%), root dry weight (134.3%), number of leaves (31.8%), root circumference (44.2%) and number of flowers (51.3%) as compared to control values (Figs. 2 and 3). On the contrary, the higher concentration of fly ash (35%) recorded lower values of foliage length (36.4%), root length (38.8%), foliage fresh weight (36.1%), foliage dry weight (14.1%), root fresh weight (55.4%), root dry weight (7.7%), number of leaves (40.9%), root circumference (52.0%) and number of flowers (51.3%) as compared to control without any treatments.

### 3.3. Interactive effect of nitrogen and fly ash on chlorophyll content and leaf characteristics

The photosynthetic parameters, including chlorophyll a, b, carotenoids and total chlorophyll, content increased maximally by the application of the low concentrations of FA (5, 10 and 15%) with N over control (Fig. 5), while the highest concentrations of FA with N (20, 25, 30 and 35%) caused a significant decrease in the photosynthetic parameters in relative to the control plants. One-way ANOVA showed that 15% FA and N was the optimum concentration among different treatments that caused an increment in Chl a (57.1%), Chl b (59.3%), carotenoids (88.9%) and total Chl (60.3%) over control, on the contrary, 35% FA and N caused the highest reduction in Chl a (34.2%), Chl b (11.2%), carotenoids (44.4%) and total Chl (37.3%). The SEM demonstrated that plants grown in various concentrations of FA have a significantly higher number of stomata on their leaves, as well as enhanced stomatal apertures (Figs. 6 and b).

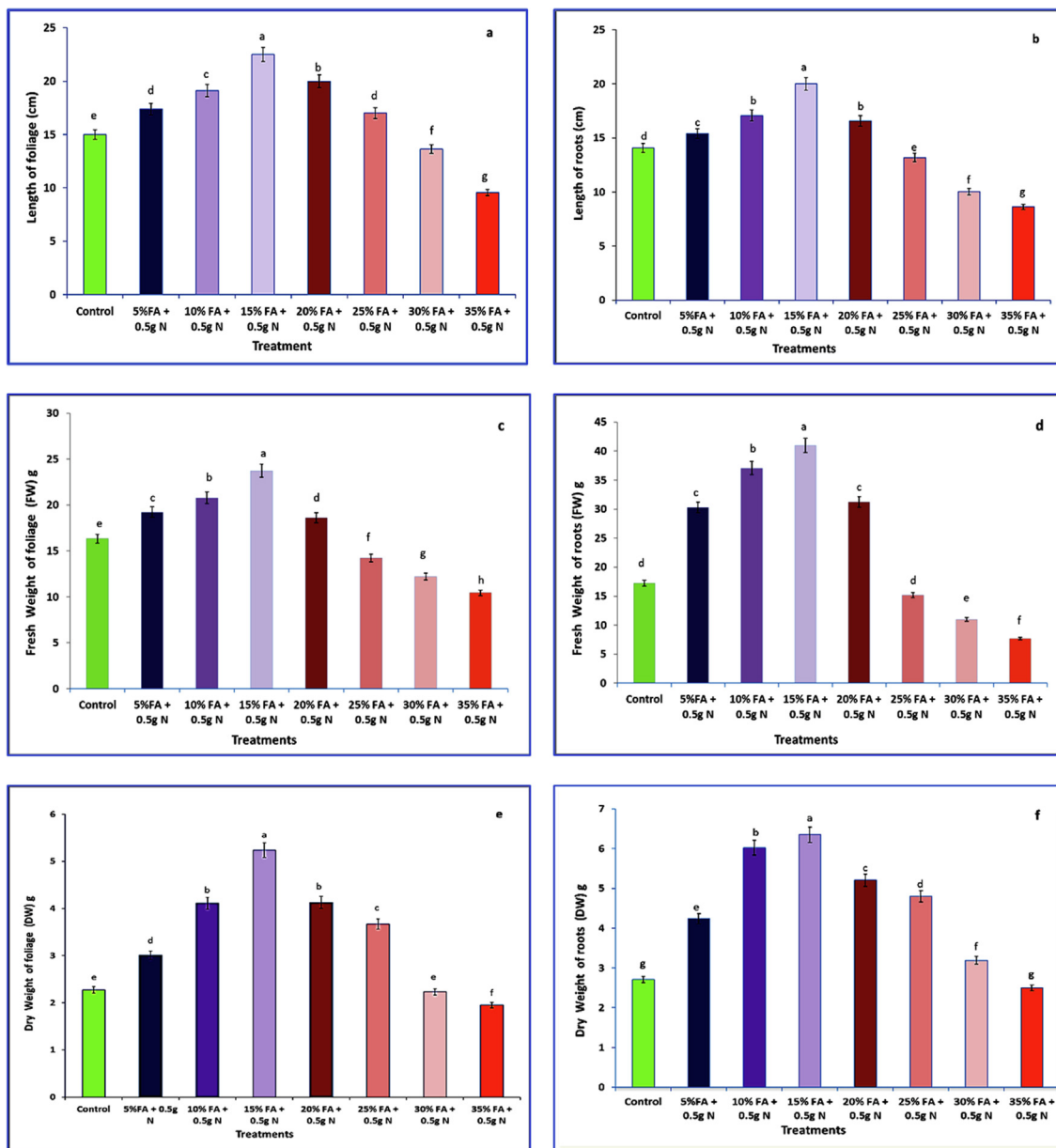


Fig. 2. Effect of different levels of fly ash and N on growth performance of radish cv. Minu Early. Data represent mean values of five replicates  $\pm$  show standard error (SE). Mean with different letters in the same bar differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

3.4. Interactive effect of nitrogen and fly ash on non-enzymatic antioxidants, osmolytes content and oxidative stress

In radish plants, amendment of soil with various FA levels with 0.5 g N resulted in a substantial increase in non-enzymatic antioxidant (ascorbic acid) activity, osmolyte content (proline and protein), and oxidative stress (MDA) as compared to untreated plants (Fig. 7). All the above components increased significantly with increasing fly ash levels compared with control plants. The most pronounced increases in ascorbic acid (189.4%), proline (145.9%), MDA (172.7%) and protein (292.5%) were found in plants grown in soil amendment with high concentrations of FA (35%) and nitrogen over control plants.

3.5. Interactive effect of nitrogen and fly ash on antioxidant enzymes activity

The activity of antioxidant enzymes such as CAT, POX, APOX, GPX, SOD, and NR in radish plants was significantly increased

when the soil was amended with varying FA levels and N (Table 2). The antioxidant activity increased with increasing FA levels with nitrogen as compared to control plants. However, at a 35% FA level, the highest enzyme activity was recorded, whereas the control plants had the lowest activity (Table 2). Plants cultivated in soil amendment with high concentrations of FA (35%) and nitrogen showed the most pronounced increases in CAT (177%), POX (35.3%), APOX (123.1%), GPX (20.3%), SOD (246.5%), and NR (88.1%) when compared to the corresponding control plants.

3.6. Interactive effect of nitrogen and fly ash on minerals content

FA added a range of critical minerals as well as some non-essential elements to the soil (Table 3). The addition of nitrogen to soil with low concentrations of FA (5, 10, and 15%) resulted in a significant increase in mineral content (N, P, K, Ca, Na, Mg, Cu, Mn, Zn, and Fe) over control plants, whereas treatment with high concentrations of FA resulted in a significant decrease in all of the above minerals (except Na, which showed a significant

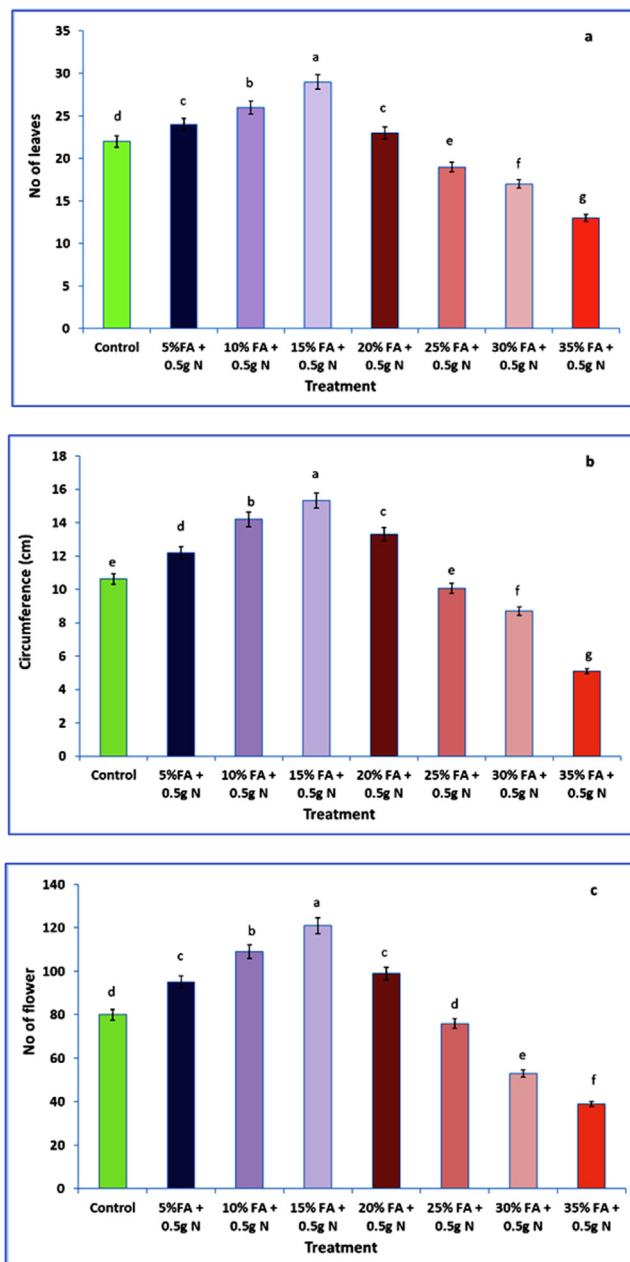


Fig. 3. Effect of different levels of fly ash and N on No of leaves (a), circumference (b) and No of leaves (c) of radish cv. Minu Early. Data represent mean values of five replicates  $\pm$  show standard error (SE). Mean with different letters in the same bar differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

increase) (Table 3). Plants grown on soil amended with 15% FA and N had higher levels of N (55.3%), P (20.8%), K (35.8%), Ca (14.7%), Mg (18.2%), Na (29.6%), Cu (4.5%), Mn (8.0%), Zn (10.7%), and Fe (9.5%). Plants grown on soil amendment with 35% FA and N, on the other hand, had lower amounts of N (33.6%), P (37.5%), K (40.0%), Ca (22.7%), Mg (34.5%), Cu (91.4%), Mn (13.1%), Zn (12.3%), and Fe (18.1%) and higher amounts of Na (50.6%).

#### 4. Discussion

Rapid industrialization and urbanization are contaminating agricultural soils with hazardous HMs to varying degrees, limiting normal metabolic activity and resulting in losses in agricultural productivity around the world (Mohamed 2011). Plants are poisonous to HMs like Cr, Pb, and Cd, and even very low concentrations cause stress, which can contribute to the production of excess ROS (El-Mahdy et al., 2021). Today, improving the agronomic features of soils for greater agricultural yield has become a serious problem for researchers in order to meet the demand for rapidly rising populations. Because it contains Si, Al, Fe, Ca, Mg, K, P, Cu, certain other micronutrients, and a low amount of C, fly ash has the ability to boost the fertility of agricultural soils based on the pore space (Ahmed et al., 2021). Absolute hollow silt-sized particles supply water molecules on a larger scale (Skousen et al., 2013). EDX profiling of FA indicated that it contains valuable plant nutrients and caused enhancements in water holding capacity, porosity, pH, organic substances, and soil permeability (Haris et al., 2019, Ahmed et al., 2021).

The results of the present study found that combining up to 25% fly ash with 0.5 g nitrogen in the form of urea improved radish plant growth and yield. However greater doses of FA combined with N (30 and 35% FA) had a negative influence on growth and yield. These findings are consistent with those of Ashfaq and Inam (2020), who found that a concentration of 60 t ha<sup>-1</sup> of FA combined with 60 or 80 kg ha<sup>-1</sup> of nitrogen reduces enzymatic activity, photosynthetic pigment levels, and yield. However, a concentration of 40 t ha<sup>-1</sup> of FA combined with 60 kg ha<sup>-1</sup> of nitrogen substantially increases crop yields in mustard plants, followed by 60 kg ha<sup>-1</sup> of nitrogen. Furthermore, FA at low doses can modify the physicochemical properties of the soil, and the availability of K, P, Mg, and Ca in the soil (Haris et al., 2021).

As previously stated, fly ash is high in Ca, which is a key component of signal transduction and works as a secondary messenger in the development and regulation of cell walls and cell division (Marschner, 2012, Helmi and Mohamed, 2016, Akladios and Mohamed, 2018). Furthermore, fly ash was enriched with Si, which plays a critical role in decreasing cell oxidation and boosting the content of chlorophyll and non-enzymatic antioxidant compounds like carotenoids, as well as reducing transpiration and boosting water use efficiency and, yield (El-Beltagi et al., 2020, Sofy et al.,



Fig. 4. Showing the effect of different levels of fly ash + 0.5 g N on radish.



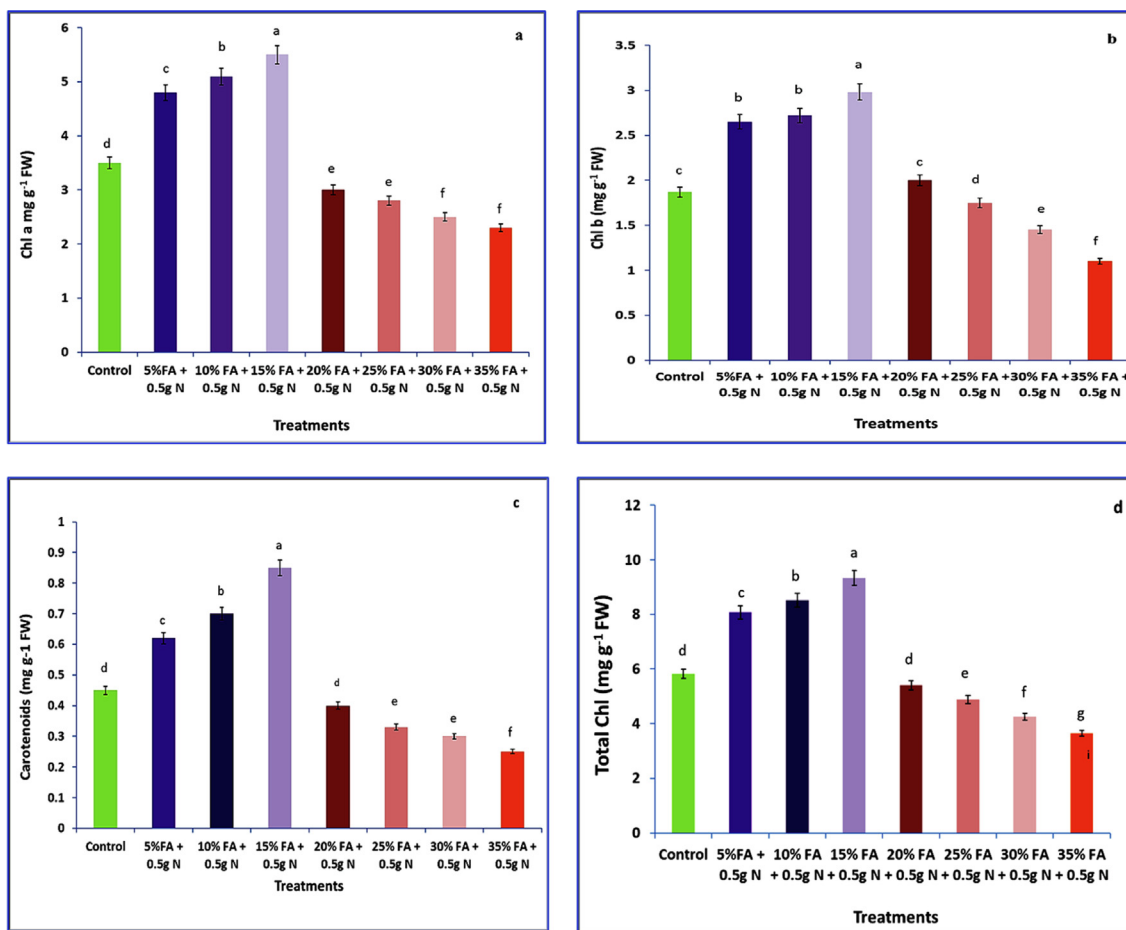


Fig. 5. Effect of different levels of fly ash and N on chl a (a), chl b (b), carotenoids (c) and total chl content (d) of radish cv. Minu Early. Data represent mean values of five replicates ± show standard error (SE). Mean with different letters in the same bar differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

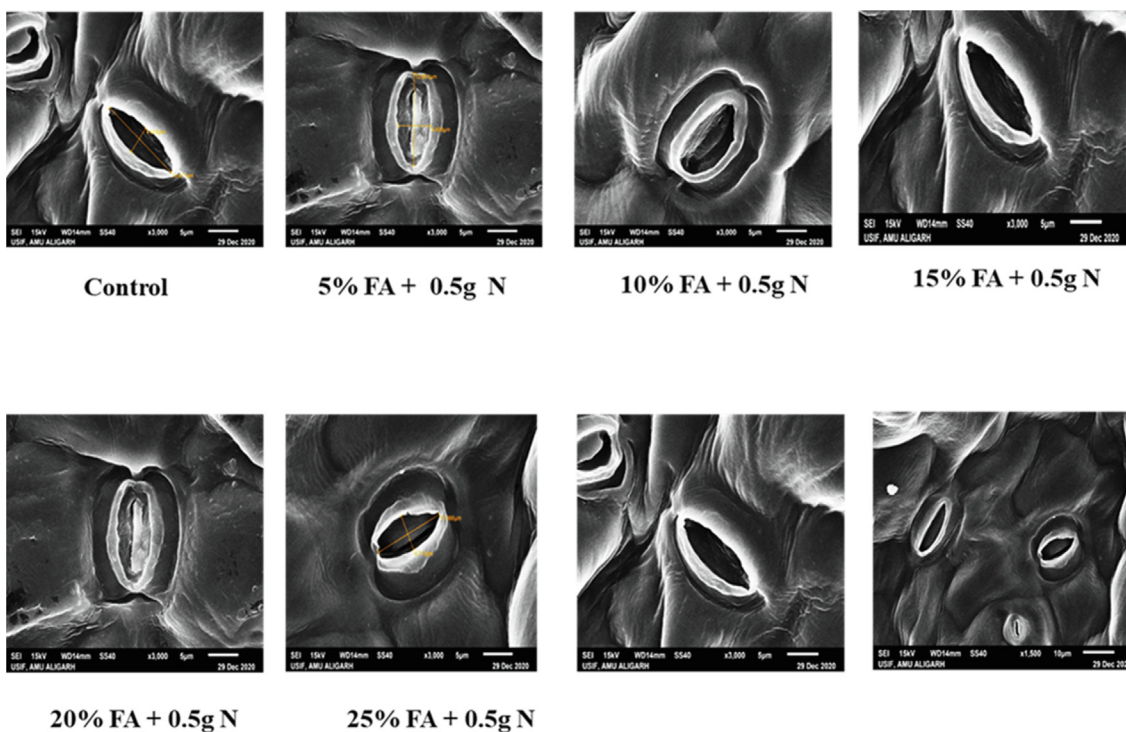
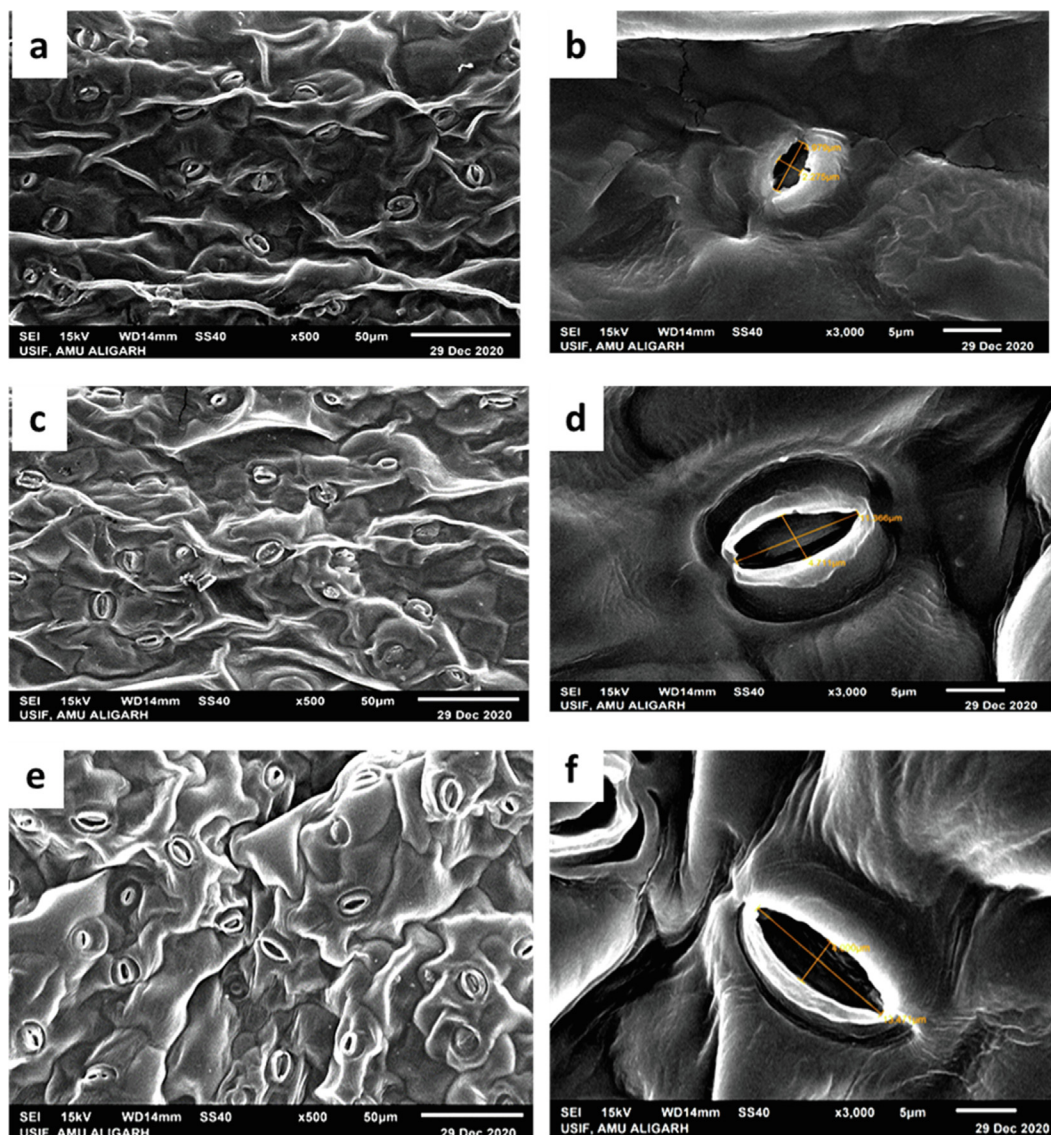


Fig. 6a. SEM images of different concentration of fly ash and N on stomatal aperture of radish cv. Early menu.



**Fig. 6b.** Scanning electron micrograph showing the structure of stomata of control (a, b), 15% FA + 0.5 g N (c, d) and 35% FA + 0.5 g N (e, f) treated plant of radish cv. Early menu.

2021a). Similarly, K has a function in reducing the detrimental effects of stress on plants by acting as a catalyst for a variety of enzymatic activities that are critical for plant production and growth (Haris et al. 2021). Furthermore, because fly ash is nitrogen-deficient, adding nitrogen with low levels of fly ash increased radish growth and yield over control plants. The rise in the number of dividing meristematic cells, which leads to the establishment of the shoot and root systems in plants, could be the cause of this improvement (Lawlor, 2002, Ghonaim et al., 2021). Additionally, increased cytokinin levels have been associated to nitrogen application, which alters cell wall extensibility and encourages cell division (Kamada-Nobusada et al., 2013, Sofy et al., 2021b).

The high concentrations of fly ash (30 and 35%) contain high concentrations of Na and heavy metals (HMs) like Al that cause a reduction in radish growth and yield. The fact is that HMs attach to the root cell wall, preventing cell division. This negative effect was caused by greater amounts of  $\text{HCO}^-$ ,  $\text{SO}_4$ ,  $\text{CO}_3$  and  $\text{Cl}^-$  in the FA-added soil, resulting in salinity (Ashfaq and Inam 2020). The drop in yield and growth characteristics may be due to a decrease in N and P availability, as well as higher levels of toxic metals (Brännvall et al., 2014). (Brännvall et al., 2014).

In radish plants, soil amendments with up to 15% fly ash and 0.5 g nitrogen resulted in a substantial increase in chl a, chl b, carotenoids, and total chl. However greater levels of FA combined with N (20, 25, 30, and 35 %) have a negative impact on the same contents when compared to untreated. Ashfaq and Inam (2020) found that applying FA at a rate of  $40 \text{ t ha}^{-1}$  with  $60 \text{ kg ha}^{-1}$  of nitrogen boosts carotenoid and total chlorophyll content in mustard plants when compared to control, whereas high concentrations of the same lower these parameters. The presence of Mg in fly ash, which is a significant plant nutrient and is required for chloroplast structural stability, could explain the rise in photosynthetic pigments in plants growing in soil amendments with lower FA concentrations (Ashfaq and Inam 2019). Furthermore, the presence of micronutrients such as Mn, Cu, K, and Fe in fly ash may have played a major role, as Mn is involved in water photolysis, Cu and Fe play a role in the photosynthesis pathway (Mohamed et al., 2016, Mohamed and Abd-El Hameed, 2014), and K is associated with enzyme activities that are critical for growth (Ashfaq and Inam, 2019). K is responsible for the creation of the pH gradient required for ATP production and thereby leads to the production of strong turgor pressure, which causes the stomata to open (Ashfaq and Inam, 2019). Due to the prevalence of HM in FA,



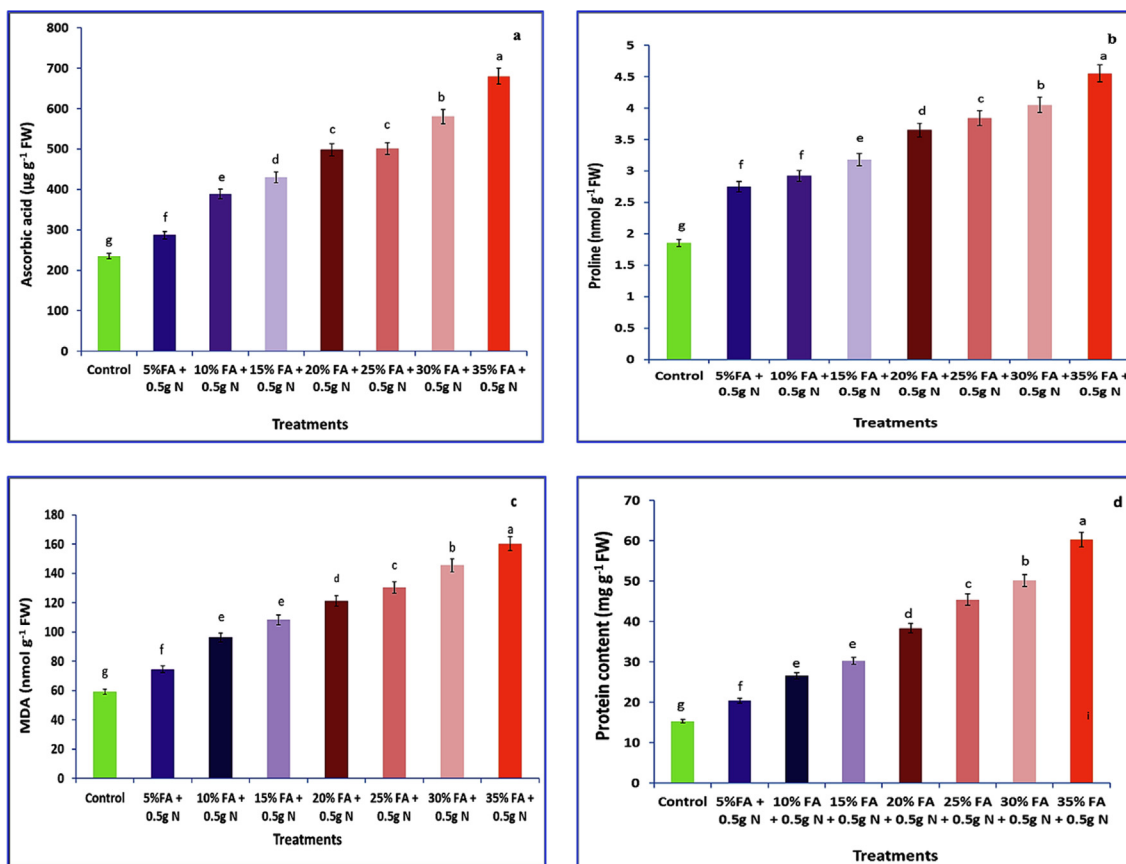


Fig. 7. Effect of different levels of fly ash and N on ascorbic acid (a), proline (b), malondialdehyde (c) and protein content (d) of radish cv. Early menu. Data represent mean values of five replicates  $\pm$  show standard error (SE). Mean with different letters in the same bar differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

Table 2  
Effect of different levels of fly ash and N on enzymatic antioxidants content of radish cv. Minu Early.

Treatment	CAT ( $\text{mmol g}^{-1}\text{FW}$ )	POX ( $\text{mmol g}^{-1}\text{FW}$ )	APOX ( $\text{mmol g}^{-1}\text{FW}$ )	GPX ( $\text{mg g}^{-1}\text{FW}$ )	SOD ( $\text{units g}^{-1}\text{FW}$ )	NR ( $\text{mg g}^{-1}\text{FW}$ )
Control	26.62 $\pm$ 1.0 <sup>g</sup>	0.68 $\pm$ 0.02 <sup>f</sup>	15.09 $\pm$ 0.2 <sup>f</sup>	15.92 $\pm$ 1.5 <sup>f</sup>	6.80 $\pm$ 0.05 <sup>g</sup>	12.28 $\pm$ 0.4 <sup>f</sup>
5% FA + 0.5 g N	37.15 $\pm$ 2.0 <sup>f</sup>	0.79 $\pm$ 0.03 <sup>e</sup>	17.86 $\pm$ 0.3 <sup>e</sup>	25.67 $\pm$ 2.0 <sup>e</sup>	10.28 $\pm$ 0.2 <sup>f</sup>	13.19 $\pm$ 0.5 <sup>e</sup>
10% FA + 0.5 g N	44.60 $\pm$ 2.0 <sup>c</sup>	0.82 $\pm$ 0.03 <sup>d</sup>	19.57 $\pm$ 0.5 <sup>d</sup>	30.19 $\pm$ 3.0 <sup>d</sup>	10.65 $\pm$ 0.2 <sup>f</sup>	15.26 $\pm$ 0.5 <sup>d</sup>
15% FA + 0.5 g N	52.06 $\pm$ 3.0 <sup>d</sup>	0.82 $\pm$ 0.04 <sup>d</sup>	20.45 $\pm$ 0.4 <sup>d</sup>	35.26 $\pm$ 3.0 <sup>c</sup>	13.90 $\pm$ 0.3 <sup>e</sup>	17.89 $\pm$ 0.6 <sup>c</sup>
20% FA + 0.5 g N	55.50 $\pm$ 3.0 <sup>c</sup>	0.83 $\pm$ 0.04 <sup>d</sup>	22.33 $\pm$ 0.5 <sup>c</sup>	41.11 $\pm$ 4.0 <sup>b</sup>	17.89 $\pm$ 0.4 <sup>d</sup>	18.99 $\pm$ 0.6 <sup>c</sup>
25% FA + 0.5 g N	55.93 $\pm$ 3.0 <sup>c</sup>	0.86 $\pm$ 0.04 <sup>c</sup>	24.19 $\pm$ 0.4 <sup>c</sup>	43.10 $\pm$ 4.0 <sup>b</sup>	19.10 $\pm$ 0.4 <sup>c</sup>	20.15 $\pm$ 0.8 <sup>b</sup>
30% FA + 0.5 g N	67.89 $\pm$ 4.0 <sup>b</sup>	0.89 $\pm$ 0.02 <sup>b</sup>	26.19 $\pm$ 0.5 <sup>b</sup>	46.29 $\pm$ 4.5 <sup>a</sup>	21.19 $\pm$ 0.5 <sup>b</sup>	22.76 $\pm$ 0.9 <sup>a</sup>
35% FA + 0.5 g N	73.69 $\pm$ 4.0 <sup>a</sup>	0.92 $\pm$ 0.03 <sup>a</sup>	33.67 $\pm$ 0.5 <sup>a</sup>	48.23 $\pm$ 4.5 <sup>a</sup>	23.56 $\pm$ 0.5 <sup>a</sup>	23.10 $\pm$ 0.8 <sup>a</sup>

Data represent mean values of five replicates  $\pm$  show standard error (SE). Mean with different letters in the same column differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

treatment with high doses of FA reduced chlorophyll production and content by limiting precursor levels or targeting the -SH group of aminolevulinic acid dehydratase (ALAD) (Ashfaq and Inam, 2020).

The presence of nitrogen also boosted photosynthetic pigments in radish due to the fact that nitrogen boosts the concentration of stroma and thylakoid proteins in the leaves, resulting in the enhancement of chlorophyll synthesis (Teixeira et al., 2011, Abd El-Rahman and Mohamed, 2014). Hong et al. (2012) explain that nitrogen fertilizer encourages the production of chloroplasts, which leads to a rise in leaf lipid content and chloroplast elements like chlorophyll and carotene. In comparison to control, fly ash and nitrogen increase the number of stomata and stomatal area (Fig. 6a, b). Similar findings were confirmed by scanning electron microscopy of carrot and beetroot leaves, which revealed that basal

nitrogen fertilizer application at U150 (436.71 mg/kg) significantly increased the stomatal area over control (Adnan et al., 2021). As a result, the observed enhancements in plant growth, yield, and stomatal characteristics could be related to the addition of K ion to the soil by FA amendment. (Shakeel et al., 2021).

The amount of oxidative stress (MDA) in fly ash-treated radish plants was higher than in control plants, and this increase persisted as fly ash levels increased. However, the use of this treatment boosted the levels of antioxidants such as ascorbic acid, proline, and protein content, which helped to alleviate the stress. Our findings are in line with previous research that found proline is implicated in removing ROS from the environment and is capable of reducing oxidative damage produced by greater FA doses, according to Haris et al. et al. (2021). In addition, Ashfaq and Inam (2020) discovered that boosting FA levels raised malondi-

**Table 3**  
Effect of different levels of fly ash and N on leaf mineral (mg 100 g<sup>-1</sup> DW) content of radish cv. Early minu.

Treatment	N	P	K	Ca	Mg	Na	Cu	Mn	Zn	Fe
Control	2.35 ± 0.2 <sup>d</sup>	0.48 ± 0.01 <sup>d</sup>	3.10 ± 0.4 <sup>d</sup>	0.75 ± 0.02 <sup>d</sup>	0.55 ± 0.01 <sup>d</sup>	0.81 ± 0.1 <sup>h</sup>	0.222 ± 0.001 <sup>d</sup>	0.137 ± 0.001 <sup>d</sup>	0.122 ± 0.002 <sup>d</sup>	0.105 ± 0.002 <sup>c</sup>
5 %FA + 0.5 g N	2.85 ± 0.2 <sup>c</sup>	0.50 ± 0.02 <sup>c</sup>	3.45 ± 0.3 <sup>c</sup>	0.78 ± 0.02 <sup>c</sup>	0.57 ± 0.01 <sup>c</sup>	0.95 ± 0.1 <sup>g</sup>	0.224 ± 0.002 <sup>c</sup>	0.139 ± 0.001 <sup>c</sup>	0.126 ± 0.001 <sup>c</sup>	0.108 ± 0.001 <sup>b</sup>
10 %FA + 0.5gN	3.05 ± 0.3 <sup>b</sup>	0.55 ± 0.02 <sup>b</sup>	3.95 ± 0.3 <sup>b</sup>	0.82 ± 0.02 <sup>b</sup>	0.61 ± 0.02 <sup>b</sup>	1.02 ± 0.2 <sup>f</sup>	0.228 ± 0.002 <sup>b</sup>	0.142 ± 0.002 <sup>b</sup>	0.128 ± 0.002 <sup>b</sup>	0.110 ± 0.001 <sup>b</sup>
15 %FA + 0.5gN	3.65 ± 0.3 <sup>a</sup>	0.58 ± 0.02 <sup>a</sup>	4.21 ± 0.4 <sup>a</sup>	0.86 ± 0.04 <sup>a</sup>	0.65 ± 0.02 <sup>a</sup>	1.05 ± 0.1 <sup>e</sup>	0.232 ± 0.002 <sup>a</sup>	0.148 ± 0.002 <sup>a</sup>	0.135 ± 0.002 <sup>a</sup>	0.115 ± 0.001 <sup>a</sup>
20 %FA + 0.5gN	2.95 ± 0.1 <sup>c</sup>	0.45 ± 0.01 <sup>e</sup>	3.05 ± 0.3 <sup>d</sup>	0.73 ± 0.03 <sup>e</sup>	0.53 ± 0.01 <sup>e</sup>	1.08 ± 0.3 <sup>d</sup>	0.220 ± 0.001 <sup>e</sup>	0.135 ± 0.001 <sup>e</sup>	0.119 ± 0.001 <sup>e</sup>	0.103 ± 0.002 <sup>c</sup>
25 %FA + 0.5gN	2.20 ± 0.1 <sup>e</sup>	0.41 ± 0.01 <sup>f</sup>	2.76 ± 0.2 <sup>e</sup>	0.69 ± 0.03 <sup>f</sup>	0.50 ± 0.02 <sup>f</sup>	1.10 ± 0.3 <sup>c</sup>	0.217 ± 0.001 <sup>f</sup>	0.131 ± 0.001 <sup>f</sup>	0.117 ± 0.001 <sup>f</sup>	0.098 ± 0.001 <sup>d</sup>
30 %FA + 0.5gN	1.90 ± 0.2 <sup>f</sup>	0.38 ± 0.01 <sup>g</sup>	2.39 ± 0.2 <sup>f</sup>	0.63 ± 0.02 <sup>g</sup>	0.43 ± 0.01 <sup>g</sup>	1.14 ± 0.3 <sup>b</sup>	0.213 ± 0.001 <sup>g</sup>	0.126 ± 0.001 <sup>g</sup>	0.110 ± 0.001 <sup>g</sup>	0.090 ± 0.001 <sup>e</sup>
35 %FA + 0.5gN	1.56 ± 0.1 <sup>g</sup>	0.30 ± 0.01 <sup>h</sup>	1.86 ± 0.1 <sup>g</sup>	0.58 ± 0.01 <sup>h</sup>	0.36 ± 0.01 <sup>h</sup>	1.22 ± 0.2 <sup>a</sup>	0.019 ± 0.001 <sup>h</sup>	0.119 ± 0.001 <sup>h</sup>	0.107 ± 0.001 <sup>h</sup>	0.086 ± 0.001 <sup>f</sup>

Data represent mean values of five replicates ± show standard error (SE). Mean with different letters in the same column differ significantly at  $p \leq 0.05$  according to Duncan's Multiple Range Test.

aldehyde (MDA) content and antioxidant levels (ascorbate and proline content) in mustard plants. The presence of HMs in a greater dose of fly ash may cause oxidative stress by creating ROS (El-Mahdy et al., 2021, Moustafa-Farag et al., 2020). ROS reacts quickly with lipids, deoxy nucleic acid, and proteins, causing disturbances in the molecules, cell membrane functions, and degradation of proteins (Tamás et al. 2014). This effect may be mitigated by an increase in antioxidant activity, which is regarded as an important strategy for reducing the poisonousness of certain metals (Ashfaq et al., 2017, Abu-Shahba et al., 2022). In our study, ascorbate, proline, and protein content were significantly boosted by increasing fly ash concentrations. These increases help the plant to boost the antioxidant enzyme activity that can remove ROS and decrease oxidative damage against free radicals (Ahmed et al., 2021, Ashry et al., 2018).

When FA levels were increased with N, antioxidant activity (CAT, POX, APOX, GPX, SOD, and NR) increased. Besides, at a 35% FA level, the highest enzyme activity was recorded, whereas the control plants had the lowest enzyme activity. Our results are in line with those of Haris et al. (2021) who observed that antioxidant enzymes increased with increasing FA concentrations in chickpea. Furthermore, Shamsi et al. (2008) revealed that K in the FA boosts the activity of antioxidant enzymes involved in photosynthesis, starch synthesis, stomatal opening, nutrient translocation, and regulation.

Similar results found that application of nitrogen increased NR and N content in carrot and beetroot (Shakeel et al., 2021). This is due to the fact that NR is a crucial enzyme in N uptake and assimilation (Shakeel et al., 2021). The application of N boosted the activity of NR, allowing it to convert more quickly into N precursors for the synthesis of amino acids and, thus, proteins and caused an increment in the protein content (Shakeel et al., 2021). An adequate supply of nitrogen reduced the formation of O<sub>2</sub> radicals and caused scavenging of superoxide anion radicals (Shakeel et al., 2021) by boosting antioxidant defence systems such as CAT, POX, APOX, GPX, SOD, and NR.

The amendment of soil with low concentrations of FA levels (5, 10, and 15%) with N resulted in a significant increase in minerals content such as N, P, K, Ca, Na, Mg, Cu, Mn, Zn, and Fe over control plants, whereas treatment with high concentrations of FA resulted in a significant decrease in all of the above minerals except Na, which showed a significant increase. A similar observation was made in pumpkin crops, where it was discovered that with lower levels of fly ash, there was an increase in NPK content (Ahmed et al., 2021).

### 5. Conclusion

The utilization of FA in agriculture may provide a feasible alternative for its safe disposal without any serious deleterious effects on the environment. Further, the utilization of FA (15%) and nitrogen could be optimized by augmentation with manure in soil, improving the physicochemical characteristics and nutrient status of the soil, which ultimately resulted in improved growth and yield of radish. The application of FA could be useful in the reclamation of the soil turning it into a cultivable land for productive agricultural practices.

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## CRediT authorship contribution statement

**Moh Sajid Ansari:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing, Project administration. **Gufuran Ahmad:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Project administration. **Abrar A. Khan:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration. **Heba I. Mohamed:** Conceptualization, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Abeer Elhakem:** Conceptualization, Investigation, Writing – review & editing, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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