Pak. J. Bot., 48(5): 1763-1774, 2016.

EFFECT OF PLANT-DERIVED SMOKE SOLUTIONS ONPHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF MAIZE (*ZEA MAYS* L.) UNDER SALT STRESS

M.A. WAHEED[1*], MUHAMMAD JAMIL[2], MUHAMMAD DAUD KHAN[2], SHAKIRULLAH KHAN SHAKIR[1], SHAFIQ-UR-REHMAN[1]

[1]Department of Botany, Kohat University of Sciences and Technology, 26000, KPK, Pakistan
[2]Department of Biotechnology & Genetic Engineering, Kohat University of Sciences and Technology Kohat, 26000, KPK, Pakistan
[*]Corresponding author's email: waheed_dawar@yahoo.com

Abstract

Among abiotic stresses, salinity is an important factor reducing crop yield. Plant-derived smoke solutions have been used as growth promoters since last two decades. The present study was conducted to investigate the effect of *Cymbopogon jwaracusa* smoke extracts (1:100 and 1:400) on physiological and biochemical aspects of maize (*Zea mays* L.) under salt stress (100, 150, 200 and 250 mM). Results showed that seed germination percentage was improved up to 93% with smoke as compared to control (70%), while seedling vigor in term of root and shoot fresh weights and dry weights were also significantly increased in seeds primed with smoke extracts. Similarly, in case of alleviating solutions, there occurred a significant alleviation in the adverse effects of salt solutions when mixed smoke in all studied end points. Application of smoke solution has also increased the level of K^+ and Ca^{+2} while reduced the level of Na^+ content in maize. In addition, the levels of photosynthetic pigments, total nitrogen and protein contents were also alleviated with the application of smoke as compared to salt. There occurred an increase in the activities of Anti-oxidant in response of salt stress but overcome with the smoke application. It can be concluded that plant-derived smoke solution has the potential to alleviate the phytotoxic effects of saline condition and can increased the productivity in plants.

Key words: Smoke, Salt stress, Cymbopogon, *Zea mays*, Germination, Growth, Biomasses.

Introduction

Salinity is affecting crop production around the globe particularly in arid and semi-arid areas. Approximately, 800 Mha of world is effected by salinity (Munns, 2005). Salt stress affect uptake and accumulation of minerals by creating osmotic stress, Ca^{+2} and K^+ imbalance and also cause Na^+ and Cl^- ions induced phytotoxocity (Grattan & Grieve, 1992; Munns, 2002, Munns *et al*., 2006). Plant extracted smoke has been proved to be used in agricultural practices for the improvements of crops productivity (Light & Van Staden, 2004). First of all De Lange & Boucher (1990) reported that smoke extract has the potential to improve germination and overall plant growth in seedlings (Tigabu *et al*., 2007; Dixon *et al*., 2009). Smoke act like plant growth hormones and also interact with plant growth regulators (PGRs) that promote cell cycle, thus speed up the radical emergence which ultimately increase the seed germination (van Staden *et al*., 2000; Jain & van Staden, 2006). Butenolide, a compound found in plant extracted smoke have been shown to increase the seedling growth in various plants (Sparg *et al*., 2006; Kulkarni *et al*., 2007; Kulkarni *et al*., 2006). As butenolide present in plant smoke can trigger plant growth and development which is controlled by PGRs that indicates its interaction with PGRs (Van Staden *et al*., 2000).

Maize (*Zea mays* L.) is an important cereal crops equally important both for animals as well as human being containing high value of food and oil. World increasing population demands an increase in areas for crops cultivation to raise food production (Anon., 2009). To increasing cropping area it is necessary to utilize the saline soil through improved agronomic practices, by the use of salt tolerant varieties and salt stress alleviating agents like smoke extracts (Allen *et al*., 1983; Mehdi & Ahsan, 2000). Seed priming is a well-known seed invigorative technique in which seeds are partially hydrated until germination related process starts (Bradford, 1976). Primed seeds shows improvement in germination rate, uniformity and percent germination (Basra *et al*., 2005). Seed priming inhibit adverse effect of various stresses by improving germination rate, reduction in germination time and better seedling growth (Farooq *et al*., 2007).

Reactive oxygen species (ROS) production increased in plant in response to abiotic and biotic stresses (Asada, 1999). The level of ROS increase in plants exposed to salt stress along with the reduction in growth and development (Rodrıguez *et al*., 2004). Antioxidants, produce in plant to control the ROS concentration (Vanacker *et al*., 1998). Smoke priming enhance the plant growth reduced by salinity. Therefore present investigation aimed to investigate the effects of smoke extracts on maize physiology and biochemistry and to study the effect of priming with smoke extracts on alleviation of salt (NaCl) stress.

Materials and Methods

Seed source: Seeds of maize (Cv. Azam) were obtained from Agricultural research station Sara-e-Nawrang, Bannu, Pakistan.

Preparation of smoke solutions: Aqueous smoke solution of *Cymbopogon jwarancusa* was prepared by the following the method of De Lange & Boucher (1990) with slight modification. For this purpose, 333 g of dried plant material was burnt in a furnace. Plant smoke produced in this way was passed and collected in 1 L water in beaker. The process was continued until the plant material was fully burnt. The smoke solutions thus prepared were filtered and stored at 4°C (Thornton *et al*., 1999).

Smoke dilutions: The concentrated smoke solutions were further diluted to 1:100 and 1:400 (v/v) and were used in experiments.

Germination experiments: Seed were primed with different smoke dilutions (1:100, 1:400) for the duration of 8 h and then incubated in Petri plates for germination. In order to assess the smoke effect on seed germination and seedling vigor, each treatment was moistened with 10 ml of respective solutions i.e., control (Distilled water), 100, 150, 200 and 250 mM of NaCl. Experiments were replicated thrice and placed 10 seeds in each replica for germination. The Petri dishes were incubated at room temperature and were examined for germination at 24 h interval for regular three days (72 h). For the determination of further parameters, the experiments were harvested after10 days and the root/shoot length and fresh/dry weights were measured. Their dry weights were taken after keeping them in an oven for 24 h, at 80°C.

Biochemical analysis: The dried plant samples were chopped in a grinder completely to get the powder and were used for further biochemical analysis.

Determination of ions (Na^+, K^+ and Ca^{+2}): Ion analysis were determined by using Awan & Salim (1997) method with lightly modified way. Plant dried material (25 mg) was digested with the addition of sulphuric acid and hydrogen peroxide in 2:1 ratio in a small beaker followed by heating for 15 minutes. After digestion, added 20 mL of distilled water and after shaking, got filtrate for determination of ions through flame photometer.

Photosynthetic pigment concentration: Chlorophyll and carotenoids were determined by using a method given by Lichtenthaler & Welburn (1985). To determine total chlorophyll concentration, 25 mg of dry plant material and 25 mg of Magnesium oxide were mixed in test tube. Magnesium oxide neutralizes the acid present in plant samples and prevents the formation of pheophytin α[127]. After that, methanol up to 5 mL was added to the test tube containing mixture, which was then homogenized on shaker for 2 hours. After 2 hours of homogenization, turbid pigment extract (methanol extract) was transferred to centrifuge tube. Samples ware centrifuge at 4000 rpm for 5 minutes at room temperature. For the determination of optical densities, the supernatant from the pigments extracts were analyzed by using the UV-VIS spectrophotometer calibrated at 3 different wavelengths i.e., 470, 653 and 666.

Determination of protein, total soluble protein and total nitrogen contents: For the determination of protein and nitrogen contents, the micro-Kjeldahl technique was used. The method consist of three steps i.e., digestion, distillation and titration. Plant samples of 100 mg, 1 gm digestion mixture and 2 mL concentrated H_2SO_4 were mixed and heated to became colorless solution. The nitrogen in the samples was transformed to ammonium sulphate through sulphuric acid (H_2SO_4 and the ammonia emitted during this process absorbed in the boric acid solution and thus its amount can be calculated in the samples which is further used for the determination of protein and nitrogen contents). Similarly, to determine the contents of total soluble protein (TSP), the method of Bradford (1990) was used.

Proline and enzymes and antioxidants analysis: Proline contents was calculated by following the method of Bates *et al*. (1973) with modification. Lipid peroxidation was analyzed in term of malondialdehyde (MDA) by using the method of Zhou & Leul (1998) while Hydrogen peroxide (H_2O_2) were analyzed by following the method of Velikova *et al*. (2000).

The method of (Giannopolitis & Ries, 1977) was used to investigate the level of Superoxide dismutase (SOD). Likewise, for the measurement of Ascorbate peroxidase (APX) activity, assay prescribed by Nakano & Asada (1981) was followed. Catalase (CAT) activity was determined by following the assay of Aebi (1984) while level of Peroxidase (POD) was measured as described by Zhou & Leul (1999) with slight changes.

Statistical analysis: For application of statistics on data, statistics 9 software was used. For this purpose, experimental data was examined by using analysis of variance (ANOVA) followed by standard deviation (SD) and $p \leq 0.05$ was considered criteria for significance relationship among the treatments.

Results

Physiological parameters

Seed germination: Significant differences were observed in germination of maize seeds exposed to various salinity levels, were subjected to smoke priming and hydro-primed. Decrease in germination percentage was recorded with increase in salinity; however seeds primed with smoke solutions showed overall high germination percentage than control. Maximum seed germination in control was found 70%, while reduction was observed with the increased level of salinity 100 to 250 mM. Among smoke dilutions, maximum germination of 93% was found with cymbopogon (1:400) while saline conditions resulted reduction in germination as 74, 70 and 66% with 100, 150 and 200 mM, respectively (Fig. 1.1).

Plant growth and development: Overall growth in maize seedlings were affected when seedlings were exposed different levels of salt stress. Increase in salt concentrations caused gradual reduction in lengths of seedling (roots and shoots). In smoke solution of 1:400 resulted shoot length of 3.08 cm while 0.6 cm in 250 mM salt stress shows that salt stress reduced the growth while can alleviate the stress. All smoke primed plantlets showed increased seedling growth in non-saline and saline primed conditions as compared to hydro primed treatments (Fig. 1.2).

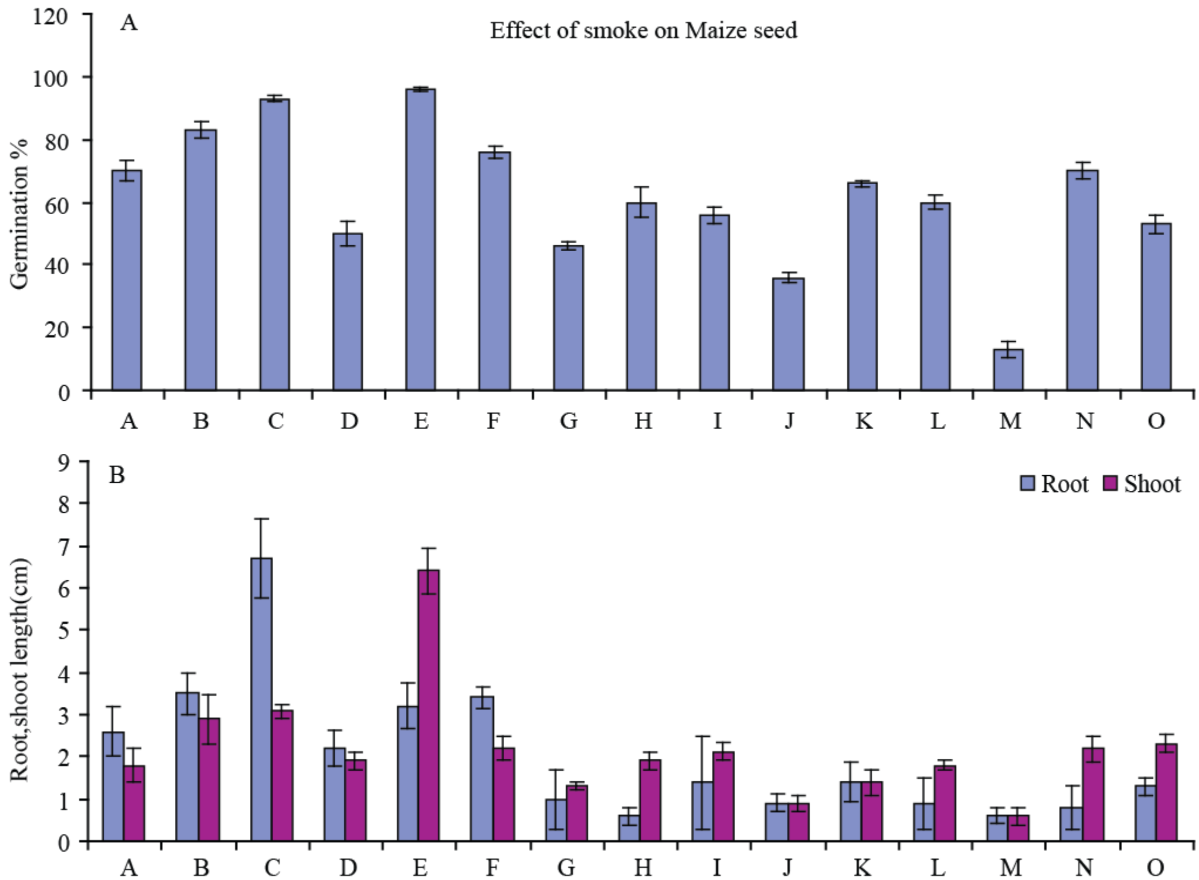


Fig. 1.1, 1.2. Effect on Effect ongermination % and root/shoot length in maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).

Root and shoot fresh and dry weight: With increase NaCl concentrations, a decrease in biomass production was found. Phytotoxicity caused by salt stress found dose dependent i.e., higher concentrations caused more toxicity than lower levels. Maximum shoot fresh weight of 4.16 g was observed in (100+150) dilution while minimum (0.15 g) at 250 mM as compared to control 0.216 g. Dry biomass (root and shoot dry weights) also showed gradual reduction with increase in salt stress. Maximum root dry weight was found in 150+400 (2.65g) as compare to control which was 0.099 g. With the increases of salinity level root dry weight decreases 0.13, 0.11, 0.09 and 0.07g respectively in 100, 150, 200 and 250 mM, respectively while in shoot dry weight maximum dry weight were found in 150+100 (2.8 g) as compare to control (0.154 g). Minimum shoot dry weight (0.052 g) was found in 250 mM (Fig. 1.3).

Ions (Na^+, K^+ and Ca^{+2}) analysis: Significant differences were found in ion analysis of plants raised under different treatments i.e., control, smoke, saline and alleviated solutions. Decreases were observed in K^+ and Ca^{+2} contents while increase in Na^2+ contents with increased level of salt (Fig. 1.4). Ion contents analysis showed that seedlings root and leaves raised under smoke dilutions, have higher K^+ and Ca^{+2} and low Na^+ as compared to control i.e., 20 µg/g in root and 17.8 µg/g in leaves of smoke treatment of (1:100). In case of root analysis high K^+ (47.56 ug g^{-1}), Ca^{+2} (38.65 ug g^{-1}) and low Na^+ (18.8 ug g^{-1}) were found in case of (1:400) dilution treatment compared to control i.e., K^+ (41.5), Ca^{+2} (33.91) and Na^+ (31.33 ug g^{-1}). Results showed that smoke extracts alleviated significantly the adverse effect of saline condition. Ions level in leaves found lower i.e., K^+ (12.4), Ca^{+2} (12.1) and high Na^+ (34.3 ug g^{-1}) when exposed to 250 mM of salts level which was significantly alleviated i.e., K^+ (18.16), Ca^{+2}(18.26) and Na^+ (26.7 ug g^{-1}) by the addition of smoke extracts of 1:100 and 1:400.

Analysis chlorophyll *a*, *b* and total carotenoids: Salt stress have also significantly reduced the contents of chlorophyll *a*, *b*. Smoke treatments improved the Chl *a* and *b* contents as compared to control while salt solutions of 100, 150, 200 and 250 mM resulted reduction in the contents. In control the Chl *a* and *b* contents were found 4.14 and 10.76 ug g-1 respectively, while salt solutions of 100, 150, 200 and 250 mM resulted reduction in Chl *a* i.e., 3.69, 3.04, 2.2, and 1.35 ugg^{-1} and in Chl *b* i.e., 8.26, 7.85, 5.83 and 3.39 ugg^{-1} respectively. Contrary to these, smoke applications of 1:100 and 1:400, improved Chl *a* (11.8 and 14 ugg^{-1}) and Chl *b* (7.26 and 6.04 ugg^{-1}), respectively. Similarly, smoke solution alleviated the stress posed by salt and resulted increase in Chl *a* and *b* (Fig. 1.5).

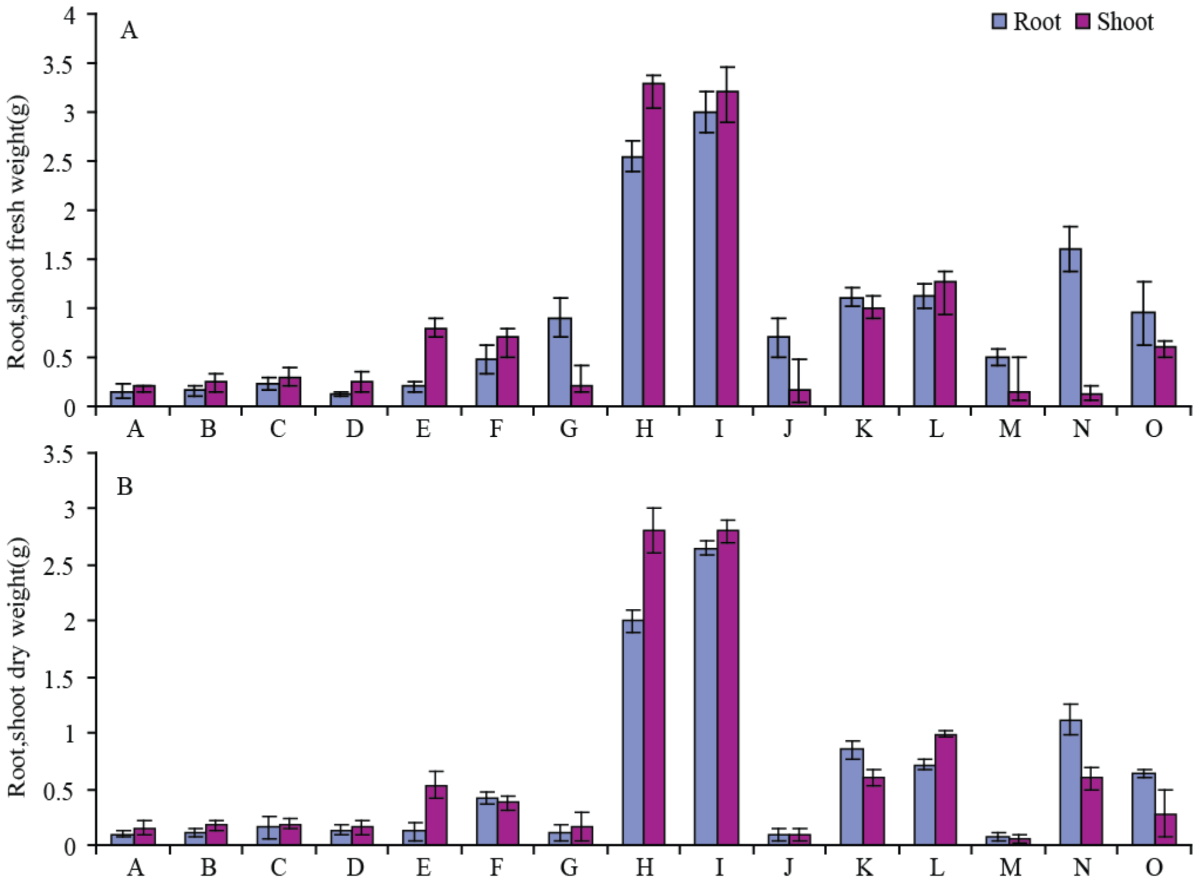


Fig. 1.3. Effect on root and shoot dry weight (A) and fresh weight (B) of maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).

Response of carotenoids contents to salt as well as alleviating solution found same as in the case of Chl *a* and *b*. application of smoke dilutions increased the carotenoids contents significantly i.e., 703 ugg^{-1} (1:100) and 655.35 ugg^{-1} (1:400) as compared to control 542.48 ugg^{-1}. Beside these, salt solutions decrease the contents with increase concentrations i.e., 0-250 mM and lowest was observed when subjected to 250 mM (194.35 ugg^{-1}). Application of alleviating solutions of smoke (1:100 and salt, resulted improvement in the contents as compared to salt solution only.

Analysis of nitrogen and protein contents: Biochemical characterization of both nitrogen as well as protein contents in maize were analyzed for various treatments i.e., control, smoke, salt and alleviating solutions. Results showed a remarkable decreased in both the parameters with increased level of salt. Saline condition drastically effect both the Nitrogen and Protein contents in roots and leaves i.e., 61.63, 44.82, 38.01 and 21.2µg g^{-1} for 100, 150, 200 and 250mM salt in root while 70.5, 57.42, 42.02 and 25.21 µg g^{-1} for 100, 150, 200 and 250mM salt in leaves nitrogen contents as compared to control 98.4 and 120.46 g g^{-1} respectively. Also 385.18, 280.12, 237.56 and 132.5 µg g^{-1} for 100, 150, 200 and 250 mM salt in root while 440.625, 358.8, 262.62 and 157.56 µg g^{-1} for 100, 150, 200 and 250mM salt in leaves protein contents as compared to control 612.75 and 752.87 µg g^{-1} respectively (Fig. 1.6).

Plants raised under smoke treatments showed significantly increase both the contents of nitrogen and protein. Highest level of both the contents was observed in smoke of 1:400 dilution which were 120.46 and 162.09 µg g^{-1} in root and leaves for nitrogen as compared to control 98.4 and 120.46 while 780.87 and 1013.06 µg g^{-1} in root and leaves for protein as compared to control i.e., 612.75 and 752.87 µg g^{-1}, respectively. Results indicate that plant derived smoke solutions also alleviated the inhibitory effects of saline condition. Decrease in nitrogen contents caused by salt 250 mM alleviated by 1:400+250 mM i.e., (21.2) to (32 µg g^{-1}) in root and (25.21) to (44.04) in leaves.

Analysis of proline contents: Analysis of proline contents revealed a gradual increase with increasing concentration of salt. While smoke dilution reduced the proline levels i.e., 1:100 (0.417) and 1:400(0.381 ug g^{-1}) as compared to control i.e., 0.372 ug g^{-1} (Fig: 1.7).

MDA and H_2O_2 activities: In order to estimate the influence of saline condition on lipid peroxidation, MDA contents in maize were determined (Fig. 1.8). The obtained results indicated that plants respond differently to different treatments of smoke and salt. Smoke dilution of 1:400 and 1:100 showed 111.21 and 94.96% of MDA level, respectively. Similarly, alleviating solutions of 150+400 (157.45%) and 200+400 (141.31%) were recorded in the treatments as compared to control.

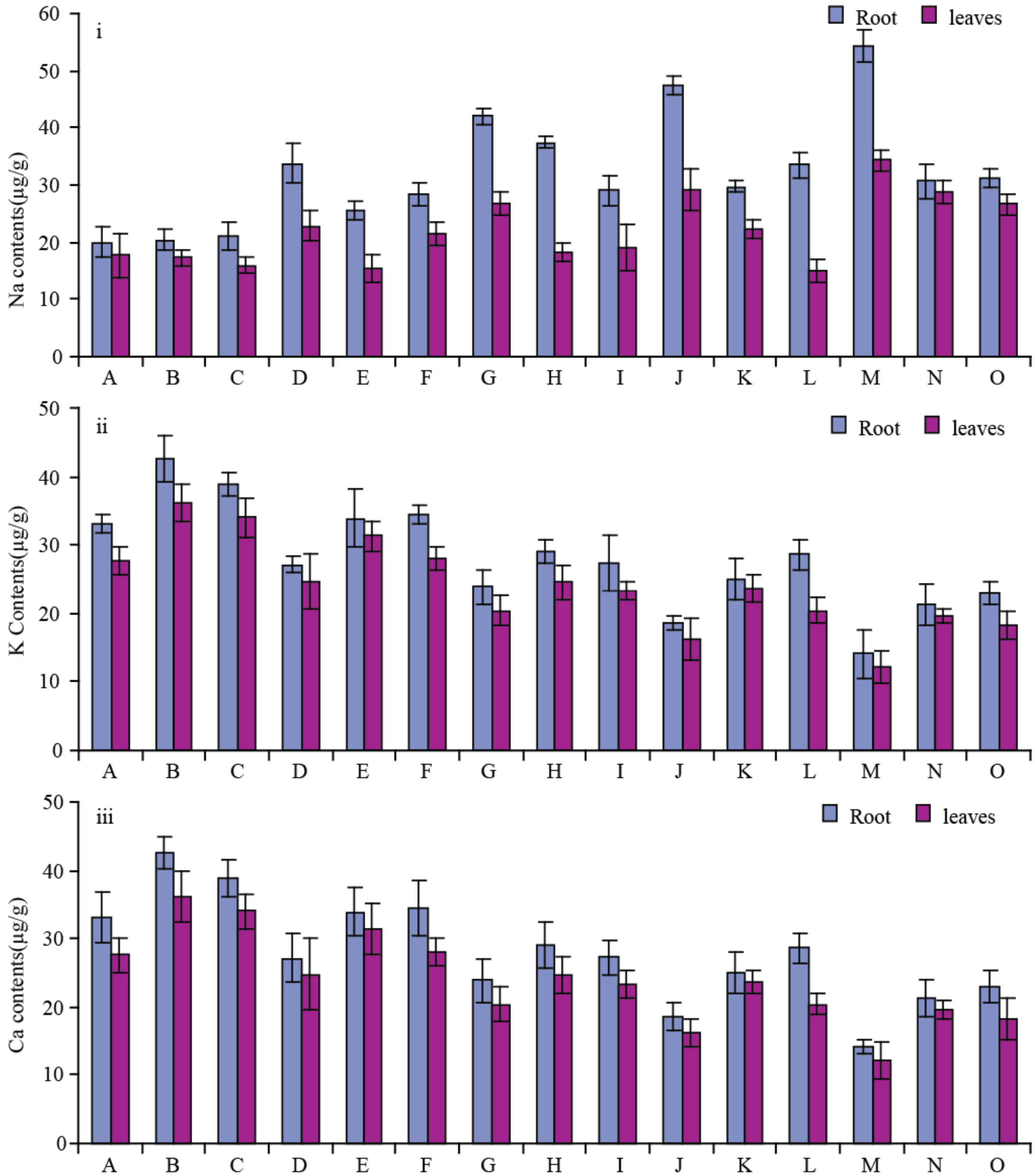


Fig. 1.4. Analysis of Na^{+}(i), K^{+} (ii) and Ca^{+2} (iii) contents in maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).

As usual H_2O_2 level increased in plants when exposed to salt stress. Results of present study also revealed percent increase in the level of H_2O_2 in plants exposed to salt stress of 100, 150 and 250 mM showed 16, 10 and 12.16% as compare to control. H_2O_2 concentration was increased in smoke solution at dilutions of 1:100 (11.16) and1:400 (8.16%) as compare to control. Alleviation solution of 150+400 showed (20.63), 250+100 (9.5) and 250+400 (4.06 %) their respective salt stress.

Soluble protein contents: Total soluble protein contents in the maize grown in pots for 21 days under salt stress were analyzed. Protein contents of maize tended to increase in smoke solution at dilution 1+400 (163.56 mgg^{-1}). Alleviated solution (salt+smoke) showed the adverse effect caused by the salt stress, by increasing protein concentration in 100+100 (110.39 mgg^{-1}), 200+100 (156.17 mgg^{-1}), 200+400 (92.04), 250+100 (117.34) and 250+400 (122.18 mgg^{-1}) respectively.

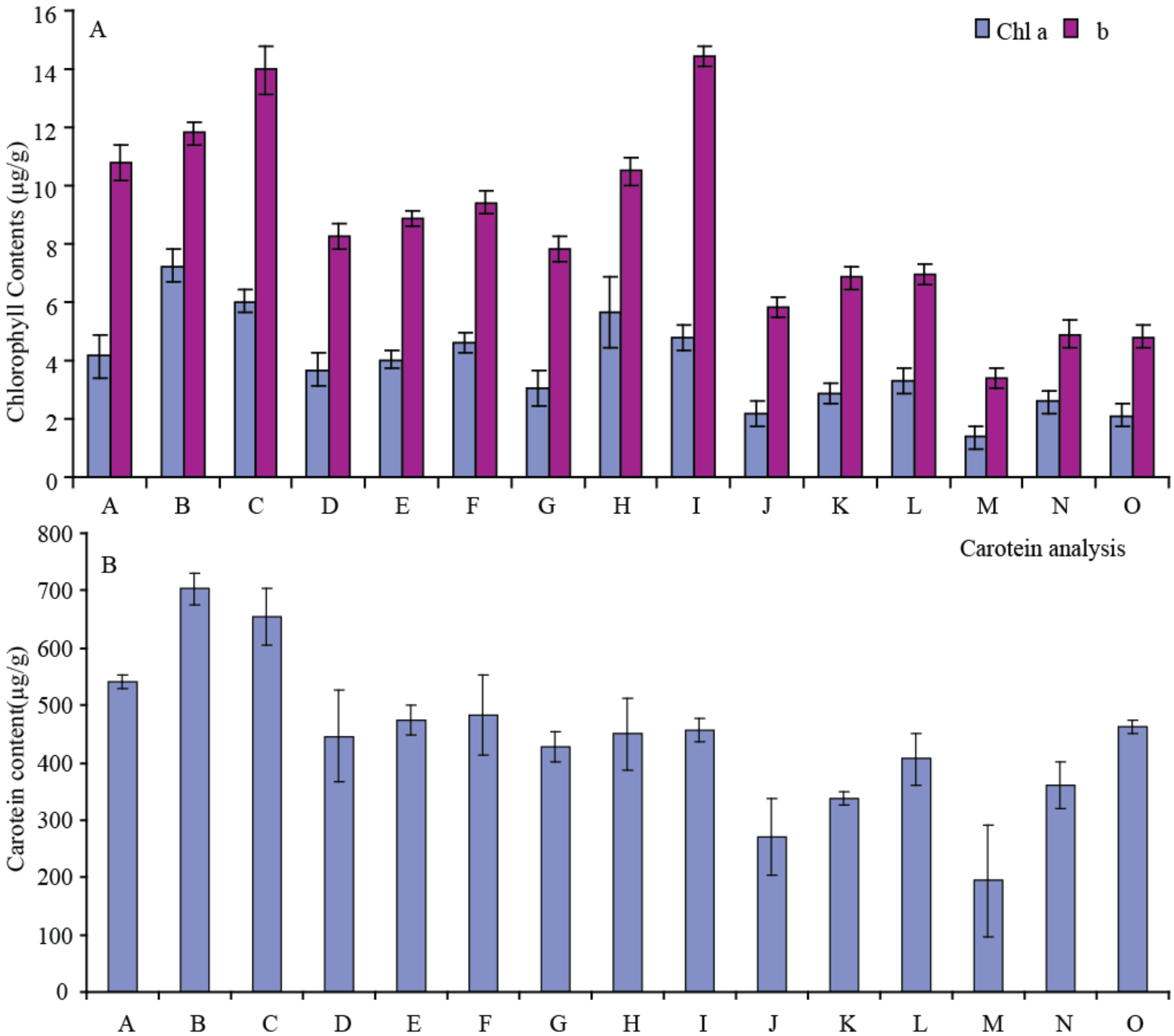


Fig. 1.5. Analysis of Chl *a* and *b* (A) and carotenoids contents (B) in maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).

Analysis of POD activity: The peroxidase activity (POD) of the maize plants under salt stress increased by raising level of salinity as compare to normal conditions (Fig. 1.8). However, different treatments of salts showed different responses upon maize plants i.e., 100 (111.05), 200 (94.31) and 250 mM (106.09%). Moreover with the application of alleviated solution of smoke and salt showed 150+100 (112.15), 200+400 (125.76) and 250+400 (141.18%) which is highly significant. Alleviated POD activity was seen as compare to the respective salt.

APX activity of maize under salt stress: The ascorbate per oxidase (APX) activity also greatly influenced when exposed to NaCl stress (Fig. 1.8). Its activity ascended in salt stress solution from 100 to 250 mM (228.80%) as compare to control. The smoke solution at dilution 1+100 (264.04) and 1+400 (246.07%) showed significantly increased in APX activity. With the application of alleviated solution of smoke and salt, smoke alleviated the APX activity i.e., 100 and 200 mM were alleviated by 100+400 (261.42), 250+400 (302.14) and 250+400 (245.59%), respectively.

CAT activity of maize under salt stress: The values CAT were found doe dependent, and different treatments showed different values increased highly at 100 (236.38) and 150 mM (92.46%) salt stress levels after 21 day treatment. At this treatment level, there was highly significant change over its control. Moreover, Smoke dilution 1+400 (306.04%) were highly significant as compare to control. Furthermore, Smoke and salt alleviation solution indicate highest catalase activity i.e., 329.62 (100+400), 98.28 (200+100) and 91.54% (250+100) as compare to 92.46% (150 mM) and 56.11 (200 mM) and 91.64% (250 mM) while other alleviation solutions have no significant change.

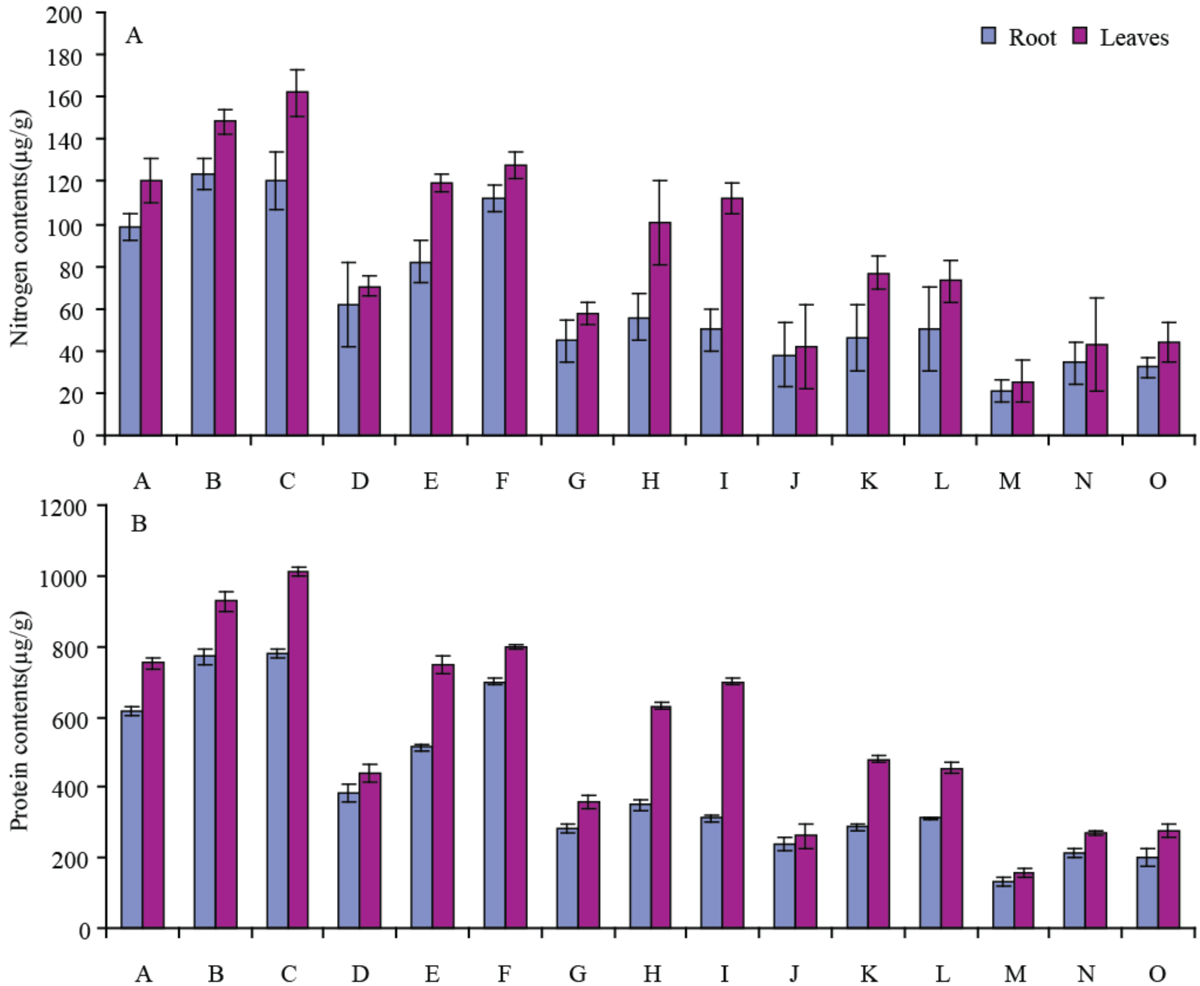


Fig. 1.6. Nitrogen and protein content in maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).

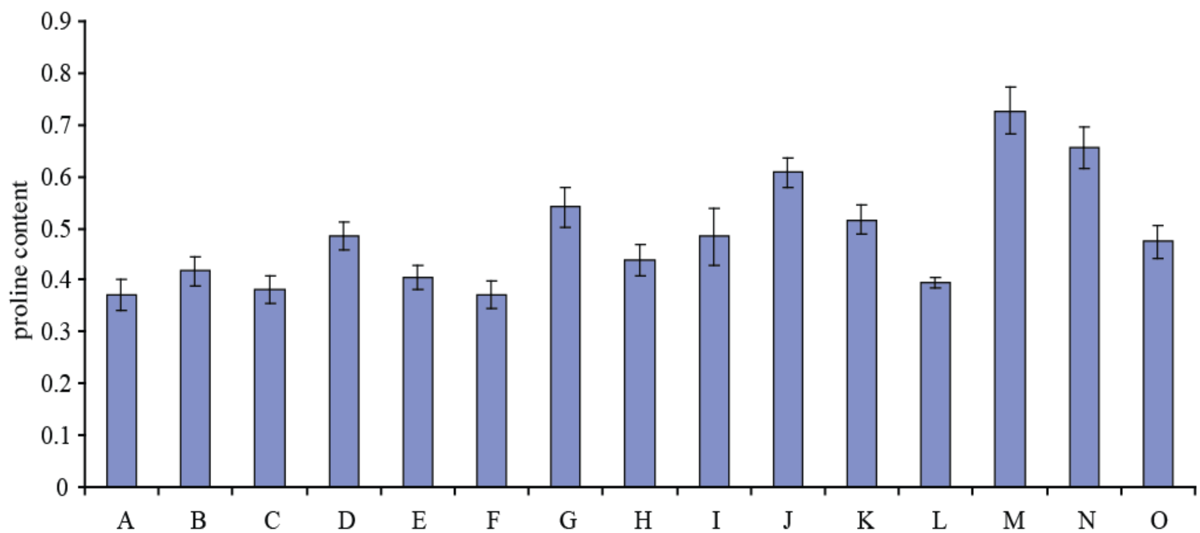
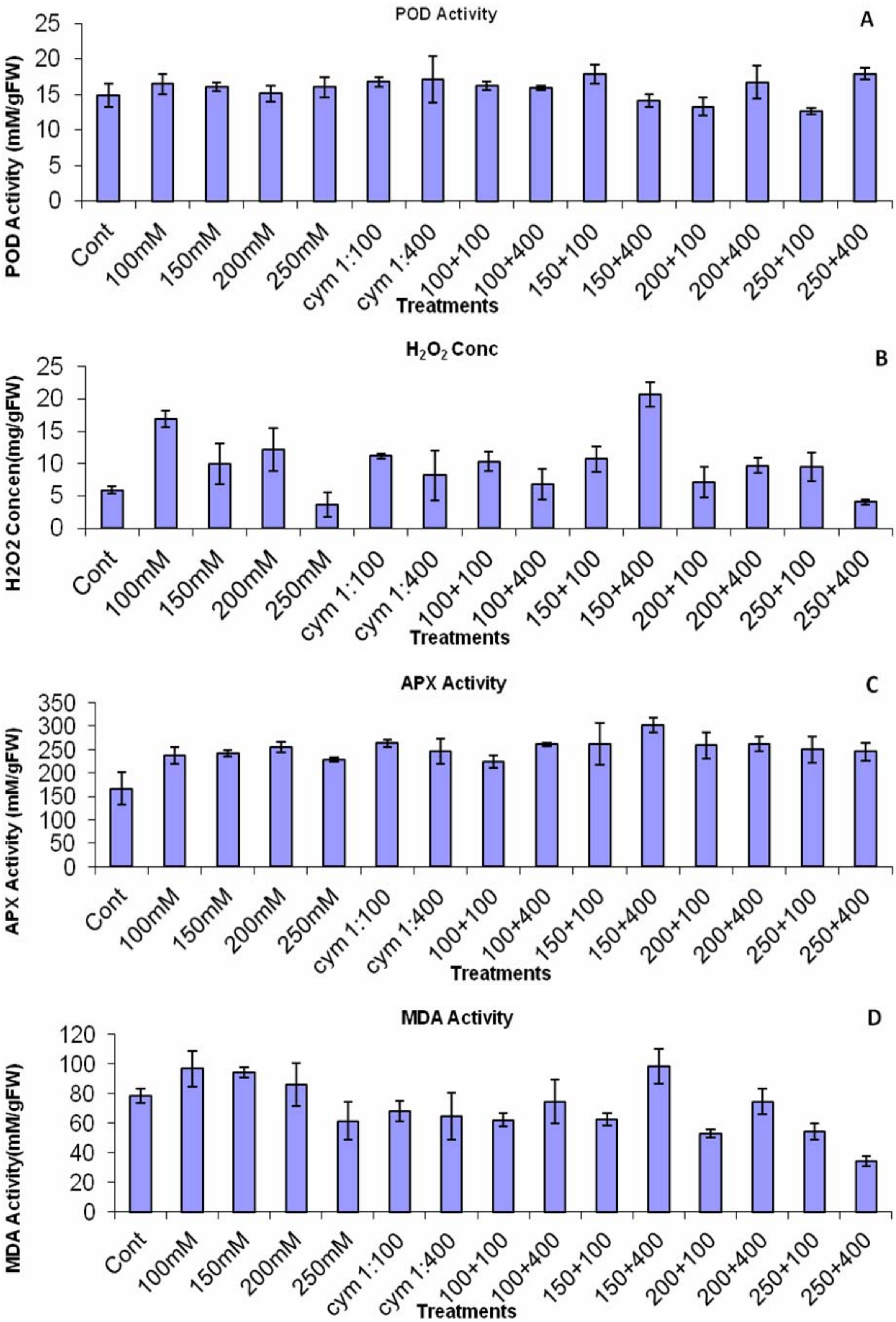


Fig. 1.7. Proline contents in maize seedlings exposed to different treatments (A-cont, B-100, C-400 are smoke dilutions , D-100, G-150, J-200, M-250 mM are salt solutions, while E-100+100, F-400+100, H-100+150, I-400+150, K-100+200, L-400+200, N-100+250 and O-400+250 mM are alleviating solutions of smoke + salt).


POD Activity
A
POD Activity (mM/gFW)
0
5
10
15
20
25
Cont
100mM
150mM
200mM
250mM
cym 1:100
cym 1:400
100+100
100+400
150+100
150+400
200+100
200+400
250+100
250+400
Treatments
H2O2 Conc
B
H2O2 Concen(mg/gFW)
0
5
10
15
20
25
Cont
100mM
150mM
200mM
250mM
cym 1:100
cym 1:400
100+100
100+400
150+100
150+400
200+100
200+400
250+100
250+400
Treatments
APX Activity
C
APX Activity (mM/gFW)
0
50
100
150
200
250
300
350
Cont
100mM
150mM
200mM
250mM
cym 1:100
cym 1:400
100+100
100+400
150+100
150+400
200+100
200+400
250+100
250+400
Treatments
MDA Activity
D
MDA Activity(mM/gFW)
0
20
40
60
80
100
120
Cont
100mM
150mM
200mM
250mM
cym 1:100
cym 1:400
100+100
100+400
150+100
150+400
200+100
200+400
250+100
250+400
Treatments

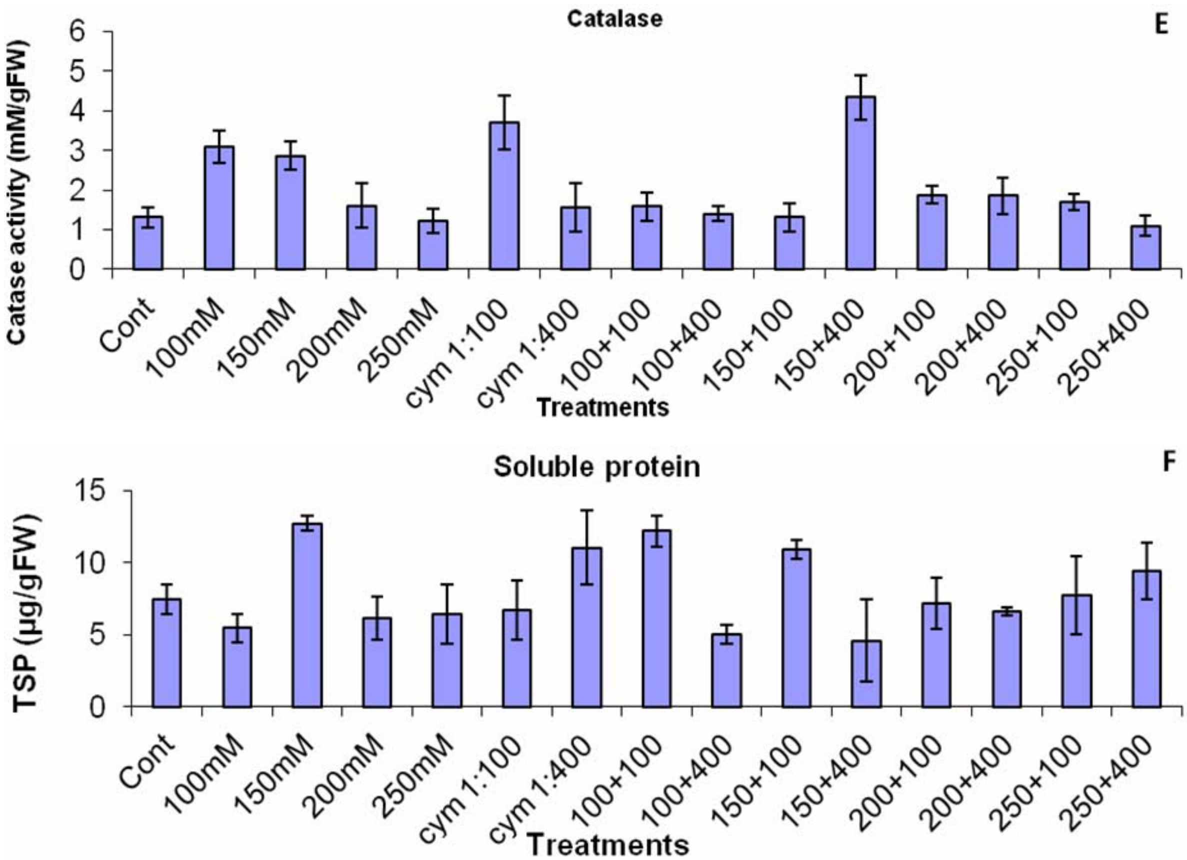


Fig. 1.8. Effect of Plant-derived smoke solutions (1:100 and1:400 dilutions) on MDA, H_2O_2,Protein contents, POD, APX and Catalas activities of smoke (Cymbo) primed maize seeds under both normal and saline conditions. (A-cont, B-100, C-400 are smoke dilutions, D-100, G-150, J-200, M-250 mM are salt solutions , while E-100+100 mM, F-400+100 mM, H-100+150 mM, I-400+150 mM, K-100+200 mM L-400+200 mM, N-100+250 mM, O-400+250 mM are alleviating solutions).

Discussion

The present experiments are the first systematic attempt to examine the phytotxicity caused by saline condition and their alleviation through plant-derived smoke solution in reference to physiological and biochemical biomarkers. Results revealed that the rate of germination is greatly affected with the application of increasing levels of salt concentrations from 100 to 250 mM as shown in (Fig. 1.1). Our results are in lined with the previous study of Alam *et al*. (2004) and Omami (2005) who studied that salt stress negatively affected overall germination with the increasing salt concentrations. Similarly, Zeng & Shannon (2000) stated that increased duration of salt stress causes reduction in the seedling growth. It has also been investigated that priming of seeds with smoke solutions improved the process of seed germination. Farooq *et al*. (2006) also reported same results which stated that, seed priming with smoke solution can improves the germination process. Priming with Cymbopogon (1:400) smoke showed 100% germination. Lower concentration of smoke showed better germination than in the higher ones. It might be due to the presence of inhibitory chemicals which become diluted at lower concentrations and cannot express the phytotoxic effect on germination and are in accordance with the previous reports of Sparg *et al*. (2006) who observed that a lower concentration plant extracted smoke increased germination of seed while at higher concentrations, reduced the seed germination. Beside these, reduced germination under saline stress was significantly alleviated with the application of plant derived smoke solution during the present research experiments.

Similarly, maize seedlings showed reduction in length when exposed to salt stress. The seedling vigor in term of seedling length significant decrease by increasing salt concentration. The decrease in seedling vigor might attributes due to the high uptake and accumulation of Na^+ which ultimately reduced the uptake of water (Chavan & Karadge, 1986). Similarly, priming of seed with smoke has improved the seedling growth which might attributes its hormone like activity (Jamil *et al.*, 2014). Likewise, high level of saline condition promotes the uptake of Na^+ and Cl^- in seedlings as described by. Seeds primed with alleviating solutions of smoke and salts, alleviated significantly the phytotoxic effects of salt stress in case of seedling vigor (Fig. 1.2).

Improvement in plant growth in response to smoke might be attributing due to the enhancement of the nutrients on metabolism and other physiological activities which in turn can improve the photosynthetic pigments and enzymes that encourage vegetative and reproductive growth in plants. On the other hand salinity posed negative impacts on photosynthetic pigments production. According to Munns (2002), with rise in the saline condition, there occur a remarkable reduction in Chl *a*, *b* and total carotenoids. The loss of chlorophyll under salt stress could be related to photo inhibition or ROS formation. Smoke treated plants of both dilutions i.e., 1:100 and 1:400 showed high level of both Chl *a*, *b* and total carotenoids while smoke with combination of salts reduced the adversity caused by saline condition. Similar results were obtained by Jamil *et al*. (2014) which indicated that smoke water can improve the chlorophyll contents in rice when subjected to salt stress.

In case of ion contents analysis, higher contents of Na^+ and lower contents of K^+ and Ca^{+2} were found in seedling exposed to salt stress while smoke alleviated the phytotoxic responses of salt stress. Katerji *et al*. (2004) demonstrated that that higher level of Na^+ in the plant tissues posing adverse effect by imbalance the nutrients and osmotic adjustment. Na^+ competes with useful ions like K^+ and Ca^{+2}, and in turn inhibits their absorption. Similar effects were reported in case of nitrogen and protein contents. Inhibition in biosynthesis of protein has been reported by Lutts *et al*. (1999). Likewise, Alfocea *et al*. (1993) investigated that higher level of nitrogen contents were found in tomato roots, but lower in the leaves and stem when exposed to saline condition. In the present literature, the level of total soluble protein has been found dose dependent and showed different response to different treatments. Protein contents of maize tended to increase in smoke solution at dilution 100 and 400. Salt stress, have been reported to increase the protein synthesis (Singh *et al*., 1987; Chandrashekar & Sandhyarani, 1995), while others suggested a reduction (Morant-Avice *et al*., 1998; Gulen *et al*., 2006) or remained unchanged (Singh *et al*., 1987).

Proline plays a key role in plant defense mechanism when exposed to stress. Present result revealed that proline accumulation level in plants remained increases in response to salt stress. Girija *et al.* (2002) reported that proline offer tolerance in plants exposed to stress like salinity. Similar trend was also observed in case of H_2O_2 activity. Hydrogen peroxide content has been shown to increase under NaCl stress in a number of studies in various plants.

As result of salt stress, antioxidant enzymes are generally produced to protect the plant from oxidative stresses. Different antioxidant enzymes i.e., ascorbate per oxidase (APX), peroxidase activity (POD) and catalase activity (CAT) showed similar response and found in increase level in the plans exposed to salt stress and alleviating solution of smoke and salt also greatly varied in the experimental of maize under the influence of NaCl stress. According to Oueslati *et al*. (2010), antioxidant enzymes plays important role by scavenging reactive oxygen species mainly SOD, POD and CAT. Likewise, Gueta-Dahan *et al.* (1997)also suggested that APX is an important enzyme and play a pivotal role in plants to tolerate saline condition.

The CAT activity was also obvious under the stressful effects of salt stress. In comparison to controls, its mean values in the salt stress increased as compared to the control treatment. Moreover, smoke dilution of 1:400 was highly significant as compare to control. Furthermore, alleviating solution showed increase level of catalase activity at 150+400 and 250+100 as compare to the control. In the present results, the MDA contents in salt stressed plants increased remarkably as also showed by Diego *et al*. (2003) and Tijen & Ismail (2005). It indicated that high salinity induced accumulation of MDA in seedlings which would be harmful for seedling growth and development. In smoke solutions and in alleviating solutions, the level of MDA remained low which indicates the alleviating potential of smoke.

Conclusions

Results of the present investigation revealed that the saline condition remarkably posed a severe stress in maize plants which reduced the overall growth and various biochemical compound productions. Likewise, the plant-derived smoke solution of 1:400 and 1:100 have been found to improve the growth and development in maize plant. However, the plant-derived smoke solutions with combination of salt solutions (alleviating solutions) significantly alleviated the adverse effects of salt stress in form of salinity-induced phytotoxicity. Priming with smoke extracts of 1:100 and 1:400 can overcome the phytotoxic effects due to salinity in diverse range of agricultural sectors which demands further research in this context to use priming of seeds with smoke water for the enhancement of crops yield under saline stress.

References

Aebi, H. 1984. Catalase *In vitro*. *Methods Enzymol*., 105: 121-126.

Alam, M.Z., T. Stuchbury, R.E. Naylor and M.A. Rashid. 2004. Effect of salinity on growth of some modern rice cultivars. *J. Agron*., 3: 1-10.

Alfocea, E.P., M.T. Estan, A.S. Cruz and M.C. Bolarin. 1993. Effect of salinity on nitrate, total nitrogen, soluble protein and free amino acid levels in tomato plants. *J. Hort. Sci.*, 68: 1021-1027.

Allen, S.E., C.C. Evans and H.M. Grimshaw. 1983. The distribution of mineral nutrients in the soil after heather burning. *Oikos.* 20: 16-25.

Anonymous. 2009. Food and Agriculture Organization of the United Nation. http://www.fao.org/news/story /en/item/ 35571/icode/

Asada, K. 1999. The water-water cycle in chloroplast: Scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant. Physiol. Plant Mol. Biol*., 50: 601-639.

Basra, S.M.A., M. Farooq, R. Tabasssum and N. Ahmad, 2005. Physiological and biochemical aspects seed vigour enhancement in fine rice (*Oryza sativa* L.). *Seed science Technology*,33: 623-628.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water studies. *Plant Soil,* 39: 205-208.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.

Bradford, K., J. Steiner and S. Trawatha. 1990. Seed priming influence on germination and emergence of pepper seed lots. *Crop Science*, 30: 718-721.

Chandrashekar, K.R. and S. Sandhyarani. 1995. Effect of salinity stress on germination, carbohydrate, protein and proline contents of *Crotalaria striata* DC seeds. *Acta Bot. Indica*, 23: 59-62.

Chavan, P.D. and B.A. Karadge, 1986. Growth, mineral nutrition, organic constituents and rate of photosynthesis in *Sesbania grandiflora* L. grownunder saline conditions. *Plant Soil*, 93: 395-404.

De Lange, J.H. andC. Boucher. 1990. Autecological studies on *Audouinia capitata* [Bruniaceae]. I. Plant-derived smoke as a germination cue*. South African Journal of Botany*, 56: 700-703.

Diego, A.M., A.O. Marco, A.M. Carlos and C. Jose. 2003. Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot*., 49: 69-76.

Dixon, K.W., D.J. Merritt, G.R. Flematti and E.L. Ghisalberti. 2009. Karrikinolide a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration, and agriculture. *Acta Horticulturae*,813: 155-170.

Farooq, M., S.M.A. Basra and H.U. Rehman. 2006. Seed priming enhances emergence, Yield and quality of direct-seeded rice. *Crop Management and Physiology*,3: 42-44.

Farooq, M. Basra, S.,H. Rehman,M. Hussain and Y. Amanat. 2007. Pre-sowing salicylicate seed treatments improve the germination and early seedling growth in fine rice. *Pak. J. Science*, 4: 56-60.

Giannopolitis C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*,59: 309-316.

Girija, C., B.N. Smith and P.M. Swamy. 2002. Interactive effects ofsodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in Peanut (*Arachis hypogaea* L.). *Environ. Exp. Bot*., 47: 1-10.

Gueta-Dahan, Y., Z. Yaniv, B.A. Zilinskas and G. Ben-Hayyim. 1997. Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. *Planta*, 203: 460-469.

Gulen, H., E. Turhan and A. Eris. 2006. Changes in peroxidase activities and soluble proteins in strawberry varieties under salt-stress. *Acta Physiol. Plant*., 28(2): 109-116.

Jain, N. and J. Van Staden. 2006. A smoke-derived butenolide improves early growth of tomato seedlings. *Plant growth Regulation.*50: 139-148.

Jamil, M., M. Kanwal, M.M. Aslam, S.K. Shakir, I. Malook, J. Tu and S. Rehman. 2014. Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. *Pak. J. Bot.*, 8: 159-170.

Katerji, N., J.W. van Hoorn, A. Hamdy and M. Mastrorilli. 2004. Comparison of corn yield response to plant water stress caused by salinity and by drought. *Agric. Water Mange*, 65: 95-101.

Kulkarni, M.G., S.G. Sparg and J. Van Staden. 2007. Germination and post germination. *J. Arid Envir.*, 69: 177-187.

Kulkarni, M.G., S.G. Sparg, M.E. Light and J. Van Staden. 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *J. Agronomy and Crop Sci.*. 192: 395-398.

Lichtenthaler, H.K. and A.R. Wellburn. 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. *Biochemical Society Transactions*, 11: 591-592.

Light, M.E. and J. Van Staden. 2004. The potential of smoke in seed technology. *South African Journal of Botany*, 70: 97-101.

Lutts, S., V. Majerus and J.M. Kinet. 1999. NaCl effects on praline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.,* 105: 450-8.

Mehdi, S.S. and M. Ashan. 2000. Coeffecient of variation, inter-relationships and heritability estimates for some seedlings traits in maize in recurrent selection cycle. *Pak. J. Biol. Sci*., 3: 181-182.

Morant-Avice, A., E. Pradier and R. Houchi. 1998. Osmotic adjustment in triticales grown in presence of NaCl. *Biol. Plant*., 41: 227-234.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*., 25: 239-250.

Munns, R., R.A. James and Lauchli. 2005. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exper. Bot.,* 5: 1025-1043.

Munns, R., R.A. James and A Lauchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot*.,5: 1025-1043.

Nakano, Y. and K. Asada. 1981. Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. *Plant Cell Physiol*., 22: 867-880.

Omami, E.N. 2005. Effects of salinity stress on amaranth seed germination and seedling growth. *New Zealand J. Crop & Horticul. Sci.,* 34: 35-59.

Oueslati S., N.H. Karray-Bouraoui, H.M. Attia, M Rabhi, R. Ksouri and M. Lachaal. 2010. Physiological and antioxidant responses of *Mentha pulegium* (Pennyroyal) to salt stress. *Acta. Physiol. Plant*., 32: 289-296.

Rodriguez, A.A, R.A. Llicia, R. Cordoba, R. Ortega and E. Taleisnik. 2004. Decreased reactive oxygen species concentration in the elongation zone contributes to the reduction in maize leaf growth under salinity. *J. Exp. Bot*., 55: 1383-1390.

Singh, N.K., C.A. Bracken, P.M. Hasegawa, A.K. Handa, S. Buckel, M.A. F. Hermodson, Pfankoch, F.E. Regnier and R. Bressan. 1987. Characterization of osmotin: A thaumatin-like protein associated with osmotic adjustment in plant cells. *Plant Physiol*., 85: 529-536.

Sparg, S.G.M., G. Kulkarni and J. Van Staden. 2006. Aerosol smoke and smoke water stimulation of seedling vigor of a commercial maize cultivar. *Crop Science*, 46: 1336-1340.

Thornton, M.A., T.H. Thomas and N.C.B. Peters. 1999. The promotive effect of combustion products from plant vegetation on the release of seeds from dormancy. *Plant Growth Regulation*, 28: 129-132.

Tigabu, M., J. Fjellstro, P.C. Ode'n and D. Teketay. 2007. Germination of Juniperus procera seeds in response to stratification and smoke treatments, and detection of insect-damaged seeds with VIS + NIR spectroscopy. *New Forests,* 33: 155-169.

Tijen D. and T. Ismail. 2005. Comparative lipid peroxidation, antioxidant defense systems and praline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot*. 53: 247-257.

Van Staden, J., N.A.C. Brown, A.K. Jager and T.A. Johnson. 2000. Smoke as a germination cue. *Plant Species Biology*, 15: 167-178.

Vanacker, H., J. Harbinson, T. Carver and C. 1998. Foyer. Antioxidant defenses of the apoplast. *Protoplasma*, 205: 129-40.

Velikova,V., I. Yardano and A. Edreva. 2000. Oxidative stress and some antioxidant system in acid rain-treated bean plant. *Sci*., 151: 59-66.

Zeng, L. and M.C. Shannon. 2000. Salinity effects on seedling growth and yield components of rice. *Crop Sciences*, 40: 996-1003.

Zhou, W.J. and M. Leul. 1998. Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. *Plant Growth Regul*., 26: 41-47.

Zhou, W.J. and M. Leul. 1999. Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. *Plant Growth Regul*., 27: 99-104.

(Received for publication 14 September 2015)