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EFFECT OF FOLIAR SPRAY OF ASCORBIC ACID ON NODULATION, GAS EXCHANGE ATTRIBUTES AND MINERAL ION CONTENTS OF *PISUM SATIVUM* UNDER ZINC STRESS

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ABSTRACT

Heavy metal toxicity is one of the major abiotic stresses leading to hazardous effects in plants. Ascorbic acid is regarded as one of the most effective antioxidants present in plants. A pot experiment was conducted to determine the effect of foliar spray of ascorbic acid on nodulation, gas exchange attributes, and mineral ion contents of pea (*Pisum sativum* L.) cultivars under zinc stress. Seeds of two pea cultivars cv. 2001-40 and cv. Meteor were sown in pots following a completely randomized design (CRD) with three replicates. Treatments of $ZnSO_4.7H_2O$ (5 and 10 mg kg-1) were applied through the rooting medium whereas ascorbic acid (5 ppm and 10 ppm) as foliar sprays after three weeks of germination. Results revealed that zinc stress reduced the growth, gas exchange attributes, potassium and calcium contents of roots and shoots of both the pea cultivars. However, foliar spray of ascorbic acid counteracted the adverse effect of zinc stress on the growth of both pea cultivars. The application of 10 mg kg-1 ascorbic acid proved to be more effective in inducing zinc stress tolerance as compared to other levels. Growth and gas exchange attributes of zinc-stressed plants of both cultivars were also enhanced due to foliar applied ascorbic acid which was positively associated with mineral ion contents. Overall, foliar-applied ascorbic acid ameliorated the adverse effect of zinc stress on nodulation and growth attributes of pea cultivars.

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INTRODUCTION

Present world is facing a main ecological problem of heavy metal pollution. Heavy metals are a very heterogeneous group of elements which considerably change in their chemical properties and biological functions (Siddika et al., 2013). Heavy metal accumulation in cultivated soils has greater concerns about food security and resultant hostile effects on human health. An extreme level of heavy metals in soils can have a detrimental influence on seed germination, root growth, root morphology and biomass production. It can also inhibit the activities of various enzymes correlated to normal metabolic processes (Vijayarengan and Mahalakshmi, 2013).

Zinc is a vital nutrient for the growth and development of plants because it acts as a microelement for the living organisms. It also acts as a cofactor for numerous enzymes such as dehydrogenase, anhydrases, oxidases, and peroxidase and plays an active role in plants via regulating the nitrogen uptake, cell partition, photosynthesis, and auxin synthesis. During seed formation, it helps in the consumption of nitrogen and phosphorous. It is also the main constituent of nucleic acids and protein synthesis (Gyana and Premananda, 2003).

Zinc plays an important role in numerous processes such as fertilization, photosynthesis, synthesis of protein, cell multiplication, and cell membrane stability. It is a vital metal and elementary component of numerous proteins and enzymes which are compulsory for the plants growth and development. It also gives advantages to plumule and radicle growth during seed germination (Salehi and Shahadneghad, 2014). Low concentration of zinc is a structural alleviating aspect of DNA-linking proteins and cell membrane and it plays a considerable role in gene expression at DNA transcription level (Farshad et al., 2013).

Greater concentration of zinc in soil leads to growth inhibition. The indications of zinc stress are limiting of shoot, death of leaf tips, twisting and rolling of immature leaves and chlorosis. It was detected that zinc stress severely damaged the root system commonly in root blunt, and also decline takes place in cell elongation and cell division (Rout et al., 2003). Excess quantity of zinc changes the physiological processes of plants due to this reason it generates toxicity in plants. The restraints of zinc injuriousness vary according to plant species, genotype, and growth stage (Elena et al., 2013).

Ascorbic acids ($C_6H_8O_6$) the main source of vitamin C is a small and water-soluble antioxidant molecule. In plants, ascorbic acid is a product of D-glucose metabolism. It is mostly present in all plant tissues, typically in photosynthetic cells, meristems, and fruits. Chloroplast comprises nearly 30 to 40% of the total ascorbic acid and stromal concentration up to 50 Mm has been perceived. The great quantity of ascorbic acid is present in the mature leaf, which have fully developed chloroplasts and greater chlorophyll concentration (Mohsen et al., 2014). It plays a significant role in numerous processes for example regulation of cell division, cell wall enlargement, photosynthesis, senescence and flowering. It helps as a vital co-factor in the development of many plant hormones, such as abscisic acid (ABA), gibberellic acid (GA) and ethylene. It contributes in nodulation and nitrogen fixation and stimulates the construction of nucleic acid contents mainly of RNA. It is a chief constituent of antioxidant defense system in plants to decontaminate ROS below numerous kinds of stresses like heavy metal, saline, ozone stress (Mangrio et al., 2020). It also plays an important role in the plant defense system to defend metabolic processes against derivatives of free radicals (El-Bassiouny et al., 2014). Thus, foliar use of ascorbic acid recovers salinity tolerance of plants in a number of ways (Popy et al., 2016). Exogenously applied ascorbic acid improved the seed sprouting and fresh mass of plants under deficiency of water, it reserved water level in plants (Noman et al., 2015).

Pea (*Pisum sativum*) belongs to family Fabaceae. It is a cool season crop grown in numerous areas of the world in winter to early summer. Its production is greater when it is cultivated in moderate areas as associated to tropical areas. The lowermost temperature for the germination of pea plants is 1-6°C and it sustains its growth at low temperature up to -5°C. It can grow in many soils, the greater yield can be attained in moist, clay-loam, deep slightly acid (pH 6.5-7.0) soils. Vegetative growth is improved in productive and moist soil (Duzdemir et al., 2009). Pea is the third main and significant legume grain in the world after soybean and common bean. Its growing areas are numerous tropical and subtropical countries including Pakistan, India, Burma, Columbia, Ethiopia, Peru, Morocco and Ecuador (Ashraf et al., 2015).

It is chiefly cultivated for its edible seeds and also for cattle feed. It has been economically cultivated for the canning manufacturing and usually for fresh fruit or dry grain. The crop is usually grown on residual moisture under rain fed environment and proficiencies common abiotic stress of variable intensity and duration throughout growth period (Hanan, 2014). Its pods have comparatively large amount of carbohydrates and protein. It is believed to be as one of the most significant sources of man diet worldwide (Hussein et al., 2006). It is a rich source of protein, sugars, carbohydrates, amino acids, vitamins C and A, phosphorus and calcium, with a small amount of iron. Pea comprises 24.6% protein as associate to wheat that comprises 9.4%. Pea is helpful for curing blood sugar illnesses since it retains the blood sugar levels (Farhana et al., 2014).

From the earlier revealed information, it is evident that ascorbic acid plays a significant role in the regulation of a number of metabolic processes in plants exposed to heavy metal stress. However, evidence on how ascorbic acid regulates physiological/biochemical processes in pea plants exposed to zinc stress is not much available in the literature. Therefore, the main objective of the present study was to detect whether the hostile effects of zinc stress on pea plants could be alleviated by exogenous application of ascorbic acid as a foliar spray and how far it regulates the plant growth and development.

MATERIALS AND METHODS

The experiment was carried out in the Botanical Garden at University of Agriculture Faisalabad, during the winter season 2016. Seeds of pea were obtained from Oil Seed Department, Ayub Agriculture Research Institute, Faisalabad. A pot experiment was conducted to assess the exogenously applied ascorbic acid on nodulation and growth attributes of two pea cultivars (2001-40 and Meteor) under Zn stress. Twelve seeds of cultivar 2001-40 and Meteor were sown in plastic pots (20 cm diameter and 24 cm depth) comprising 9 kg dry soil on 14 November, 2016. Sufficient amount of compost was also mixed in the soil to make it well airy and suitable for pea growth. The experiment was arranged in a completely randomized design with three replications. After germination the plants were thinned to maintain seven seedlings in each pot.

Treatments

Treatments of $ZnSO_4.7H_2O$ (5 ppm and 10 ppm) through the rooting medium and ascorbic acid (5 ppm and 10 ppm) as foliar spray were applied after three weeks of germination. Optimum irrigation was supplied according to the need of plants.

Harvesting and measurement of nodule growth attributes

The plants were harvested after ten days of treatments. For each treatment, two plants were up-rooted carefully; rinsed with distilled water and the numbers of nodules of per plant were calculated with common observation and mean values were recorded. Fresh weights of nodules were determined immediately after uprooting the plants with the help of an electrical balance and mean values were calculated. The dry weights of nodules were recorded after drying them in an oven at 75^{o}C for three days to get constant dry weight. Their dry weights were taken in grams and means values were calculated.

Gas exchange parameters

A portable, open Infra-Red Gas Analyzer (IRGA), Model LCA-4 ADC, Analytical Development Company, Hoddeson, England) was used to determine CO_2 assimilation rate (*A*), transpiration rate (*E*), water use efficiency (*A/E*), stomatal conductance (*gs*) and sub-stomatal or intercellular CO_2 (*Ci*) of the youngest fully developed leaf (usually 2nd leaf from top) of each plant. To ensure uniform light and temperature conditions for all the plants, the measurements were made in December, 2016 during 11:00 a.m. to 1:00 p.m. at ambient temperatures ranging from 22°C to 27°C.

Determination of mineral ions

Mineral ions were determined following the Allen and Rae (1986) method with slight modifications. The dried leaves and roots were crushed and placed in digestion flasks containing 2 mL sulphuric acid for one day. On next day, the material was heated until the loss of fumes at 250^{o}C. 0.5 ml of H_2O_2 (35%) was poured after cooling to decolorize the material. The extract was diluted up to 50 mL by using distilled water. Impurities were removed by filtration and Na^+, K^+ and $Ca2^+$ ions were determined by using flame photometer (Model PFPI-7, Jenway, UK). The amount of zinc was determined by using Atomic Absorption spectrophotometer.

Statistical analysis

The data were statistically analyzed using COSTAT computer software.

RESULTS

Number of nodules/plant, fresh and dry weight (g) of nodules

Imposition of zinc stress reduced the number of nodules/plant, nodule fresh and dry weights of both pea cultivars (Figure 1a, b and c). However, exogenous application of ascorbic acid as a foliar spray improved the reduced number of nodule/plant, nodule fresh and dry weights of both pea cultivars. Foliar spray with 5 or 10 ppm ascorbic acid enhanced the reduced number of nodule/plant, nodule fresh and dry weights of both pea cultivars under non-stress or zinc stress conditions. However, in non-stressed plants of cultivar 2001-40 foliar spray with 10 ppm ascorbic acid caused a maximum increase in nodule growth attributes.

Photosynthetic rate

Zinc stress caused a significant reduction in photosynthetic rate of both the cultivars (Figure 2). Both the pea cultivars did not differ significantly in photosynthetic rate under non-stress or zinc stress conditions. Although exogenous application of both the levels of ascorbic acid at all growth stages significantly enhanced the photosynthetic rate of both cultivars under control or zinc stress conditions, 10 ppm ascorbic acid showed to be more effective in improving photosynthetic rate in both cultivars.

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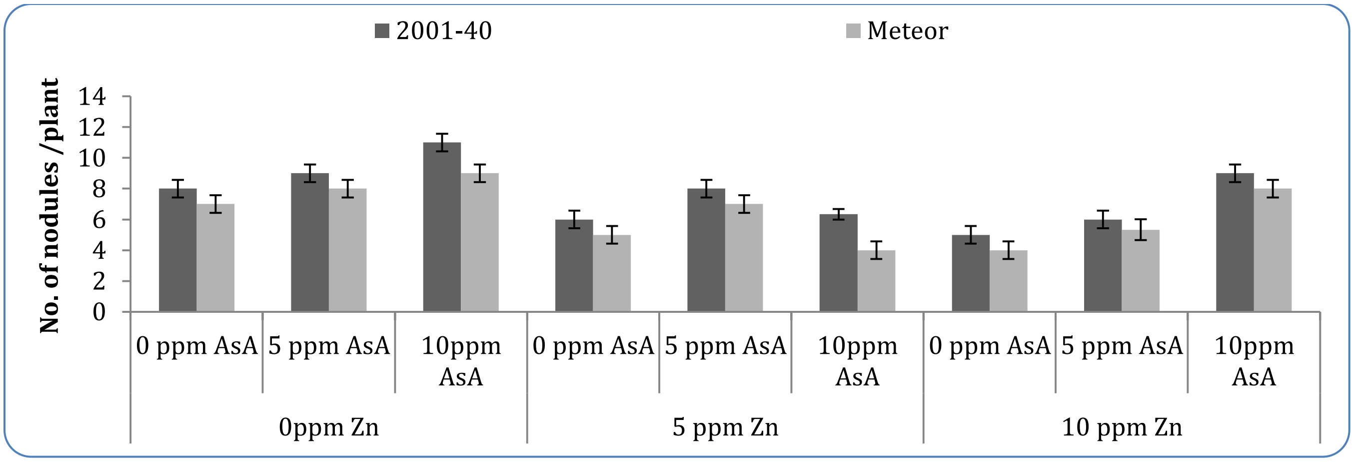

Figure 1a: Effect of various levels of ascorbic acid and zinc on number of nodules/plant of two pea (2001-40 and Meteor) cultivars.

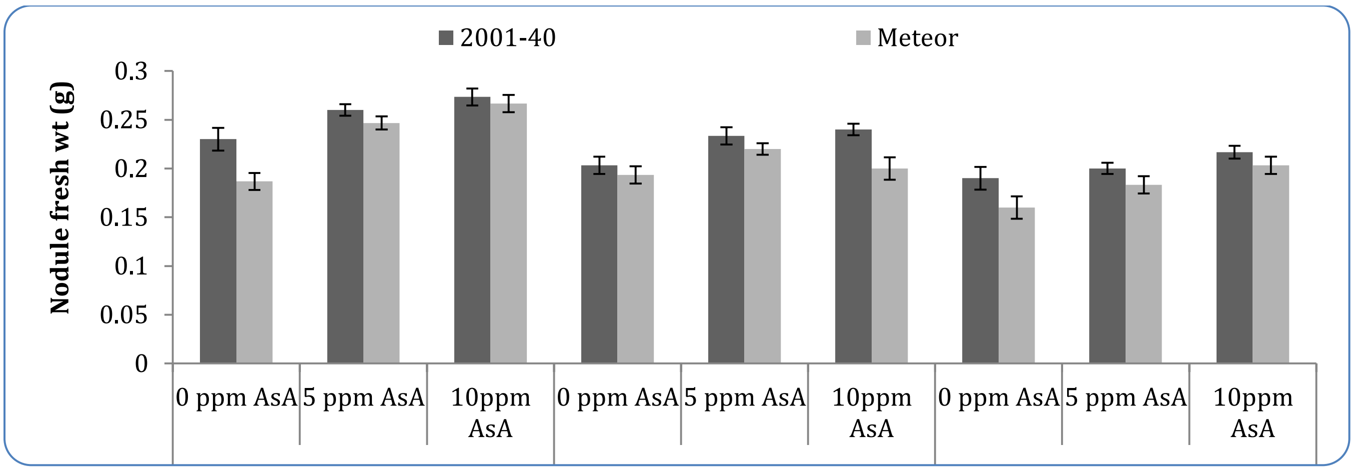

Figure 1b: Effect of various levels of ascorbic acid and zinc on fresh weight of nodule of two pea (2001-40 and Meteor) cultivars.

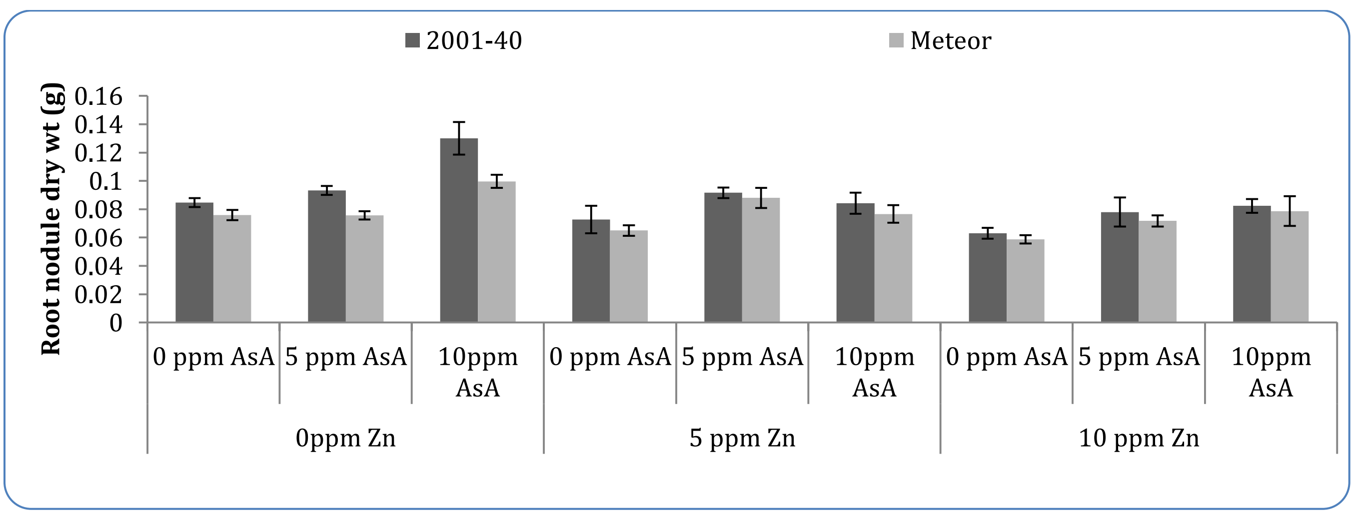

Figure 1c: Effect of various levels of ascorbic acid and zinc on dry weight of nodule of two pea (2001-40 and Meteor) cultivars.

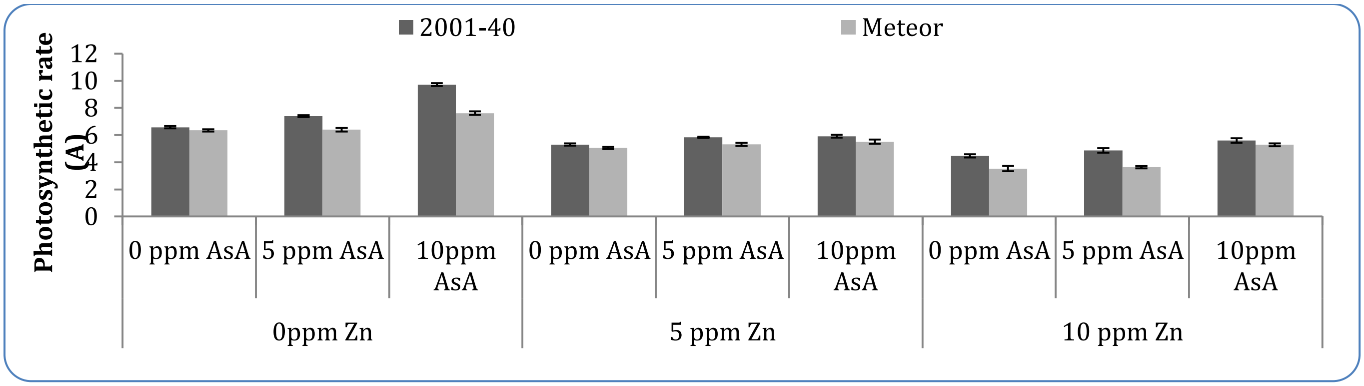

Figure 2: Effect of various levels of ascorbic acid and zinc on photosynthetic rate of two pea (2001-40 and Meteor) cultivars.

Transpiration rate

Transpiration rate of both cultivars was markedly suppressed due to zinc stress (Figure 3). Both cultivars of pea did not vary in transpiration rate under zinc stress conditions. But, externally applied ascorbic acid increased the transpiration rate of zinc stressed plants of both cultivars.

Stomatal conductance

A marked reduction in stomatal conductance in both pea cultivars was observed due to zinc stress (Figure 4). Both the cultivars did not differ in the gas exchange characteristic. On the other hand, the foliar spray of ascorbic acid significantly improved stomatal conductance under stressed and non-stressed environments, 10 ppm ascorbic was more effective in enhancing stomatal conductance in zinc stressed plants of both cultivars.

Sub-Stomatal CO_2 concentration

Imposition of zinc stress significantly reduced the sub-stomatal CO_2 concentration of both pea cultivars (Figure 5). However, foliar applied ascorbic acid pointedly improved the internal CO_2 concentration in both pea cultivars.

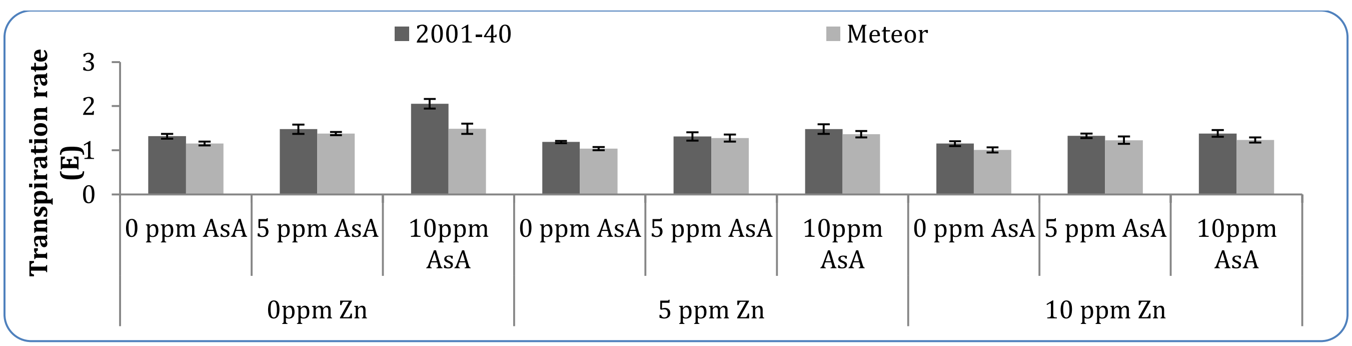

Figure 3: Effect of various levels of ascorbic acid and zinc on transpiration rate of two pea (2001-40 and Meteor) cultivars.

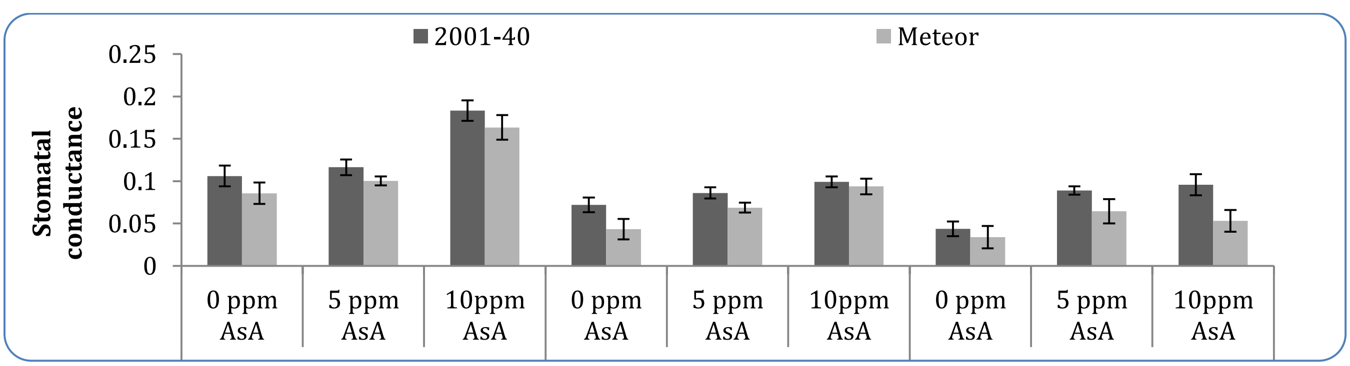

Figure 4: Effect of various levels of ascorbic acid and zinc on stomatal conductance of two pea (2001-40 and Meteor) cultivars.

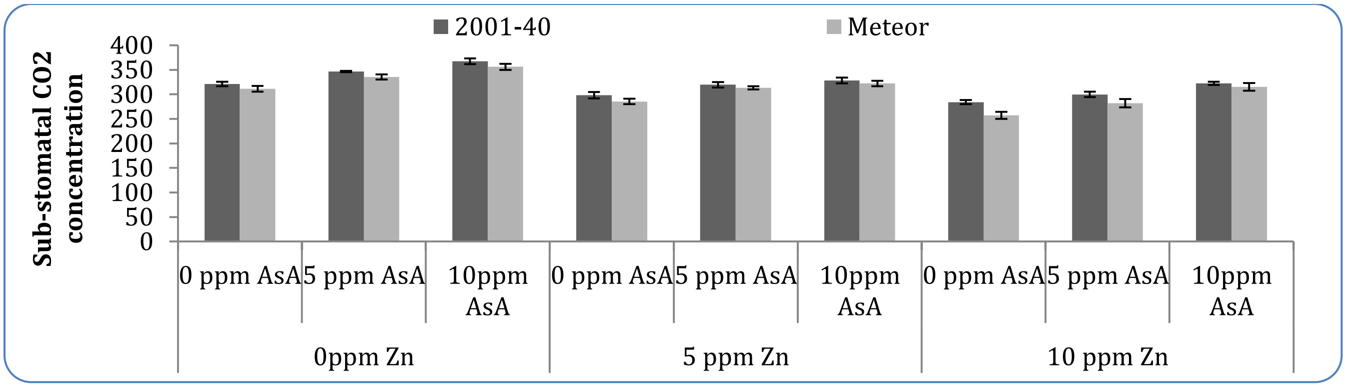

Figure 5: Effect of various levels of ascorbic acid and zinc on sub- stomatal CO_2 concentration of two pea (2001-40 and Meteor) cultivars.

Water use efficiency

Although water use efficiency of both the pea cultivars (Figure 6) was significantly reduced due to zinc stress, foliar applied ascorbic acid improved WUE in zinc stressed plants of both cultivars.

Root and shoot sodium contents

Imposition of zinc stress caused a significant increase in root and shoot sodium contents of both the pea cultivars (figure 7a and 7b). Foliar spray of ascorbic acid at 10 ppm minimizes the damaging effects of zinc in zinc stressed plants of both cultivars.

Root and shoot potassium contents

A marked reduction was observed in root and shoot potassium contents of both the pea cultivars under zinc stress (Figure 8a and 8b). Damaging effects of zinc were decreased when ascorbic acid was applied as foliar spray. Maximum increase in root potassium content was observed in both cultivars at a level of 10 ppm of ascorbic acid.

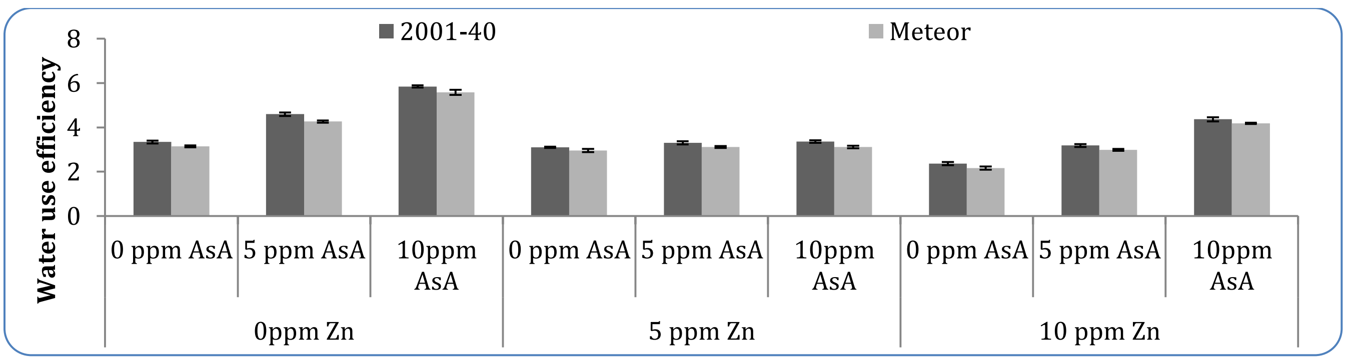

Figure 6: Effect of various levels of ascorbic acid and zinc on water use efficiency of two pea (2001-40 and Meteor) cultivars.

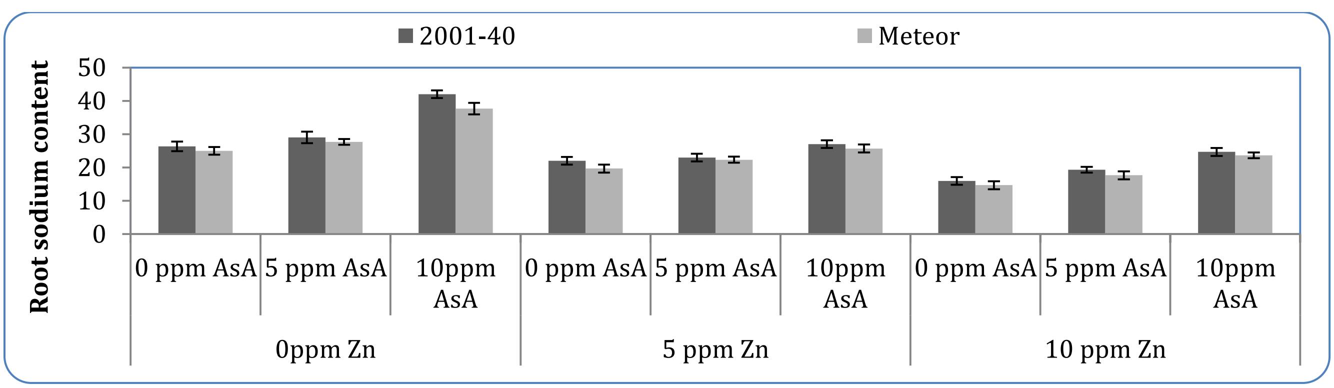

Figure 7a: Effect of various levels of ascorbic acid and zinc on root sodium content of two pea (2001-40 and Meteor) cultivars.

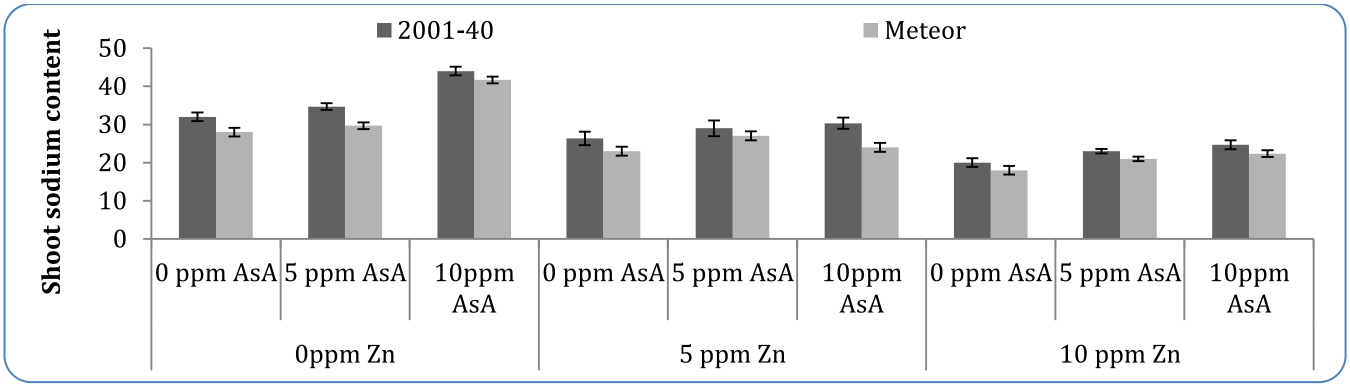

Figure 7b: Effect of various levels of ascorbic acid and zinc on shoot sodium content of two pea (2001-40 and Meteor) cultivars.

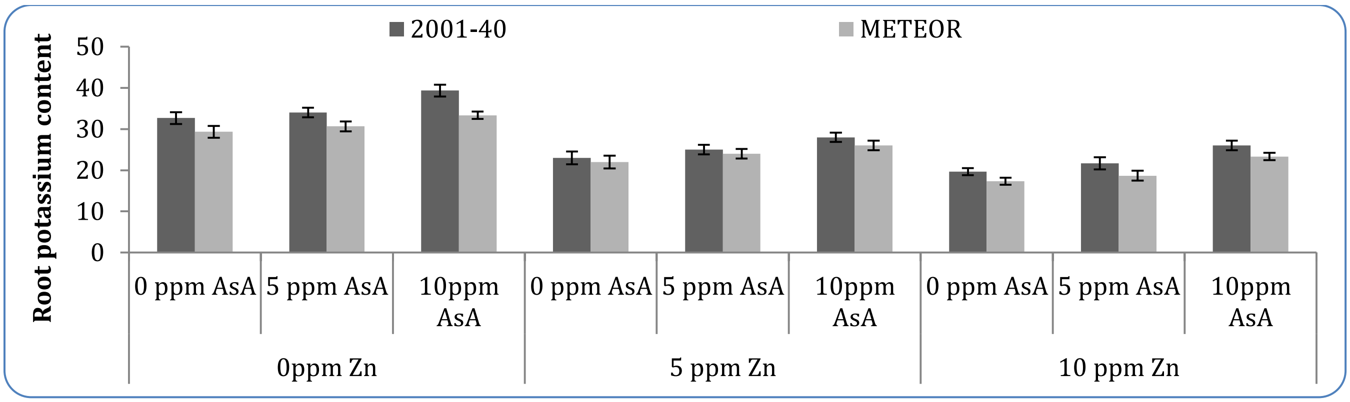

Figure 8a: Effect of various levels of ascorbic acid and zinc on root potassium content of two pea (2001-40 and Meteor) cultivars.

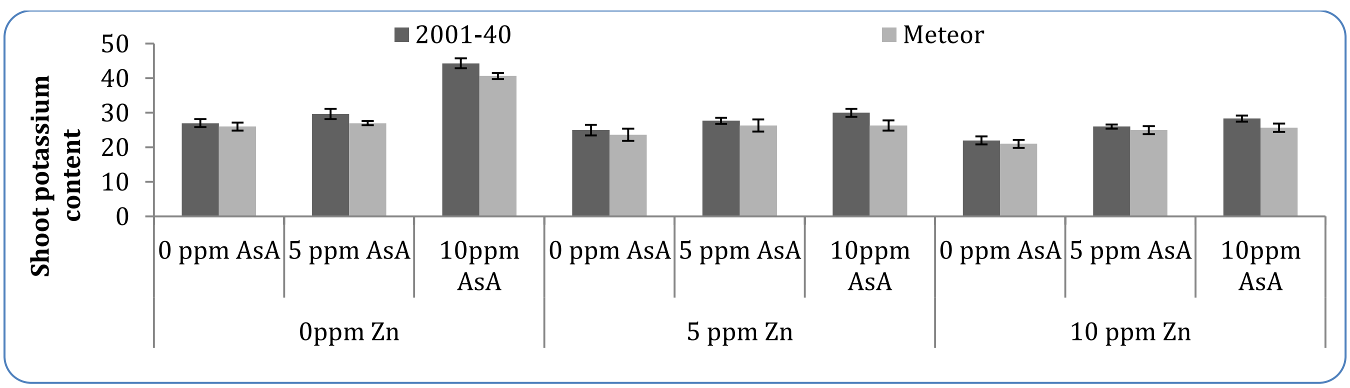

Figure 8b: Effect of various levels of ascorbic acid and zinc on shoot potassium content of two pea (2001-40 and Meteor) cultivars.

Root and shoot calcium content

Zinc stress decreased the calcium contents of root and shoot of both the pea cultivars (Figure 9a and 9b). Ascorbic acid reduced the destructive effects of zinc when it was applied as foliar spray. Maximum increase in shoot potassium content was noted at concentration of 10 ppm of ascorbic acid in both cultivars. Intermediate root calcium content was observed in the combined treatment of zinc and ascorbic acid (5 ppm AsA, 5 ppm Zn and 10 ppm AsA, 5 ppm Zn).

Root and shoot zinc contents

Zinc contents of root and shoot of both cultivars were markedly increased due to zinc stress (Figure 10a and 10b). Ascorbic acid overcomes the injurious effects of zinc when it was applied as foliar spray. Intermediate root and shoot zinc content was observed in the combined treatment of zinc and ascorbic acid (5 ppm AsA, 5 ppm Zn and 10 ppm AsA, 5 ppm Zn). The cultivar 2001-40 showed greater root zinc content as compared to meteor.

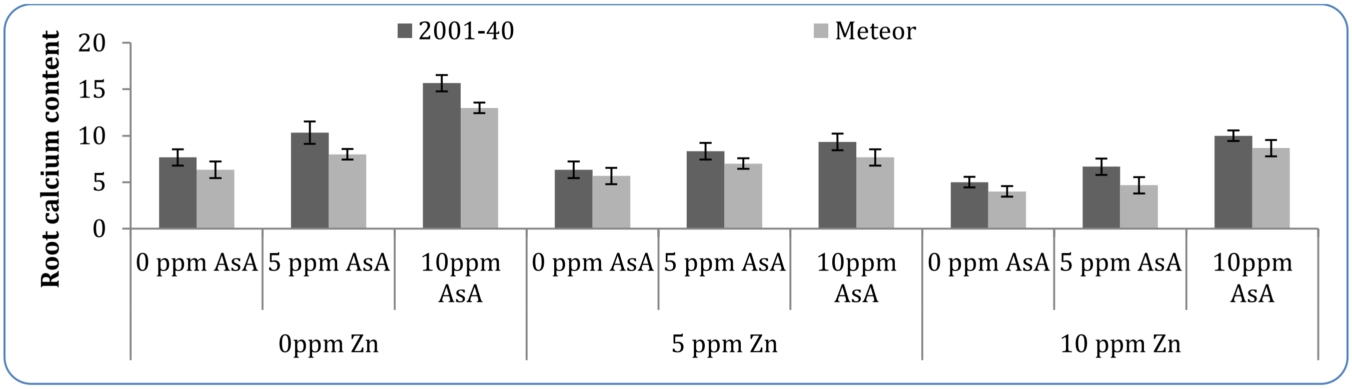

Figure 9a: Effect of various levels of ascorbic acid and zinc on root calcium content of two pea (2001-40 and Meteor) cultivars.

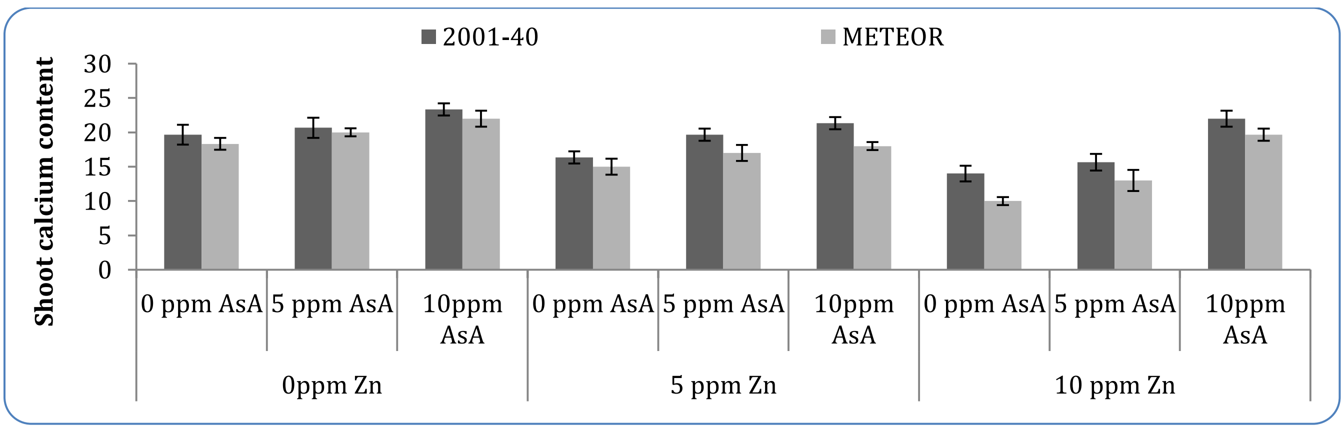

Figure 9b: Effect of various levels of ascorbic acid and zinc on shoot calcium content of two pea (2001-40 and Meteor) cultivars.

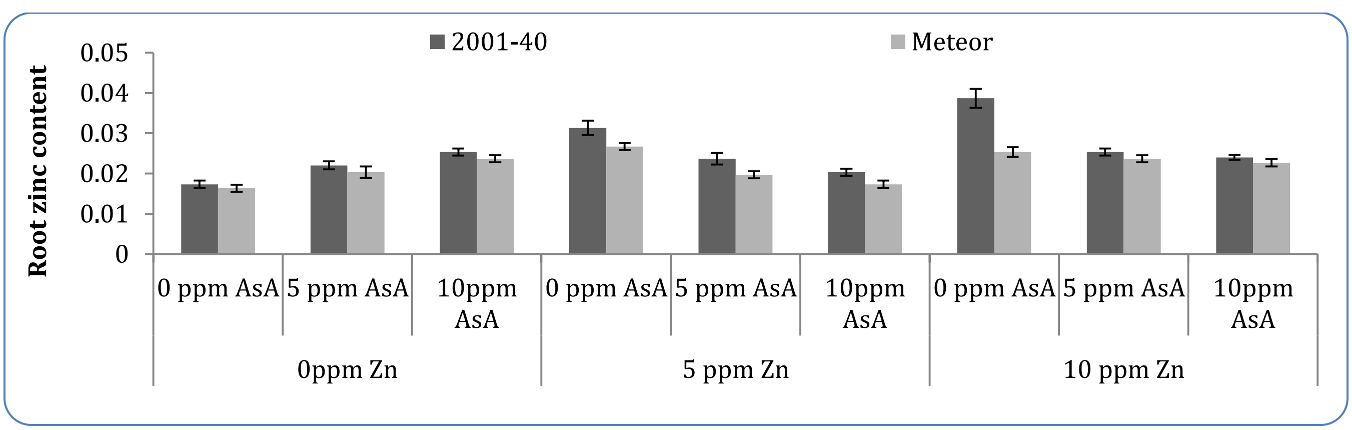

Figure 10a: Effect of various levels of ascorbic acid and zinc on root zinc content of two pea (2001-40 and Meteor) cultivars.

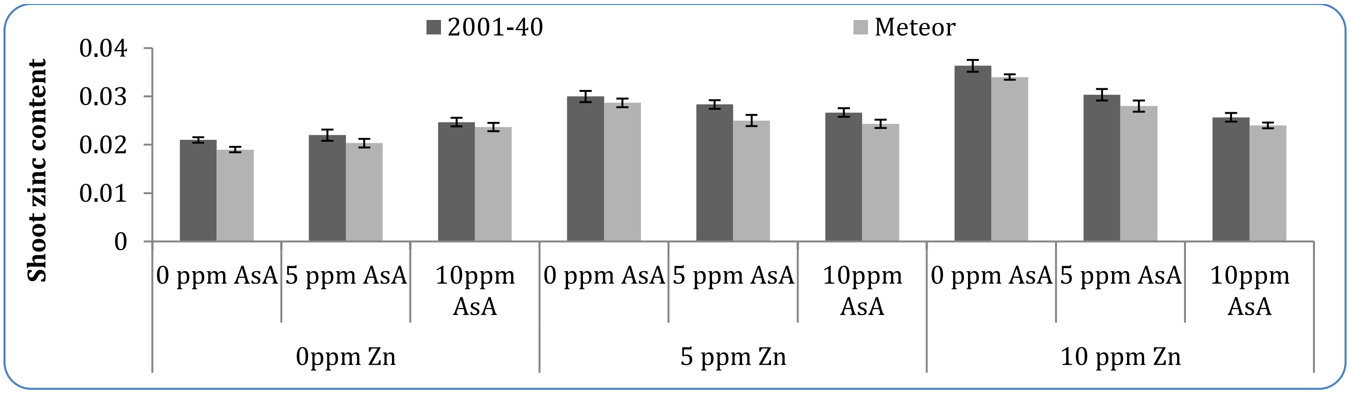

Figure 10b: Effect of various levels of ascorbic acid and zinc on shoot zinc content of two pea (2001-40 and Meteor) cultivars.

DISCUSSION

There is limited existing literature regarding the effect of foliar application of ascorbic acid on pea plant under zinc stress. So, the present study was planned to check the response of growth, physiological and nodulation attributes of pea plants under zinc stress.

In the study, number of nodules per plant and fresh and dry weight of nodules decreased under zinc stress. Our results are parallel with some other studies in which significant growth inhibition was reported in *Phaseolus vulgaris* and pea plants under zinc stress (Romero-Puertas et al., 2004; Van Assche et al., 1998). Zinc toxicity in plants limited the growth of both root and shoot (Choi et al., 1996). However, foliar applied ascorbic acid significantly improved the nodulation attributes i.e. number of nodules per plant and fresh and dry weight of nodules of both the pea cultivars under zinc stress and these findings are in accordance with those of Ewais (2003) who reported that application of ascorbic acid improved growth of broad bean plants.

In the present study, gas exchange attributes such as net photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal conductance and water use efficiency were reduced under zinc stress. Other researchers also found similar decrease in pea plants (Doncheva et al., 2001; Mohsen et al., 2014). Contrarily, foliar spray of ascorbic acid improved the gas exchange attributes under zinc stress. The present findings agree with those obtained by Smirnoff (2000) and Pastori et al. (2003).

In this study, mineral ion contents such as calcium and potassium were reduced due to deleterious effect of zinc stress as well as the contents of sodium and zinc were enhanced. But foliar application of ascorbic acid improved the calcium and potassium contents of root and shoot. Similar results were reported by Sheteawi (2007).

CONCLUSION

Through the discussion it can be accomplished that heavy metal stress caused reduction in growth and can be ameliorated by foliar application of ascorbic acid. Quite simply ASA possibly used as any growth regulator to improve plant heavy metal threshold. Nevertheless, amount and time of exogenous application must be developed with further analysis distinctly for each and every crop.

AUTHORS' CONTRIBUTION

MAA, MS, AA, and MW designed, planned and prepared layout of the study, MAA and MS conducted greenhouse experiments and recorded the data, MAA, MS and AI compiled and organized the data, MAA, MS and MU analyzed the data, MU made the graphs, all the authors helped in manuscript write up and formatting and MAA, MS, AA and MU proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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