



## Research article

## Effect of soil amendments on antioxidant activity and photosynthetic pigments in pea crops grown in arsenic contaminated soil



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## ABSTRACT

The mechanism of arsenic (As) immobilization in soils is crucial for improving photosynthetic pigments and antioxidants in food crops. The effects of soil amendments with arbuscular mycorrhizal fungi (AMF), biochar (BC), selenium (Se), sulfur (S) and Si-gel on the concentrations of chlorophyll, carotenoid, proline, malondialdehyde (MDA), and the activity of ascorbate peroxidase (APX), guaiacol peroxidase (POD), and catalase (CAT) were studied in BARI pea (*Pisum sativum*) under As stress. Soil amendments with AMF, Se, Si-gel and S enhanced chlorophyll a and total chlorophyll contents by 31–35% and 60–75%, respectively. Likewise, CAT activity was increased by 24–46% in BC, AMF, Se, Si-gel and S-treated pea, respectively. APX and POD activity was also found to be enriched with the treatment of BC, AMF and Se. In contrast, the content of MDA and proline was found lower than that of control in peas. These findings indicate that oxidative damage, osmotic stress and cell injury were possibly reduced in As-stressed peas. Particularly, AMF and Se both were comparatively more potential in comparison to BC. Thus, soil amendments with AMF, BC and Se are significantly important for improving antioxidant enzyme activity of food crops grown in soil with elevated As levels.

## 1. Introduction

Antioxidants have great potential to prevent the toxic effects of metalloids (Gupta and Sharma, 2006). Many of the plant species have been found to be pharmaceutically essential, and nearly all of these have excellent antioxidant potential (Krishnaiah et al., 2011). Antioxidants reduce the degeneration of cells under stress in food crops (Gupta and Sharma, 2006). Antioxidant activities can neutralize the harmful effects of free radicals. Free radical production is generated by abiotic stress, which enhances the injury to plant tissues (Gupta and Sharma, 2006).

Proline is recognized as a stress indicator in food crops (Shamsul and Ahmad, 2012). It highlights a dynamic role in the biomass growth of plants under stress conditions. It accumulates proteogenic amino acid in plants grown under stress and non-stress conditions (Kavi Kishor et al., 2015). Chlorophyll and carotenoids both represent photosynthetic pigments in plants. Carotenoids are universal and essential pigments in photosynthesis. Carotenoids act to protect photosynthetic organisms under stress conditions (Hashimoto et al., 2016).

Reactive oxygen species (ROS) are a group of unstable molecules that contain oxygen and easily react with other molecules. Research shows that ROS (OH<sup>-</sup>, O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>) also work as signaling molecules in plants grown in stress (Uarrotta et al., 2016). Stress conditions enhance oxidative damage in food crops (Ahammed et al., 2020a; Sharma et al., 2012). For instance, CAT functions for the reduction of H<sub>2</sub>O<sub>2</sub> (Kasote et al., 2013) and thus reduces cell injury (Gill and Tuteja, 2010; Cheyner et al., 2013). Ascorbate (APX) and guaiacol (POD) both are the key peroxidase in H<sub>2</sub>O<sub>2</sub> detoxification (Uarrotta et al., 2016). In contrast, MDA is a biomarker of oxidative stress and recognized to be a “mutagenic substance” (Giera et al., 2012).

Field pea is a vital leguminous crop due to its high antioxidant, protein and mineral content for the human diet (Wu et al., 2007). Starch, protein, fiber, vitamins, minerals and phytochemicals provide human benefits in peas. Pea contributes to the improvement of gastrointestinal function. Pea protein increase peptides with bioactive compounds, and activities of antioxidant (Tidona et al., 2009). Peas might have significant

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roles in the prevention of diseases. Peas have also been shown to increase anti-carcinogenic activity for human beings (Dahl et al., 2013).

Different food crops, including peas act as a medium for transferring As from irrigated soils and eventually into living organisms through the food chain (Santra et al., 2013; Alam et al., 2020a). Arsenic stress increases ROS (Mishra et al., 2008; Srivastava et al., 2009a, b; Talukdar, 2013) and decreases antioxidant activity in food crops (Srivastava et al., 2009a, b). Human beings experienced the formation of ROS, including peroxy radicals (ROO•), hydrogen peroxide, dimethyl arsenic radical, and dimethyl arsenic peroxy radical due to As stress in the food chain (Jomova et al., 2011). The critical balance between the formation of ROS and the suppressing activity of different antioxidants is distressed, when pea crops experienced As stress (Alam et al., 2011, 2019a; Gousul et al., 2017; Ahammed et al., 2020b).

Arbuscular mycorrhizal fungi, BC, Se, Si-gel and S might have potential effects to improve antioxidant activity in food crops grown in As soil (Alam et al., 2019b, 2020b). AMF can endorse the host plant's resistance to stress by using the regulation of plant physiology (Aroca et al., 2013; Bharti et al., 2013), the appearance of stress-related proteins and genes (Ruiz-Lozano et al., 2012), enriched nutrient and water uptake (Chandrasekaran et al., 2014), and the production of much of external mycelia that increase the soil exploration capacity. As a result, AMF are considered to be a potential tool for increasing phytostabilization efficacy in heavy metal-contaminated soils (Giri et al., 2007). Consequently, AMF increase the activity of superoxide dismutase (SOD), ascorbate peroxidases (APX), catalase (CAT), glutathione peroxidase (GPX), and reduce the levels of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) in food crops grown in soil with elevated stress (Sharma et al., 2017a,b; Yang et al., 2015).

Similarly, BC enhances catalase, and peroxidase activities as well as increases photosynthetic pigments. Biochar also increases gas exchange parameters in leaves of food crops. Furthermore, the application of BC reduces oxidative stress in food crops grown in As soil. Thus, soil amendment with BC validated optimistic results for stabilizing heavy metals in hazardous soil (Rehman et al., 2019). In this perspective, proline, MDA and H<sub>2</sub>O<sub>2</sub> content reduced in food crops grown in soils with stress conditions (Farhangi-Abriz and Torabian, 2017). In contrast, Se, S and Si-gel (Si) reduce MDA content and oxidative damage and osmotic stress in plants grown in heavy metal-contaminated soils (Fariborz et al., 2017; Ahmad and Haddad, 2011; Gill and Tuteja, 2011). Selenium is highly potential to increase bioactive compounds in food crops grown in As soil. Selenium increases biomass production besides improving the photosynthetic pigments and protein content in food crops (Pandey and Gupta, 2015).

Much research has been conducted on the fluctuations of antioxidant and photosynthetic pigments under environmental stress in food crops. However, the effects of soil amendments with BC, AMF, Se, S and Si-gel on photosynthetic pigments and antioxidant enzyme activity in BARI released pea genotypes grown in As soils remain elusive. It is assumed that the use of AMF, Se, S, Si-gel and BC would significantly increase the biomass growth, photosynthetic pigments and antioxidant defense mechanism under As stress in food crops.

## 2. Materials and methods

### 2.1. Experimental soil and seeds

The experimental soil was collected from the As-contaminated crops field in Faridpur district of Bangladesh. The collected soil was silty loam. The experiment with As contaminated soil was conducted in the Department of Environmental Science at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). Collected soil samples were ground homogeneously for sowing of the pea. In background soil (5.058 mg kg<sup>-1</sup>), the concentration of As was increased to 30 mg As kg<sup>-1</sup> soil through adding the required amount of sodium arsenate dibasic heptahydrate (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O) solution with water (Alam et al., 2019a).

BARI Motor1, 2, and 3 pea varieties were collected from the pulse research center in Bangladesh. The average yields of these varieties are 10–14 tons per ha (Digital Herbarium of crop plants, 2020). The total length needed from seed to maturity is about 14 weeks.

### 2.2. Sodium arsenate dibasic heptahydrate, BC, Se, Si-gel, S and other materials

Sawdust and rice husk were collected for the preparation of biochars. Biochars were manufactured using a biochar stove (Mia et al., 2015). The five percent BC was mixed with soil in each treated pot for growing of pea. Nutrients were added from Urea, Triple Super Phosphate (TSP), Muriate of Potash (MOP) and Trichoderma enriched biocompost. Also, pots, and pesticides were collected from the native market in Bangladesh. On the other hand, selenium metal powder (Se) Qualikems-India, silica gel (Si-gel) Loba chemie-India, sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O) Sigma-India, and hydrated ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O), Scharlau, Spain were used in this pot experiment.

### 2.3. Samples preparation for chemical analysis

First soil samples of 250g were collected from each combined with the recommendations of the Bangladesh Agricultural Research Council (BARC, 2012). Samples were ground and sieved with a ≤250 μm mesh and kept in polythene bags with appropriate tagging. Other samples were also prepared for chemical analysis as well as the soil samples. The percentages of total nitrogen (N), phosphorus (P), exchangeable potassium (K), and Organic Carbon (OC) were detected by Kjeldahl method (Jackson, 1973), by Olsen method (Olsen and Sommers, 1982), by Ammonium Acetate Extraction method (Jackson, 1973), and by wet oxidation method (Walkley and Black, 1935) in biofertilizer, soil, and biochars, respectively. Digestion was completed for the detection of total As concentration through the heating block digestion technique (Rahman et al., 2007). The total As in soils, biofertilizer, and biochars samples was analyzed by flow injection hydride generation atomic absorption spectrophotometry (FI-HG-AAS, Perkin Elmer A Analyst 400, USA) using external calibration (Welsch et al., 1990) (Table 1).

### 2.4. Arbuscular mycorrhizal fungi (AMF)

A mixture of AMF in soil and roots were collected from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University (WV), USA. The collected AMF was cultured as a source of AMF with the host plant of Sorghum. AMF content soils (5%) were used in pea crop grown in As soil. Mycorrhizal spores in the soil and vesicle, hyphae, arbuscules in the root samples were observed by the following methods (Gerdemann and Nicolson, 1963; Giovanetti and Mosse, 1980).

### 2.5. Nutrient added in pot soils

Two hundred (200) g of Trichoderma enriched biofertilizers with 4 kg soils were mixed together in each pot. According to the recommendations of the Bangladesh Agricultural Research Institute (BARI), Urea 90 mg, TSP 180 mg, and MOP 70 mg were incorporated into the soil in each pot. At that time, 5 pea seeds of each variety were spread in each pot. The dimension of pots was 10/10 inch and soil depth was 8/8 inch in each pot for growing pea.

### 2.6. Treatments

BARI Motor 1, 2, and 3 pea varieties and ten (10) treatments were included such as, T<sub>1</sub> = rice husk BC, T<sub>2</sub> = saw dust BC, T<sub>3</sub> = AMF, T<sub>4</sub> = selenium (20 mg kg<sup>-1</sup>), T<sub>5</sub> = selenium (30 mg kg<sup>-1</sup>), T<sub>6</sub> = silica-gel (Si) 5 g kg<sup>-1</sup> soil, T<sub>7</sub> = silica-gel (Si) 10 g kg<sup>-1</sup> soil, T<sub>8</sub> = sulfur (S) FeSO<sub>4</sub>·7H<sub>2</sub>O 50 mg kg<sup>-1</sup> (S), T<sub>9</sub> = sulfur (S) FeSO<sub>4</sub>·7H<sub>2</sub>O 100 mg kg<sup>-1</sup> (S), and T<sub>10</sub> =

**Table 1.** Total arsenic (As), percentage of OC, N, P, and K in background soil, trichoderma enriched biofertilizer and biochars.

Samples	Total arsenic (As) in mgkg <sup>-1</sup>	% Organic carbon (OC)	% Nitrogen (N)	% Phosphorus (P)	% Potassium (K)
Trichoderma enriched biofertilizer	0.044	14.50	1.28	1.20	0.87
Background soil	5.058	0.48	0.03	0.0008	0.0016
Saw dust biochar (BC)	0.003	24.60	0.33	0	0.77
Rice husk biochar (BC)	0.024	6.32	0.30	0.10	0.33

control. Five replications were used and the total number of pots was 150 in this pot experiment. The concentration of As in soils was 30 mg kg<sup>-1</sup>. These three pea varieties were also grown in 5.058 mg As kg<sup>-1</sup> background soils in pots. Five replications were also used for a total number of 15 pots in this stage.

### 2.7. Collection of leaves for the analysis of photosynthetic pigments and antioxidants

Leaves of pea varieties were collected for the analysis of photosynthetic pigments and antioxidants. The collected leaves from each treated pot were kept in Ziploc bags with proper labeling. Then leaf samples with ice boxes were brought into the laboratory for the extractions. After extraction of samples were preserved in a (-) 20 °C refrigerator.

### 2.8. Chlorophyll and carotenoids contents

Chlorophyll a, b and total chlorophyll contents of pea leaves were calculated using the following formula: i) Chlorophyll a (mg g<sup>-1</sup> FW) = (0.0127 x A<sub>663</sub>) - (0.00269 x A<sub>645</sub>) (Arnon, 1949); ii) Chlorophyll b (mg g<sup>-1</sup> FW) = (0.0229 x A<sub>645</sub>) - (0.00468 x A<sub>663</sub>) (Arnon, 1949); and iii) Total Chlorophylls (mg g<sup>-1</sup> FW) = (0.0202 x A<sub>645</sub>) + (0.00802 x A<sub>663</sub>) (Arnon, 1949). The carotenoids of pea leaves were determined as per the formula of (mg g<sup>-1</sup> FW) = (1000 x A<sub>470</sub> - 2.270 x Chl. a - 81.4 x chl. b)/227 (Khan et al., 2017).

### 2.9. Proline and malondialdehyde (MDA) contents

The proline content of pea leaves was expressed using the following formula as the fresh weight basis- μmol/g fresh weight = (μg/ml proline x volume of toluene x volume of sulfosalicylic acid)/(0.5 x 115.5), where 115.5 is the molecular weight of proline and 0.5 is the sample weight. The absorbance was recorded at 520 nm (Bates et al., 1973). The MDA content of pea leaves was calculated using an extinction co-efficient (ε) of 155 mM<sup>-1</sup> cm<sup>-1</sup>. The following formula was used for the calculation of MDA content= 3 x 5x (ΔA/) x 2 μmol/gFW. The absorbance of the colored supernatant was taken at 532 nm (Heath and Packer, 1968).

### 2.10. Catalase and peroxidase activity

Catalase activity in pea leaves was estimated by the method of Aebi (1984). The disappearance of H<sub>2</sub>O<sub>2</sub> was monitored by measuring a decrease in absorbance at 240 nm for 2 min. The activity of catalase was calculated by using an extinction coefficient of 40M<sup>-1</sup>cm<sup>-1</sup>.

The activity of ascorbate peroxidase (APX) of pea leaves was determined by the following formula- mM/min/g FW= changes in absorbance/min. x Total volume (ml)/Extinction coefficient x volume of samples (ml). The APX was calculated using an extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup> (Nakano and Asada (1981). The activity of Guaiacol peroxidase (POD) was determined by the following formula- mM/min/g FW= changes in absorbance/min. x Total volume (ml)/Extinction coefficient x volume of samples (ml) (Putter, 1974).

### 2.11. Statistical analysis

Completely Randomized Design (CRD) was followed in this experiment. Analysis of variance (ANOVA) and interaction effect of variety and

treatment on photosynthetic pigments and antioxidants activities under arsenic stress in pea crops were analyzed using R Software (version 3.6.3).

## 3. Results

### 3.1. Chlorophyll

The effects of treatment and interaction result of treatment and varieties on chlorophyll were found to be statistically different ( $p \leq 0.001$ ,  $p \leq 0.05$ ) in 30 mg As kg<sup>-1</sup> soils in BARI Motor 1, 2 and 3 pea varieties (Table 2). In BC, AMF, Se, Si-gel, and S treatments, chlorophyll a and b both were found statistically similar with control. In BARI Motor 1 pea, Rice husk BC and AMF treated total chlorophyll was found higher than that of control. Total chlorophyll was found significantly higher in Se (T<sub>5</sub>) treated BARI motor 2 pea as compared to other treatments. Soil amendments with AMF, Se, Si-gel, S and saw dust BC treated total chlorophyll was found higher than that of control in BARI Motor 3 pea grown in As soil (Table 3). Chlorophyll a and total chlorophyll was increased 31–35% and 60–75% respectively with the treatment of AMF, Se, Si-gel and S (on an average) in pea (Table 3). Chlorophyll a and total chlorophyll in BARI motor 1, 2 and 3 pea varieties were found statistically similar ( $p \leq 0.05$ ) in 5.058 mg As kg<sup>-1</sup> uncontaminated soil. Amendment with BC, AMF, Se, Si-gel and S enhanced total chlorophyll content in pea varieties grown in high As soil (Table 4; Figure 1).

### 3.2. Carotenoid

The effects of treatment and interaction results of treatment and variety on carotenoid were found to be statistically different in pea ( $p \leq 0.001$ ) (Table 2). AMF treated carotenoid was found statistically similar to BC, Se, Si-gel and S treated carotenoid in BARI Motor 1, 2 and 3 pea grown in high As soil. AMF, Se and Si-gel treated carotenoids in BARI Motor 3 were found significantly higher than that of control. Carotenoid in BARI Motor 2 was found statistically higher with the treatment of Si-gel and S than control (Table 3). Carotenoid in leaves of pea varieties was increased 15% by BC, 17% by AMF, 13% by Se, 17% by Si-gel and 14% by S on average (Table 3; Figure 1). Carotenoid content was found similar to AMF treated pea grown in high As soils as compared to uncontaminated soil (Tables 3 and 4). Carotenoid was found to be higher in BARI Motor 1 as compared to other pea varieties in uncontaminated soils (Table 4).

### 3.3. Proline

The interaction effects of treatment and variety on proline content were to be found significantly different ( $p \leq 0.001$ ) in pea (Table 2). Treatments with BC, AMF, Se, Si-gel and S significantly reduced proline in BARI Motor 1 & 2 pea variety grown in As soils. BC and AMF both were found highly effective for the lessening of proline content compared to control in BARI Motor 3 pea (Table 3). Proline was reduced 32 %, 40%, 41%, 26% and 17% by BC, AMF, Se, Si-gel and S treated pea crops (Table 3; Figure 1). Amendment with AMF in pea grown in high As soil produced a similar amount of proline in contrast with uncontaminated soil (Tables 3 and 4).

### 3.4. Catalase

The treatment effect on catalase was found to be statistically ( $p \leq 0.001$ ) different in BARI Motor 1, 2 & 3 peas grown in As soils (Table 2). AMF and S treated catalase activity was found to be significantly higher in BARI Motor 1 pea than that of control. BC treated catalase activity in BARI Motor 1 pea was found to be statistically similar to the treatment of AMF, Se, Si-gel and S treated pea. AMF, Se, Si-gel and S treated catalase activity in BARI Motor 2 pea was found to be significantly higher than that of control. In control, catalase activity was found to be significantly lower as compared to Se treatment in BARI Motor 3 pea (Table 3). Catalase activity was increased 24%, 38%, 40%, 38% and 46% by BC, AMF, Se, Si-gel and S treated pea grown in As soils, respectively (on an average) (Table 3; Figure 1). Catalase activity was found similar to amendment of AMF and Se in pea grown in high As soil in contrast with uncontaminated soil (Tables 3 and 4). On the other hand, catalase activities in BARI Motor1, 2 & 3 pea were found to be statistically similar in background soils (Table 4). BC, AMF, Se, S and Si-gel enhanced the catalase defense activity under As stress in pea.

### 3.5. Malondialdehyde

Interaction effects of treatment and variety on the MDA content was found to be statistically dissimilar ( $p \leq 0.001$ ) in BARI Motor 1, 2 and 3 pea grown in 30 mg As kg<sup>-1</sup> soils (Table 2). BC, AMF, Se, Si-gel and S treated MDA content was found significantly lower in pea varieties as compared to control (Table 3). MDA content was found to be decreased 35%, 59%, 59%, 63% and 48% by BC, AMF, Se, Si-gel and S in pea grown in 30 mg As kg<sup>-1</sup> soil, respectively (Table 3; Figure 1). However, MDA content in pea grown in high As soil with AMF amendment was found 24% higher in contrast with pea grown in uncontaminated soils (Tables 3 and 4). Amendment with AMF showed 59% lower of MDA content in pea grown in high As soil as compared to its control (Table 3). In BARI Motor 1 pea, MDA content was found significantly higher than other pea varieties in background soils with 5 mg As kg<sup>-1</sup> soil (Table 4).

### 3.6. Ascorbate peroxidase (APX)

Interaction results of treatment & variety were found to be significantly different ( $p \leq 0.001$ ) on APX activity in BARI Motor 1, 2 and 3 pea

grown in soils with an As concentration of 30 mg kg<sup>-1</sup> (Table 2). AMF and Se both treated APX were found to be significantly higher than that of control and other treatment in BARI Motor 1 pea. In BARI Motor 2 & 3 pea, APX amended with BC, AMF, and Se were found to be significantly higher than that of control (Table 3). APX content was found to be increased 56%, 38%, and 70%, by BC, AMF and Se in pea grown in 30 mg As kg<sup>-1</sup> soil, respectively (Table 3; Figure 1). Amendment with AMF and Se in pea grown in high As soil showed similar APX activity in contrast with pea grown in uncontaminated soil (Tables 3 and 4). The APX activity was found to be lower in BARI motor 3 pea variety in uncontaminated soil (Table 4).

### 3.7. Guaiacol peroxidase (POD)

The effects of treatment and interaction result of treatment and variety on POD activity in pea variety were found to be statistically dissimilar ( $p \leq 0.001$ ) at 30 mg As kg<sup>-1</sup> soils (Table 2). POD activity amended with AMF, Se and S was found to be significantly higher than that of control in BARI Motor 1, 2 & 3 pea grown in high As soils. Among these treatments, Se was found to be highly effective for increasing the activity of POD in pea crops (Table 3). Amendment with AMF, Se and S showed 84% higher of POD activity in pea grown in high As soils (Table 3&4; Figure 1). The POD activity was found to be significantly higher in BARI motor 2 than other pea varieties in uncontaminated soils (Table 4).

## 4. Discussion

Arsenic is a hazardous substance that inhibits the growth and development of food crops. Soil amendments with BC, AMF, Se, Si-gel, and S enhanced photosynthetic pigments and antioxidant enzyme activity in pea grown in As soil. The stress induced by As can attenuate antioxidant defense and biomass production in food crops (Alam et al., 2019a).

Biochars contained carbons with aromatic rings that show functional groups. This functional group works as an adsorbent for the immobilization of toxic metalloid uptake in food crops grown in contaminated soils (Uchimiya et al., 2013). BC amendment immobilizes As metalloid and enhances microbial activity in food crops grown in high As soil (Vithanage et al., 2017). Literature showed that BC enhances biomass

**Table 2.** ANOVA on changes of photosynthetic pigments and antioxidants enzyme activities in BARI Motor 1, BARI Motor 2 and BARI Motor 3 pea varieties grown in 30 mg As kg<sup>-1</sup> soils.

Chlorophyll a mgg <sup>-1</sup> FW				Chlorophyll b mgg <sup>-1</sup> FW				Total Chlorophyll mgg <sup>-1</sup> FW				
	df	Sum of squares (SS)	Mean sum of square (MSS)	Pr (>F)	df	SS	MSS	Pr (>F)	df	SS	MSS	Pr (>F)
Variety	2	0.00007	3.65e-05	0.0007 ***	2	0.004	0.002	<2.2e-16 ***	2	6.03e-05	3.01e-05	<2.2e-16 ***
Treatment	9	0.0002	2.65e-05	2.10e-06 ***	9	0.02	0.0026	<2.2e-16 ***	9	3.25e-05	3.61e-06	<2.2e-16 ***
Variety: Treatment	18	0.0001	9.11e-06	0.020*	18	0.04	0.0025	<2.2e-16 ***	18	1.35e-04	7.50e-06	<2.2e-16 ***
Residuals	120	0.0005	4.76e-06		120	0.005	0.00004	<2.2e-16 ***	120	1.19e-05	9.95e-08	<2.2e-16 ***
Proline µgg <sup>-1</sup> FW				Catalase (CAT) mM min <sup>-1</sup> g <sup>-1</sup> FW				Carotenoid mgg <sup>-1</sup> FW				
	df	SS	MSS	Pr (>F)	df	SS	MSS	Pr (>F)	df	SS	MSS	Pr (>F)
Variety	2	40.23	20.11	<2.2e-16 ***	2	0.005	0.002	0.0001 ***	2	1.58	0.79	0.0002 ***
Treatment	9	114.85	12.76	<2.2e-16 ***	9	0.04	0.004	<2.2e-16 ***	9	6.90	0.76	5.10e-10 ***
Variety: Treatment	18	62.35	3.46	<2.2e-16 ***	18	0.02	0.001	9.73e-09 ***	18	19.79	1.09	<2.2e-16 ***
Residuals	120	3.54	0.02	<2.2e-16 ***	120	0.03	0.0002	0.0001***	120	10.53	0.08	
Malondialdehyde (MDA) µmole g <sup>-1</sup> FW				Ascorbate peroxidase (APX) mM min <sup>-1</sup> g <sup>-1</sup> FW				Guaiacol Peroxidase (POD) mMmin <sup>-1</sup> g <sup>-1</sup> FW				
	df	SS	MSS	Pr (>F)	df	SS	MSS	Pr (>F)	df	SS	MSS	Pr (>F)
Variety	2	0.022	0.0112	<2.2e-16 ***	2	0.084	0.0422	<2.2e-16 ***	2	0.138	0.069	<2.2e-16 ***
Treatment	9	0.277	0.0308	<2.2e-16 ***	9	0.541	0.0602	<2.2e-16 ***	9	1.313	0.145	<2.2e-16 ***
Variety: Treatment	18	0.191	0.0106	<2.2e-16 ***	18	0.334	0.0185	<2.2e-16 ***	18	0.350	0.019	<2.2e-16 ***
Residuals	120	0.004	0.00003	<2.2e-16 ***	120	0.013	0.0001	<2.2e-16 ***	120	0.007	0.00006	<2.2e-16 ***

(\*\*\*) indicate significant difference at 0.1% ( $p \leq 0.001$ ) level of significance.

(\*) indicate significant difference at 5 % ( $p \leq 0.05$ ) level of significance.

**Table 3.** Effects of BC, AMF, Se, S and Si-gel on photosynthetic pigments and antioxidant activities in pea varieties grown in soils with an As concentration of 30 mg kg<sup>-1</sup>.

Variety	Treatment	Chlorophyll a mgg <sup>-1</sup> FW	Chlorophyll b mgg <sup>-1</sup> FW	Total Chlorophyll mgg <sup>-1</sup> FW	Proline µgg <sup>-1</sup> FW
BARI Motor 1	Rice husk -BC (T <sub>1</sub> )	0.013 ± 0.0005 ab	0.001 ± 3.74e-05 a	0.005 ± 6.63e-05 k	3.75 ± 0.07 bcd
	Saw dust -BC (T <sub>2</sub> )	0.013 ± 0.0003 ab	0.003 ± 4.83e-04 a	0.002 ± 8.60e-05 abcd	5.50 ± 0.04 i
	AMF (T <sub>3</sub> )	0.014 ± 0.0001 b	0.010 ± 2.15e-04 a	0.003 ± 3.74e-05 efghi	4.62 ± 0.07gh
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.015 ± 0.0001 b	0.011 ± 1.12e-04 a	0.002 ± 5.09e-05 bcdef	3.62 ± 0.05 bc
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.015 ± 0.0006 b	0.010 ± 5.83e-05 a	0.002 ± 6.78e-05 cdefg	2.98 ± 0.06a
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.014 ± 0.0003 b	0.013 ± 3.74e-04 a	0.002 ± 3.74e-05 defgh	3.73 ± 0.02bc
	Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.015 ± 0.0005 b	0.013 ± 1.52e-04 a	0.001 ± 2.44e-05 abcd	3.41 ± 0.04 b
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 50 mg kg <sup>-1</sup> (T <sub>8</sub> )	0.015 ± 0.0003 b	0.012 ± 1.11e-04 a	0.002 ± 7.071e-05 abcd	3.83 ± 0.02cde
	Sulfur FeSO <sub>4</sub> . 7H <sub>2</sub> O 100 mg kg <sup>-1</sup> (T <sub>9</sub> )	0.014 ± 0.0003 b	0.012 ± 1.50e-04 a	0.002 ± 5.83e-05 abcd	5.55 ± 0.03i
	control (T <sub>10</sub> )	0.013 ± 0.0001 ab	0.002 ± 1.26e-04 a	0.001 ± 1.16e-04 abcd	6.76 ± 0.25k
BARI Motor 2	Rice husk-BC (T <sub>1</sub> )	0.011 ± 0.0003 ab	0.002 ± 2.42e-04 a	0.002± 2e-05 a	5.49 ± 0.08 i
	Saw dust-BC (T <sub>2</sub> )	0.012 ± 0.001 ab	0.002 ± 1.07e-04 a	0.002 ± 3.16e-05 abcd	4.15 ± 0.03def
	AMF (T <sub>3</sub> )	0.014 ± 0.0014 b	0.003 ± 6.78e-05 a	0.002 ± 1.09e-04 abcd	4.20 ± 0.12ef
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.014 ± 0.0004 b	0.002 ± 7.07e-05 a	0.002 ± 1.319e-04 abcde	4.71 ± 0.02gh
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.014 ± 0.0003 b	0.002 ± 4.47e-05 a	0.004 ± 1.019e-04 j	3.70 ± 0.04 bc
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.015 ± 0.0012 b	0.002 ± 9.27e-05 a	0.002 ± 8.60e-05 defg	6.75 ± 0.01 k
	Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.014 ± 0.0019 b	0.004 ± 1.50e-04 a	0.002 ± 6.78e-05 abcd	6.67 ± 0.02 k
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 50 mg kg <sup>-1</sup> (T <sub>8</sub> )	0.014 ± 0.0007 ab	0.003 ± 1.15e-04 a	0.002 ± 2.57e-04 abcd	6.62 ± 0.02 jk
	Sulfur FeSO <sub>4</sub> . 7H <sub>2</sub> O 100 mg kg <sup>-1</sup> (T <sub>9</sub> )	0.012 ± 0.001 ab	0.003 ± 1.35e-04 a	0.001 ± 1.63e-04 abcd	6.54 ± 0.01jk
	control (T <sub>10</sub> )	0.011 ± 0.0006 ab	0.002 ± 1.36e-04 a	0.001 ± 1.16e-04 ab	7.54 ± 0.03 L
BARI Motor 3	Rice husk-BC (T <sub>1</sub> )	0.013 ± 0.001 ab	0.012 ± 2.84e-04 a	0.0012 ± 1.82e-04a	4.41 ± 0.03 fg
	Saw dust-BC (T <sub>2</sub> )	0.013 ± 0.001 ab	0.004 ± 1.77e-04 a	0.005 ± 8.60e-05 k	4.34 ± 0.03 fg
	AMF (T <sub>3</sub> )	0.013 ± 0.001 ab	0.003 ± 1.16e-04 a	0.004 ± 5.09e-05 ij	3.59 ± 0.10bc
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.012 ± 0.0007 ab	0.127 ± 1.63e-02 b	0.0033 ± 2.50e-04 fghij	4.56 ± 0.08fgh
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.013 ± 0.001 ab	0.005 ± 1.07e-03 a	0.003 ± 1.11e-04 ghij	4.51 ± 0.02fg
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.013 ± 0.0013 ab	0.002 ± 1.59e-04 a	0.004 ± 6.0e-05 ij	4.76 ± 0.01gh
	Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.011 ± 0.0002 ab	0.002 ± 6.78e-05 a	0.003 ± 1.39e-04 hij	4.95 ± 0.01 h
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 50 mg kg <sup>-1</sup> (T <sub>8</sub> )	0.017 ± 0.0002 b	0.003 ± 8.36e-05 a	0.006 ± 9.27e-05 L	5.73 ± 0.02 i
	Sulfur FeSO <sub>4</sub> . 7H <sub>2</sub> O 100 mg kg <sup>-1</sup> (T <sub>9</sub> )	0.014 ± 0.0018 ab	0.002 ± 8.60e-05 a	0.004 ± 4.59e-04 ij	5.86 ± 0.01 ij
	control (T <sub>10</sub> )	0.007 ± 0.0004 a	0.0008 ± 5.09e-05 a	0.001 ± 8.12e-05abc	6.21 ± 0.18 jh

Variety	Treatment	CAT mM min <sup>-1</sup> g <sup>-1</sup> FW	Carotenoid mgg <sup>-1</sup> FW	MDA µmole g <sup>-1</sup> FW	APX mM min <sup>-1</sup> g <sup>-1</sup> FW	POD mMmin <sup>-1</sup> g <sup>-1</sup> FW
BARI Motor 1	Rice husk-BC (T <sub>1</sub> )	0.14 ± 0.004bcdefgh	5.0 ± 0.17 fghi	0.15 ± 0.006 ij	0.21 ± 0.004 efghi	0.03 ± 0.0005 de
	Saw dust-BC (T <sub>2</sub> )	0.13 ± 0.01 abcdef	5.07 ± 0.32 ghi	0.15 ± 0.002 i	0.22 ± 0.005 fghij	0.02 ± 0.0005 cd
	AMF (T <sub>3</sub> )	0.15 ± 0.003 cdefghi	4.65 ± 0.06 cdefgh	0.05 ± 0.0031 bc	0.25 ± 0.003 kl	0.08 ± 0.0010 f
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.14 ± 0.007 bcdefg	4.52 ± 0.15 cdefg	0.10 ± 0.001 efg	0.37 ± 0.003 n	0.15 ± 0.0008 h
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.14 ± 0.014 bcdef	4.32 ± 0.06 bcdef	0.10 ± 0.001 efg	0.19 ± 0.003 de	0.13 ± 0.002 g
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.16 ± 0.006 fghi	4.05 ± 0.02 abcd	0.06 ± 0.00073 cd	0.19 ± 0.004 de	0.02 ± 0.0007 bcd
	Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.13 ± 0.010 abcdef	4.88 ± 0.04 efghi	0.07 ± 0.0008 d	0.16 ± 0.005 bc	0.01 ± 0.001 abc
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 50 mgkg <sup>-1</sup> (T <sub>8</sub> )	0.16 ± 0.007 cdefghi	4.61 ± 0.12 cdefgh	0.11 ± 0.0013 gh	0.16 ± 0.004 bc	0.04 ± 0.0008 e
	SulfurFeSO <sub>4</sub> .7H <sub>2</sub> O100 mgkg <sup>-1</sup> (T <sub>9</sub> )	0.16 ± 0.008 efghi	4.57 ± 0.02 cdefg	0.19 ± 0.002 L	0.11 ± 0.004 a	0.33 ± 0.0005 L
	control (T <sub>10</sub> )	0.10 ± 0.003 ab	4.29 ± 0.13 abcdef	0.21 ± 0.0005 m	0.20 ± 0.002 ef	0.006 ± 0.0007 ab
BARI Motor 2	Rice husk-BC (T <sub>1</sub> )	0.12 ± 0.001 abcd	4.50 ± 0.09 cdefg	0.17 ± 0.003 k	0.36 ± 0.006 n	0.01 ± 0.0008 abcd
	Saw dust -BC (T <sub>2</sub> )	0.13 ± 0.005 abcdef	4.50 ± 0.13 cdefg	0.01 ± 0.00086 a	0.36 ± 0.005 n	0.01 ± 0.0007 abc
	AMF (T <sub>3</sub> )	0.14 ± 0.004 abcdef	4.49 ± 0.26 cdefg	0.07 ± 0.0013 d	0.26 ± 0.005 L	0.09 ± 0.001 f
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.12 ± 0.0007 abc	4.38 ± 0.207cdefg	0.10 ± 0.0013 efg	0.26 ± 0.005 kl	0.18 ± 0.003 i
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.18 ± 0.0005 hi	4.06 ± 0.018 abcd	0.05 ± 0.0007 bc	0.24 ± 0.005 ijk	0.11 ± 0.001 g
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.14 ± 0.003 abcdef	5.09 ± 0.009 ghi	0.06 ± 0.001cd	0.22 ± 0.003 fghi	0.12 ± 0.003 g
	Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.19 ± 0.002 i	4.13 ± 0.01 abcd	0.10 ± 0.003 fg	0.17 ± 0.001 cd	0.13 ± 0.001 g
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 50 mg kg <sup>-1</sup> (T <sub>8</sub> )	0.14 ± 0.01 bcdefgh	5.29 ± 0.04 hi	0.05 ± 0.0008 bc	0.23 ± 0.001 ghij	0.18 ± 0.001 i
	Sulfur FeSO <sub>4</sub> .7H <sub>2</sub> O 100 mgkg <sup>-1</sup> (T <sub>9</sub> )	0.18 ± 0.003 ghi	4.71 ± 0.07 defgh	0.12 ± 0.004 gh	0.20 ± 0.001 efg	0.38 ± 0.002 m
	control (T <sub>10</sub> )	0.10 ± 0.002 a	3.98 ± 0.05 abc	0.16 ± 0.001 jk	0.19 ± 0.004 de	0.002 ± 0.0005 a
BARI Motor 3	Rice husk -BC (T <sub>1</sub> )	0.14 ± 0.013 abcdef	4.12 ± 0.04 abcd	0.09 ± 0.0017 ef	0.31 ± 0.004 m	0.05 ± 0.002 e
	Saw dust-BC (T <sub>2</sub> )	0.11 ± 0.0005 ab	4.20 ± 0.03 abcde	0.22 ± 0.005 n	0.22 ± 0.003 fghi	0.03 ± 0.0006 de
	AMF (T <sub>3</sub> )	0.13 ± 0.003 abcdef	4.67 ± 0.3cdefgh	0.13 ± 0.005 h	0.23 ± 0.008 hijk	0.13 ± 0.014 g
	Selenium 20 mg kg <sup>-1</sup> (T <sub>4</sub> )	0.13 ± 0.011abcdef	4.74 ± 0.08 defgh	0.10 ± 0.001 efg	0.52 ± 0.004°	0.37 ± 0.0009 m
	Selenium 30 mg kg <sup>-1</sup> (T <sub>5</sub> )	0.16 ± 0.0008 efghi	4.66 ± 0.07 cdefgh	0.04 ± 0.0008 b	0.27 ± 0.005 L	0.28 ± 0.0007 k
	Silica gel 5 g kg <sup>-1</sup> soil (T <sub>6</sub> )	0.14 ± 0.013 abcdef	4.13 ± 0.008 abcd	0.05 ± 0.0008 bc	0.19 ± 0.005 de	0.22 ± 0.0029 j
Silica gel 10 g kg <sup>-1</sup> soil (T <sub>7</sub> )	0.12 ± 0.0008 abcde	5.46 ± 0.02 l	0.09 ± 0.0007 e	0.21 ± 0.00006 efg	0.13 ± 0.001 g	

(continued on next page)

Table 3 (continued)

Variety	Treatment	CAT mM min <sup>-1</sup> g <sup>-1</sup> FW	Carotenoid mg g <sup>-1</sup> FW	MDA μmole g <sup>-1</sup> FW	APX mM min <sup>-1</sup> g <sup>-1</sup> FW	POD mMmin <sup>-1</sup> g <sup>-1</sup> FW
	Sulfur FeSO <sub>4</sub> ·7H <sub>2</sub> O 50 mgkg <sup>-1</sup> (T <sub>8</sub> )	0.14 ± 0.005 abcdef	3.69 ± 0.17 ab	0.05 ± 0.0007 bc	0.27 ± 0.0058 L	0.12 ± 0.009 g
	Sulfur FeSO <sub>4</sub> ·7H <sub>2</sub> O 100 mgkg <sup>-1</sup> (T <sub>9</sub> )	0.13 ± 0.007 abcdef	4.25 ± 0.01 abcde	0.10 ± 0.0006 efg	0.25 ± 0.007 jkl	0.23 ± 0.005 j
	control (T <sub>10</sub> )	0.10 ± 0.001ab	3.58 ± 0.04 a	0.25 ± 0.004 <sup>c</sup>	0.14 ± 0.002 b	0.004 ± 0.0004 a

Mean ± SE with different lower case letter(s) indicate significant difference at  $p \leq 0.05$ .

production and reduces oxidative stress in food crops grown in abiotic stress (Hussain et al., 2017; Abbas et al., 2018). BC amendment in food crops grown in metalloid stress enhances photosynthetic pigments and antioxidant activity (Beesley et al., 2013). BC significantly reduces proline and MDA content in rice, mung bean, and wheat crops grown in contaminated soils; however, amendments enhance the activities of CAT, APX and POD (Kanwal et al., 2018; Zhang et al., 2014; Alam et al., 2019a). Similarly, proline and MDA contents were found to be significantly lower in BARI released pea grown in As soil in this study. Antioxidants and photosynthetic pigments with BC amended were higher as compared to control in these pea crops grown in As contaminated soils (Table 3; Figure 1). As a result, the addition of BC reduced reactive oxygen species (ROS) formation and cell membrane peroxidation and also enhance the ascorbate pool in food crops (Quartacci et al., 2017).

Mycorrhizal symbiosis immobilizes As accumulation to different plant tissues through blocking the phosphate transporter (Spagnoletti et al., 2018). AMF maintain P: As ratio in soil and decrease As accumulation in plant tissue. AMF amendment reduced the generation of H<sub>2</sub>O<sub>2</sub> and lipid peroxidation and increased the concentration of the antioxidant molecules (carotenoids, proline, and α-tocopherol) in food crop grown in high As soils. AMF colonization increased the concentrations of Glutathione-S-transferase that facilitated sequestration of As uptake in food crops (Sharma et al., 2017a,b; Caser et al., 2019).

The formation of hyphal network by the AMF with plant roots significantly enhances the access of roots to a large soil surface area (Bowles et al., 2016). Subsequently, AMF increase As sequestration in intraradical hyphae, reducing metalloid uptake by roots through symbiosis association with its host plant (Wu et al., 2006; Evelin and Kapoor, 2014). Due to the symbiotic relation of AMF with food crops, biomass growth and photosynthetic pigments increased significantly (Elahi et al., 2010; Garg and Singla, 2012; Garg et al., 2015; Elhindi and Elgorban, 2017). Conversely, AMF reduces proline, H<sub>2</sub>O<sub>2</sub> toxicity and MDA

content, and increases the activities of CAT, APX and POD in food crops under abiotic stress (Alam et al., 2019a; Sharma et al., 2017a,b). Similarly, AMF inoculation to pea crops grown in As soils provides high amount of chlorophyll and carotenoid, and increases the activities of catalase, APX, and POD. Conversely, MDA and proline content both were found to be lower in pea crops grown in high As soils as compared to the control (Table 3; Figure 1).

Furthermore, AMF-inoculated soil forms more persistent masses and significantly higher extra-radical hyphal mycelium (Syamsiyah et al., 2018). Hyphae of AMF can accelerate the decomposition process of soil organic matter (Paterson et al., 2016). Glomalin-related soil protein (GRSP) is supposed to maintain water holding capacity in soils under stress conditions (Wu et al., 2014; Sharma et al., 2017a,b). As a result, AMF escalate the availability of plant nutrient as well as translocation of different nutrients (Rouphael et al., 2015; Alam et al., 2017a, 2017b; Haque et al., 2018). Consequently, biomass growth as well as photosynthetic pigments and antioxidant activities are increased under abiotic stress in food crops (Ahmad et al., 2018; Das and Sarkar, 2018). Besides, AMF increased the phytostabilization efficiency in food crops grown in heavy metal contaminated soils (Yang et al., 2015; Hoque et al., 2016).

Antagonistic interaction was found between Se and As in rice (*Oryza sativa*) crops. As uptake is reduced significantly in rice crops and biomass production is increased due to the incompatible relations between As and Se (Kaur et al., 2017). Se supplementation retrieved As-induced nutrient shortage. Se decreased H<sub>2</sub>O<sub>2</sub> content, ROS and superoxide radical (O<sub>2</sub><sup>-</sup>) along with cell injury in rice varieties (Pandey and Gupta, 2018). Se acted as an antioxidant, inhibiting lipid peroxidation through increased levels of thiols and glutathione. These results suggest that Se alleviated oxidative stress and reducing As uptake in *Pteris vittata* (Srivastava et al., 2009a, b). In addition, Se can reduce the bio-concentration factor of As in root, shoot and grain that reduce the translocation of As in food crops. This mechanism can be endorsed to the increase of iron plaques outside

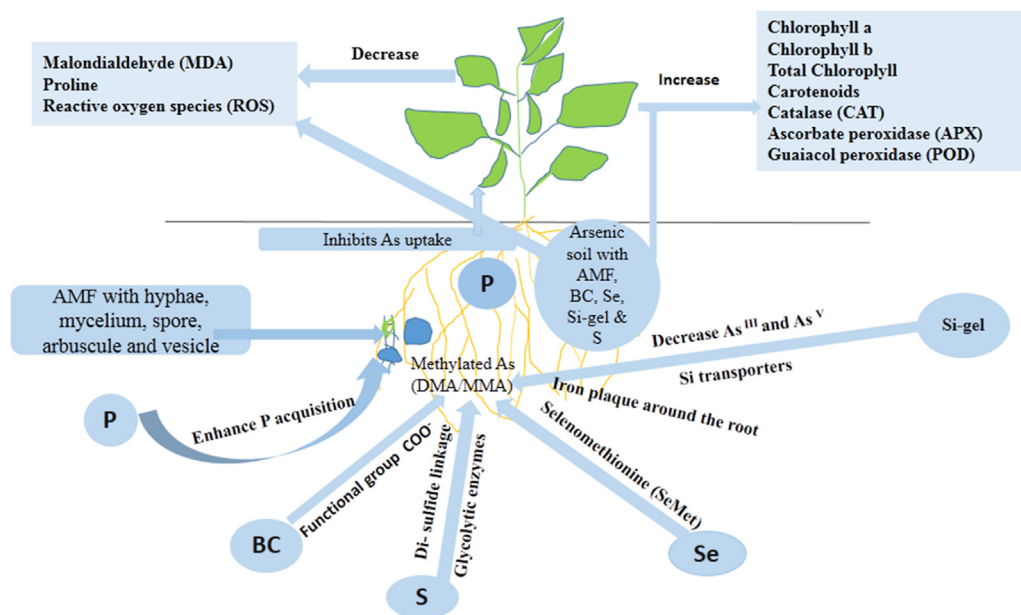


Figure 1. Schematic diagram representing the role of arbuscular mycorrhizal fungi (AMF), selenium (Se), silica (Si-gel), sulfur (S) and biochar (BC) for improving photosynthetic pigments and antioxidant enzyme activity under arsenic stress in pea.

Table 4. Photosynthetic pigments and antioxidant activities in pea grown at back ground As (5.058 mgkg<sup>-1</sup>) soils.

Varieties	Chlorophyll a mgg <sup>-1</sup> FW	Chlorophyll b mgg <sup>-1</sup> FW	Total chlorophyll mgg <sup>-1</sup> FW	Proline μg g <sup>-1</sup> FW	CAT mM min <sup>-1</sup> g <sup>-1</sup> FW	Carotenoid mgg <sup>-1</sup> FW	MDA μ mole g <sup>-1</sup> FW	APX mM min <sup>-1</sup> g <sup>-1</sup> FW	POD mM min <sup>-1</sup> g <sup>-1</sup> FW
BARI Motor 1	0.014 ± 0.002a	0.014 ± 0.0003a	0.002 ± 0.0003a	4.35 ± 0.27a	0.16 ± 0.02a	5.31 ± 0.26 a	0.095 ± 0.0023 a	0.313 ± 0.004 a	0.08 ± 0.011b
BARI Motor 2	0.014 ± 0.002a	0.002 ± 0.0003 b	0.002 ± 0.0003a	3.63 ± 0.11b	0.17 ± 0.02 a	4.15 ± 0.05c	0.044 ± 0.0019 c	0.301 ± 0.010 a	0.12 ± 0.013 a
BARI Motor 3	0.015 ± 0.004a	0.002 ± 0.0003 b	0.002 ± 0.0002a	4.48 ± 0.15a	0.17 ± 0.02a	4.43 ± 0.19b	0.063 ± 0.0037 b	0.169 ± 0.011 b	0.09 ± 0.004 b

Mean ± SE with different lower case letter(s) indicate significant difference at  $p \leq 0.05$ .

the roots, and the glutathione concentration in the leaves. The addition of Se also significantly increased the concentration of selenomethionine (SeMet) in grains. Therefore, Se contained fertilizer can be used to progress the Se nutrition as well as reduce the accumulation of As in grains (Zhou et al., 2017).

Consequently, Se increases antioxidant activities in crops grown-up in abiotic stress. There is evidence that Se reduces abiotic stress with enhancing growth and antioxidant activities in food crops (Hasanuzzaman et al., 2010; Rios et al., 2009). Oxidative stress is increased due to the As stress in crops. The oxidative stress can be substantially reduced with the amendment of Se in food crops (Zhou et al., 2017). For many years, research has been conducted on the role of Se in heavy metal stress in food crops. For instance, Se increases  $\alpha$ -tocopherol and reduces free oxygen species and lipid peroxidation under abiotic stress in crops (Pedrero et al., 2008). Se increased the activity of POD, APX and SOD enzymes in the roots of ryegrass (Cartes et al., 2010). The application of Se in As stress condition in pea crops significantly reduced MDA and proline content and increased the activity of catalase, APX, POD, carotenoids and chlorophyll content (Table 3; Figure 1).

The addition of Si from silica-gel in As soil forms an iron plaque around the root surface that reduces As uptake by plant tissues (Gang et al., 2018). Silicon as a form of silica-gel is considered as a beneficial element for plant growth. It increases biomass and decreases abiotic stress in food crops (Malhotra et al., 2016). Studies found that Si enhanced the activities of photosynthetic pigments and antioxidants such as, chlorophyll, carotenoid, CAT, APX and POD under abiotic stress conditions in wheat crops (Ahmad and Haddad, 2011; Ahammed et al., 2020c). The oxidative stress can be reduced by enhancing the activity of several enzymatic antioxidants like CAT, POD, APX and SOD in food crops under abiotic stress (Gill and Tuteja, 2011; Zhang et al., 2019). Similarly, Si enhanced biomass production as well as improved antioxidant enzyme activity in pea crops grown in As contaminated soils as compared to control. At the same time, proline and MDA content reduced under As stress in pea crops (Table 3; Figure 1). Consequently, in recent years, a significant relation was detected between Si concentration and uptake of inorganic As species in food crops (Tripathi et al., 2013; Sanglard et al., 2014; Bakhat et al., 2017).

Sulfur is involved in di-sulfide linkage in many proteins and plays a crucial role in As detoxification. Glycolytic enzymes play a major role in amino acid biosynthesis that leads to reduce As uptake in plant tissue. Similarly, the supplementation of S reduces the As accumulation in shoot positively skewed thiol metabolism and glycolysis towards amino acid accumulation under As stress in food crops (Dixit et al., 2015). As a result, S restricted the effects of As in food crops with increased antioxidant activities (Mallick et al., 2013). In addition, antioxidant activities increased in rice crops under As stress and reduced As induced ROS (Dixit et al., 2016; ). In this instance, the MDA and proline content also reduced because of the amendment of BC, AMF, Se, S and Si-gel while the antioxidant activity improved, supporting our current observation in pea crops grown in As-free background soils (Tables 3 and 4; Figure 1). It can be recommended that endophytic fungi and their practical approach might be significant for sustainable food and agriculture (Rai et al., 2014). The decontamination of heavy metals over endophytic fungi decreases H<sub>2</sub>O<sub>2</sub> content and enhances the activities of CAT, peroxidases, and chlorophyll content in food crops (Mallick et al., 2014; Alam et al., 2019c). Soil amendments with AMF, BC, and Se are considerably important for the decline of oxidative damage, osmotic stress and cell injury to food crops grown in As soils.

## 5. Conclusions

Arsenic accumulation in food crops induced oxidative stress. Consequently, reduced antioxidant enzyme activities along with decreased biomass growth in food crops. Soil amendments with AMF, Se, BC, Si-gel and S in pea grown in As-soil reduced ROS and enhanced the antioxidant enzyme activity. Among these soil amendments, AMF, Se and Si-gel were

found to be significantly increased chlorophyll, carotenoids, CAT, and peroxidase activities in pea. In contrast, proline and MDA (lipid peroxidation) contents were found to be reduced significantly in pea in comparison to the control. Catalase activity was increased 24–46% by BC, AMF, Se, Si-gel and S treated pea grown in 30 mg As kg<sup>-1</sup> soils, respectively. In contrast, MDA content was found to be decreased through soil amendments with BC, AMF, Se, Si-gel and S treated pea grown in As soil. BARI motor 2 produced lower proline and MDA as compared to BARI motor 1 and 3 pea grown in uncontaminated soils. It is concluded that Se, and AMF both were found extremely effective for increasing antioxidant enzyme activities along with reducing ROS levels in As-stressed pea. It is recommended that the choice of pea variety and soil amendments with AMF and Se have great potential for improving antioxidant enzyme activity of pea grown in As-contaminated field soil.

## Declarations

### Author contribution statement

Mohammad Zahangeer Alam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lynne Carpenter-Boggs: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md. Anamul Hoque, Golam Jalal Ahammed: Performed the experiments; Analyzed and interpreted the data.

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### Data availability statement

Data included in article.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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