Pak. J. Weed Sci. Res. 18(1): 51-64, 2012

GROWTH RESPONSES OF *Parthenium hysterophorus* L. GROWING UNDER SALT STRESS

Saba Khurshid[1], Ghazala Nasim[1], Rukhsana Bajwa[1] and Steve Adkins[2]

ABSTRACT

The present study conducted during 2009-10 prescribed the potential of varyingly devised osmotic constrains on germination and growth as well as mycorrhizal association of Parthenium hysterophorus, an alien and highly invasive weed species. The effects of differential salinity concentrations of three selected salts viz. NaCl, KCl, and $MgSO_4$ were evaluated on germination and growth of P. hysterophorus in lab as well as field trials at Institute of Agricultural Sciences, University of the Punjab, Lahore. The different concentrations of test salts for lab experiments were 10, 20, 30, 40 mM, and for field experiments were 500, 750, 1000, 1250 mM. The control treatment with simple water irrigation was marked as 0 (zero). The parthenium plants were allowed to grow in silty loam textured soils in pots. Twenty five days old seedlings were allowed to establish in the pots. Saline sprays with test salts were carried out one week after transplantation. In the results, different salts had different mechanisms of affecting the plants. Average decline of 86% was observed in germination of parthenium against maximum concentrations. In both of the lab and field experiments, shoot and root fresh weights were severely affected in comparison to the dry weights. Mycorrhizal colonization was higher in the control treatments against all the saline conditions. Significant decline in chlorophyll content was evidenced in case of KCl. The salinity threshold of P. hysterophorus was estimated up to 0.75 M, growth was strongly decreased and growth was significantly reduced at higher salinity regimes.

Key words: Alien; Invasive; Mycorrhizal; Transplantation.

INTRODUCTION

Parthenium (*Parthenium hysterophorus* L.) is an aggressive invasive alien weed of 30-150 cm height (Adkins e*t al.,* 1996, Kohli *et al*., 2006) is native to the America but now widely spread in Asia, Africa and Australia (Javaid and Anjum, 2005; Evans, 1997). Being an aggressive colonizer of wasteland, road sides, railway sides, water courses, cultivated fields and overgrazed pastures (Wiesner *et al*., 2007) it has been arrived in Pakistan through India (Adkins and Navie, 2006).

[1] Institute of Plant Pathology, University of the Punjab, Lahore, Pakistan.
[2] Tropical and Subtropical Weeds Research Unit, School of Land, Crop and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia.
Correspondence email: drsaba16@gmail.com

In nature all living organisms including weeds are frequently exposed to unfavorable environmental conditions that have negative effects on their survival. Thus these natural limitations limit proliferation of weeds up to a certain extent. The recent research based on management strategies to check growth and development of Parthenium include application of synthetic chemical herbicides (Javaid, 2007), natural herbicides from plants (Javaid *et al.,* 2010; 2011) biological control agents like insects and pathogens (Adkins and Navie, 2006). (Javaid and Adrees, 2009; Javaid, 2010; Javaid and Ali, 2011a). However among the abiotic factors limiting the plant germination and seedling growth, water stress due to drought and salinity plays vital role worldwide (Kaya *et al*., 2006). All plants tolerate salinity up to a certain threshold level with no decline in crop yield (Ozturk *et al.,* 2004).

Plants can respond to salt stress at morphological, anatomical and cellular levels with modifications that allow the plant to avoid stress or to increase its tolerance (Al-Karaki, 2000). Generally salt stress induces both osmotic and ionic stress. Osmotic stress is created by an excess of salts in the soil, while the ionic stress is caused by the over accumulation of salts in the cells.

These stresses individually affect the physiological status of the soil (Ueda *et al*., 2004). Germination percentage and rate of germination of seeds have been reported to be significantly reduced due to increasing intensity of salt stress (Noureen *et al.,* 2007). Increased concentrations of salt ions ($Na+$, Ca^{2+}, $Cl-$, and SO^{4-}) in the soil cause water deficit.

This is followed by growth reduction, nutrient deficiencies, injury of the foliage, eventual shrinkage of leaf size, wilting of the whole plant and finally death of the plant. (Marschner, 1986; Maas 1989; Allen *et al*., 1994) Salinity may also cause reduced ATP and growth regulators in plants; however management practices must be selected to ensure that the levels of salinity in the soil are not harmful to crop growth. This is usually done by applying enough water to satisfy crop requirements and leach out salts from the root zone (Ozturk *et al.,* 2004).

Herbicidal activity of certain fungi, weeds, allelopathic plants and insects against *P. hysterophorus* have been reported to manage its proliferation (Javiad and Anjum 2006; Javaid *et al.,* 2011) however investigation regarding salt stress has still not being taken under consideration.

The presently planned study aimed at inducting variously devised osmotic stress conditions on germination and growth of *P. hysterophorus* in view of evolving a potent strategy to curtail its fast infestation in Pakistan.

MATERIALS AND METHODS

Collection of Seeds

The capitula of *Parthenium* flowers were collected, air and sun dried, then stored in polythene bags at $4^{O}C$. Seeds of Parthenium before sowing on double layer of filter paper were surface sterilized by dipping the seeds in 1% sodium hypochlorite solution for 5 minutes to avoid surface microorganisms. Viability test was done to ensure the number of viable seeds, and it was estimated up to 90%.

Experimental Setup

Three salts viz., NaCl, KCl, and $MgSO_4$ were selected for osmotic shock experiment during 2009-10. The selection of the salts was made on the pervious experimentation regarding physiological stress (Khan and Gulzar, 2003; Rehman *et al.,* 2008). Chloride salts were chosen for high solubility (Ajmal and Khan, 1986) to prepare irrigation regimes of varying salinity levels. 1.5 molar solution of each test salt was made by dissolving the weighed amount (grams equivalent to molecular weight of the salt) in one liter of distilled water. Further dilutions of 10, 20, 30, 40 mM for lab trials and 0.5, 0.75, 1.0, 1.25 for field experiment were made by diluting the stock solution. To evaluate the potential of each salt on plant metabolism, solutions of different strengths were made. Assessment of germination and early seedling growth was done by performing set of experiments. For lab experiment 10 seeds per plate were placed on the double layer of moist filter paper in a covered Petri plate. And different salinity regimes @ 10 ml per plate were applied. For greenhouse experiments 10X5 inches polythene bags were used. Seedlings were raised on a sand bed in the greenhouse. Solar sterilized silty loam soil was selected for planting seedling. Four weeks old seedlings were transferred to the already filled bags.

Plants were allowed to establish for one week after transplantation and then subjected to five salt levels of three different salts i.e. NaCl, KCL and $MgSO_4$ as salt spay. Different concentrations of all three salts as, 0 (control without salt stress), and 0.5M, 0.75M, 1M and 1.25 M @ 100 ml solution per pot were employed to evaluate the potential stress of salinity on growth of *Parthenium.* Plants were watered at field capacity daily with tap water until harvest. The plants were harvested after they had been grown under salt stress conditions for 1 week. Dig out the whole plant shoots and roots were separated. Shoot and root lengths of all the seedlings were carefully measured in centimeters just after the harvesting. Root and shoot fresh weights were taken just after harvest and oven-dried weights were taken after drying at 70°C for 48 h.

Seedling Analysis

The total chlorophyll contents were determined according to method of Arnon (1949). Absorbance of the supernatant was recorded at 645 and 663 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan). Arbuscular mycorrhiza was estimated following the method of Phillips and Hayman 1970.

$$\text{Chlorophyll a} = 12.7A_{663} - 2.69A_{645} \quad \text{Chlorophyll b} = 22.9A_{645} - 4.68A_{663}$$

Total chlorophyll content = Chlorophyll a + Chlorophyll b (mg/g)

All the data were statistically analyzed by applying Duncan's New Multiple Range (DMR) Test (Steel and Torrie, 1980) at 5% level of significance to separate the treatment means.

RESULTS AND DISCUSSION

Seed germination in early seedling experiment was significantly reduced as compared to control presented in Fig. 1. Reduction in germination from 90 to 6.6, 16.6 and 6.6% was observed at maximum concentration for NaCl, KCl and $MgSO_4$, respectively. In general, $MgSO_4$ among all the tested salts resulted in the highest depression in germination. Salinity can affect seed germination through osmotic effects (Welbaum *et al*., 1990). Salts also interfere with seed germination and crop establishment (Esechie, 1996) however the degree of salt stress can affect the different crops differently. Strong reduction was observed mainly at the higher level of salt concentrations against control. Germination delayed as the level of salinity increased (Jamil *et al.,* 2006).

Growth in shoot lengths of the plants was also severely affected by the salt spray and effect became more pronounced at higher concentrations. Maximum decline in shoot length was recorded due to NaCl salinity i.e. 21% and 53% in early seedling experiments (Fig. 1-B) and pot experiments (Fig. 3-A), respectively. At maximum concentration of 40 mM KCl shows significant decline of about 57% in terms of root length (Fig. 1-C). However in pot experiments all the salts exhibited almost similar depression i.e. 18% in root length at 0.75M concentration against control. Maximum reduction in root length was recorded under $MgSO_4$ salinity at maximum concentration of 1.25 M (Fig. 4-A). These findings are also in line with the previous results that salinity generally has been accepted to reduce shoot growth (Tattini *et al*., 1992; Klein *et al.,* 1994), pollen viability and germination, number of flowers and fruits (Cresti *et al.,* 1994). Salinity also affects the yield depending on the concentration (Klein *et al.,* 1992, Wiesman *et al.,* 2004)

In our study, the root and shoot biomasses were also affected by the action of various salinity regimes. Adequate reduction in shoot fresh biomass was recorded in case of KCl and $MgSO_4$ in comparison with NaCl (Fig.2-A, B, 4-B). These results are aligned with the pervious results that salt stress inhibited the whole seedling growth however root growth in length was more affected than shoots. Inhibition of plant growth by salinity may be due to the inhibitory effect of ions. Indicated that salinity may rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil (Neumann, 1995). Data recorded in terms of dry biomass showed that the level of inhibition was concentration dependent (Fig. 2-A, 3-C, 4-C). Similar kind of results were observed by Jeannette *et al*. (2002) that total fresh weight of root and shoot of cultivated accessions was significantly reduced with increased salt stress. These results are also in line with Shannon *et al.* (2000) who indicated salinity induced reduction in fresh weight (FW) of vegetables. Chlorophyll content presently found to be affected due to salinity stress caused by NaCl, KCl and $MgSO_4$ as photosynthesis was reduced and respiration was increased. Lower concentration significantly reduced chlorophyll content however at maximum concentration of 1.25 M 10%; 36% and 17% reduction was recorded for NaCl, KCl and $MgSO_4$ respectively. (Fig. 5-A) According to Lewis, (2002) increasing salinity inhibits nitrate reductase activity, decreases chlorophyll content and photosynthetic rate, and increases the respiration rate. Auge *et al.* (2008) also concluded that mycorrhizal colonization may be inhibited due to allelopathic nature; releasing allelochemicals in the rooting zone; or it may not require mycorrhiza for its successful establishment. Number of vesicles in mycorrhizal association were also reduces due to salinity stress. The maximum concentration of 1.25 M NaCl and $MgSO_4$ exhibited highest depression i.e. 82% in terms of no. of vesicles, where as 80% inhibition was recorded for KCl (Fig. 5-B, C).

CONCLUSION

Salinity regimes of 30 and 40 mM NaCl in lab experiments were found effective in causing 66% and 88% depression in seed germination respectively. Likewise other growth parameters indicated suppression potential of the test salts. NaCl and $MgSO_4$, both were found effective in field experiments, however mortality recorded at higher concentrations of 1.0 and 1.25M for all test salts. Higher salinity threshold of *P. hysterophorus* indicated that its likeliness to infest our salinity hit fallow lands where other plants cannot normally survive. As this is a preliminary study to check the salinity threshold of *Parthenium hysterophorus,* extension program must be planned in such a way that further proliferation of *Parthenium* be suppressed by spraying the saline solution of NaCl specifically even at lower concentration of 0.5-0.75M to weed infested barren lands along with cultivated lands as it is cost effective, less laborious and less toxic to environment. However, salinity threshold of crops must be estimated before commercial application.

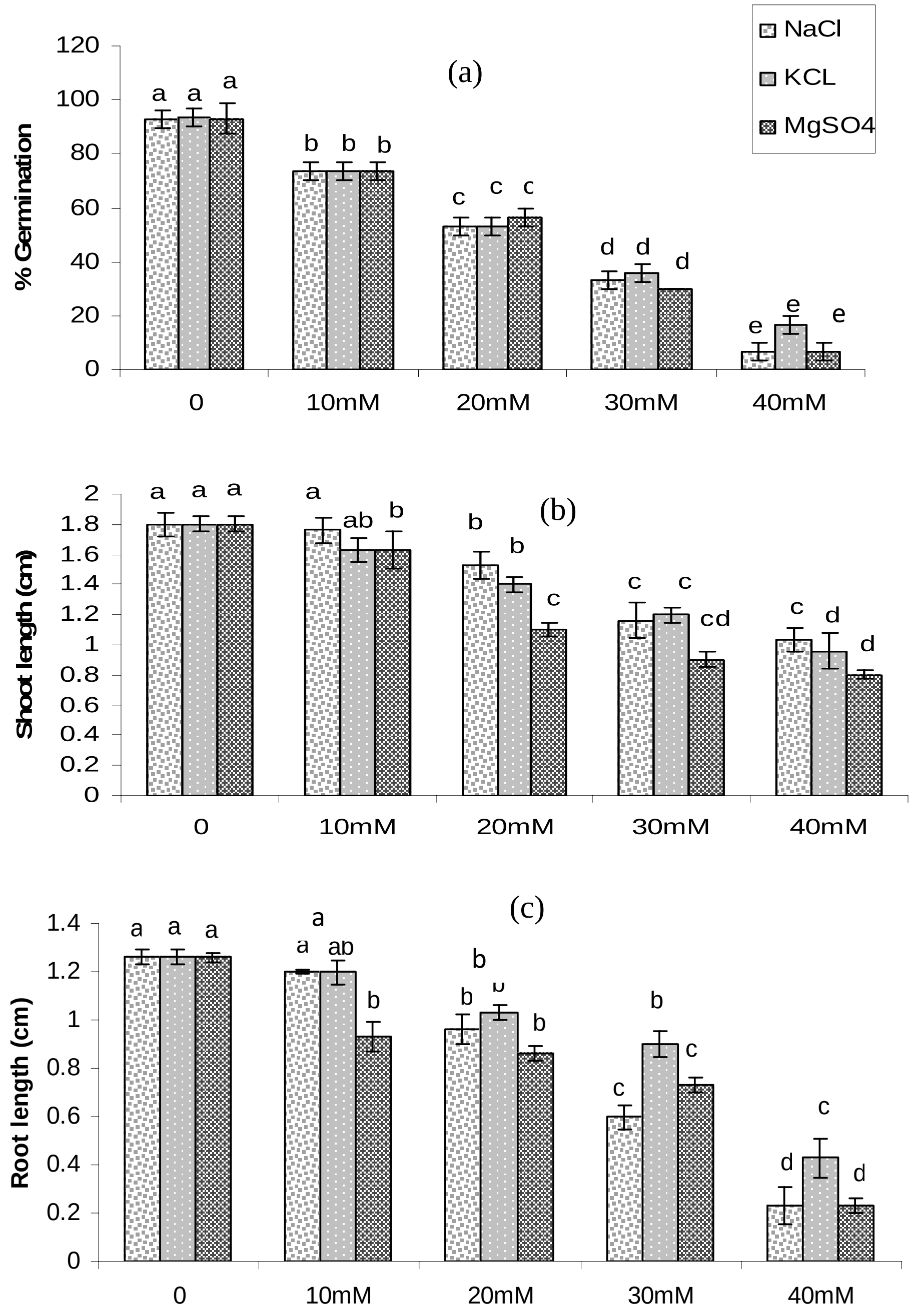

Figure 1. Effect of NaCl, KCl and $MgSO_4$ salinity on germination (a), shoots (b) and root growth (c) of *Parthenium hysterophorus* in pertiplates.

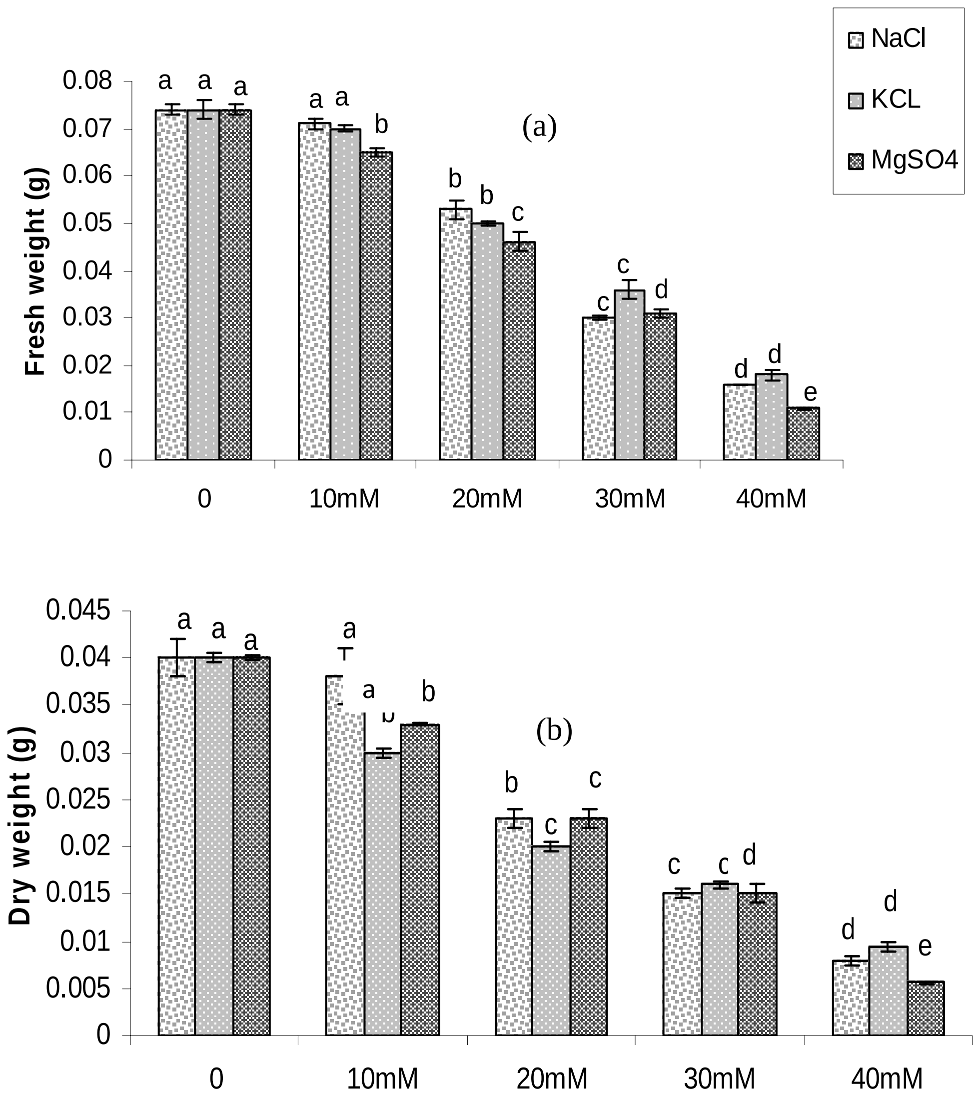

Figure 2. Effect of NaCl, KCl and $MgSO_4$ salinity on fresh biomass (a) and dry biomass (b) of *Parthenium hysterophorus* in pertiplates.

Vertical bars show standard errors of means of three replicates; Values with different letters show significant difference (P = 0.05) according to DMRT.

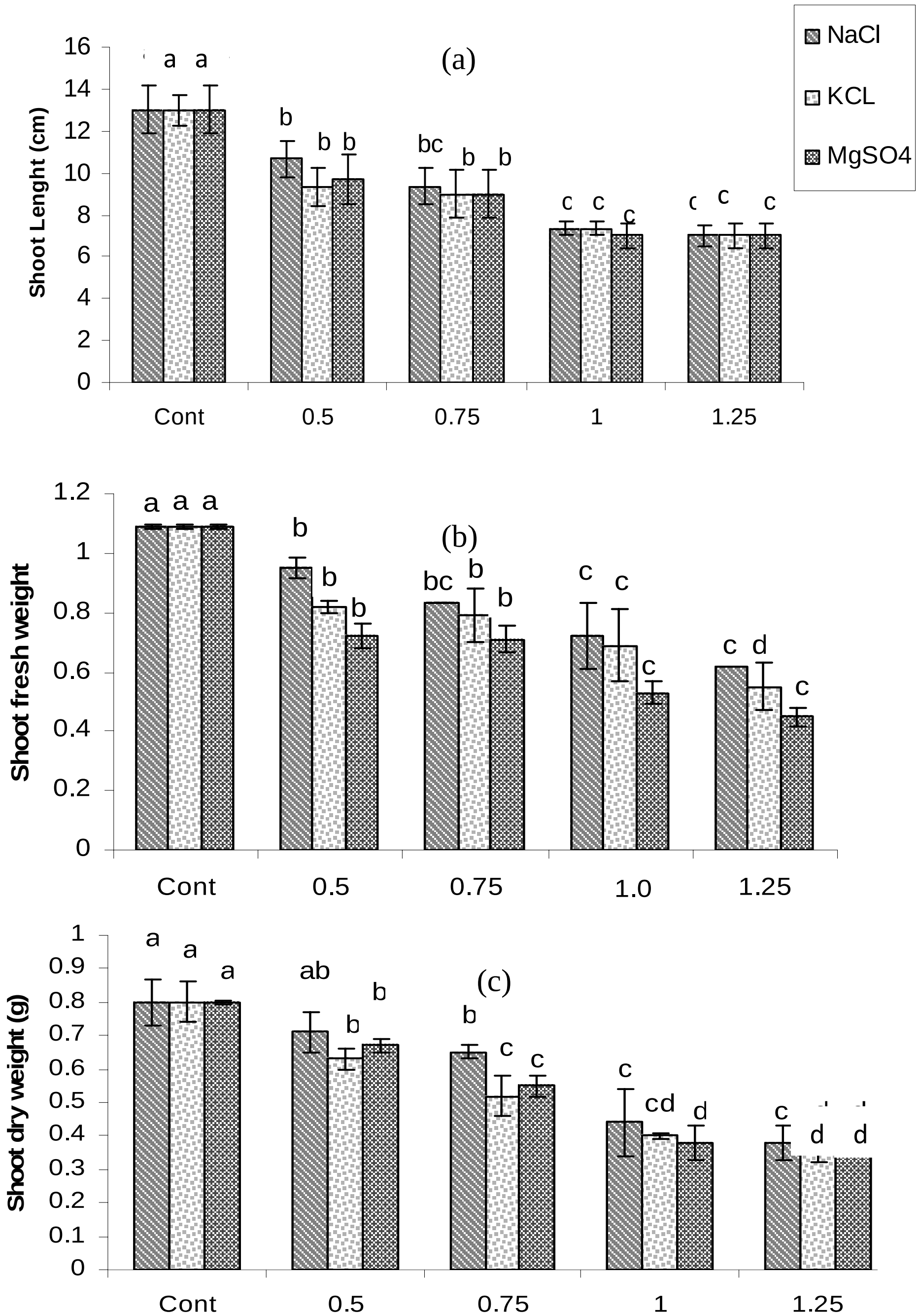

Figure 3. Effect of NaCl, KCl and $MgSO_4$ salinity on shoot length (a), shoot fresh weight (b) and shoot dry weight (c) of *Parthenium hysterophorus* in pot experiment.

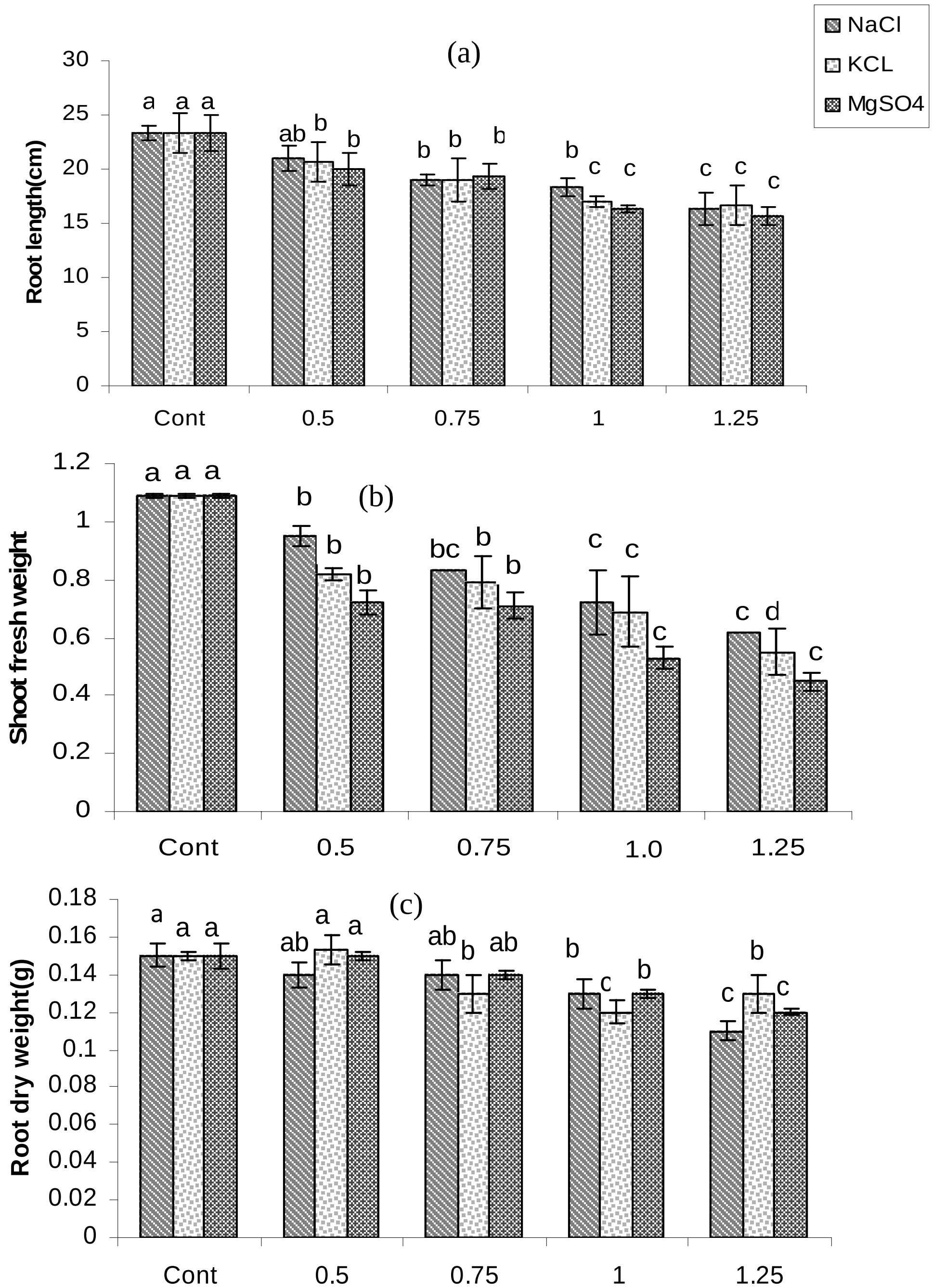

Figure 4. Effect of NaCl, KCl and $MgSO_4$ salinity on Root length (a), shoot fresh weight (b) and root dry weight (c) of *Parthenium hysterophorus* in pot experiment.

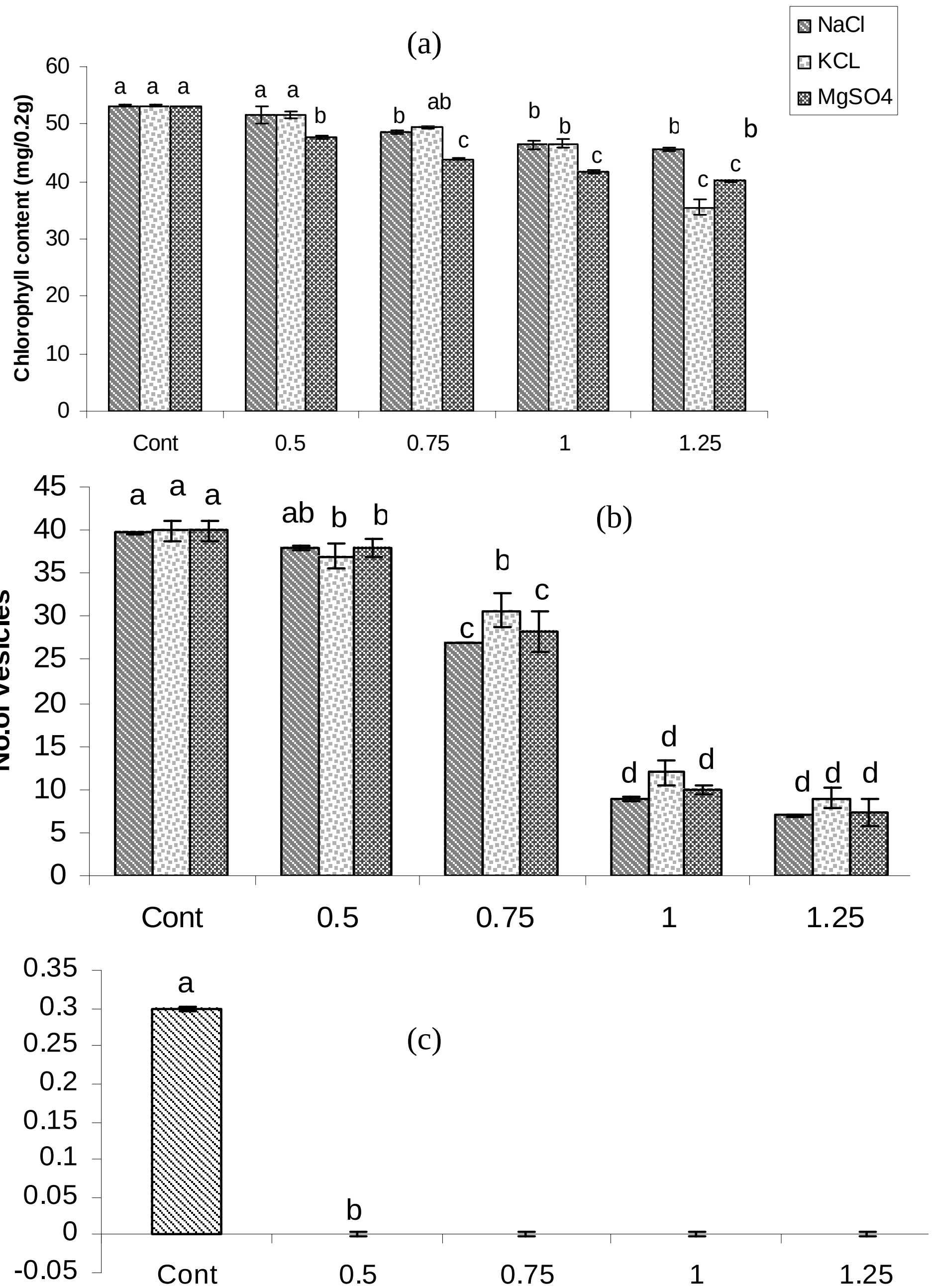

Figure 5. Effect of NaCl, KCl and $MgSO_4$ salinity on chlorophyll content of leaves (a), No. of vesicle (b) and arbuscules in mycorrhizal association (c) of *Parthenium hysterophorus* in pot experiment.

Vertical bars show standard errors of means of three replicates. Values with different letters show significant differences (0.05) according to DMRT.

ACKNOWLEDGEMENTS

The present study is a part of HEC linkage project with foreign universities. The project is being carried out in collaboration with the Khyber Pakhtunkhwa Agricultural University Peshawar, University of the Punjab, and Queensland University, Australia.

REFERENCES CITED

Adkins, S.W., S.C. Navie and R.E. McFadyen. 1996. Control of Parthenium weed (Parthenium hysterophorus L.): A centre for tropical pest management team effort. In R.C.H. Shepherd (Ed.). Proc. 11th Aust. Weeds Conf., Weed Sci. Soc. Victoria, Frankston 573-578 pp.

Adkins, S. W. and S.C. Navie. 2006. Parthenium weed: a potential major weed for agro ecosystems in Pakistan. Pak J. Weed Sci. Res. 12(1-2): 19-36.

Allen, J. A., J. L. Chambers and M. Stine. 1994. Prospects for increasing salt tolerance of forest trees: a review. Tree Physiol. 14: 843-853.

Al-Karaki, G.N. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza. 10: 51–54.

Ajmal, M. and M. Khan. 1986. Effects of coal-fired thermal power plant discharges on agricultural soil and crop plants. Environ. Res. 39(2): 405–417.

Auge, R. M., H. D. Toler, C. E. Sams and G. Nasim. 2008. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza induced increases in stomatal conductance. Mycorrhiza. 18(3): 115-121.

Cresti, M., F. Ciampolini, M. Tattini and A. Cimato. 1994. Effect of salinity on productivity and oil quality of olive (*Olea europaea* L.) plants. Adv. Hortic. Sci. 8: 211–214.

Esechie, H. A. 1996, Interaction of Salinity and Temperature on the Germination of Sorghum. J. Agron. Crop Sci. 172(3):194-199.

Evans, H.C. 1997. *Parthenium hysterophorus*: A review of its weed status and the possibilities for biological control. Biocon. N. Info. 18: 89-98.

Jamil, M., D.B. Lee, W.Y. Jung, M. Ashraf, S.C. Lee and E.S. Rha. 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. J. Europ. Agric. 7: 273-282.

Javaid, A. 2007. Efficacy of some chemical herbicides against *Parthenium hysterophorus* L. Pak. J. Weed Sci. Res. 13: 93-98.

Javaid, A. 2010. Herbicidal potential of allelopathic plants and fungi against *Parthenium hysterophorus* – a review. Allelopathy J. 25: 331-344.
Javaid, A. and H. Adrees. 2009. Parthenium management by cultural filtrates of phytopathogenic fungi. Natural Product Res. 23: 1541-1551.
Javaid, A. and S. Ali. 2011a. Herbicidal activity of culture filtrates of Trichoderma spp. against two problematic weeds of wheat. Natural Product Res. 25: 730-740.
Javaid, A. and S. Ali. 2011b. Alternative management of a problematic weed of wheat *Avena fatua* L. by metabolites of Trichoderma. Chilean J. of Agric Res. 71: 205-211.
Javaid, A. and T. Anjum. 2005. Parthenium hysterophorus L. – A Noxious Alien Weed. Pak. J. Weed Sci. Res. 11(3-4): 81 – 87.
Javaid, A. and T. Anjum. 2006. Control of Parthenium hysterophorus L., by aqueous extracts of allelopathic grasses. Pak. J. Bot. 38(1): 139-145.
Jeannette, S., R. Craig and J. P. Lynch. 2002. Salinity tolerance of phaseolus species during germination and early seedling growth. Crop Sci. 42: 1584-1594.
Kaya, M. D., G. Okçu, Atak, M. Çıkılı and Y. Kolsarıcı. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur. J. Agron. 24: 291- 295.
Kaymakanova, M. 2009. Effect of salinity on germination and seed physiology in Bean (*Phaseolus vulgaris* L.) Biotechnol. & biotechnol. Eq. 23/2009/se special edition/on-line
Keck, T.J., R. J. Wagnet, W. F. Champbell and R. E. Knighton. 1984. Effect of water and salt stress on growth and acetyl reduction in alfalfa. Soil Sci. Soc. Am. J. 6: 1310-1316.
Khan, A. and S. Gulzar. 2003. Light, salinity, and temperature effects on the Seed germination of perennial grasses. American J. Bot. 90(1): 131–134.
Klein, I., Y. Ben-Tal, S. Lavee and I. David. 1992. Olive irrigation with saline water (in Hebrew). Volcani Center Report, Bet Dagan, Israel.
Klein, I., Y. Ben-Tal, S. Lavee, Y. De Malach and I. David. 1994. Saline irrigation of cv. Manzanillo and Uovo di Piccione trees. Acta Hortic. 356: 176–180.
Kohli, R. K., D. R. Batish, H. P. Singh and K. Dogra. 2006. Status, invasiveness and environmental threats of three tropical American invasive Weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.). Biol. Invasions 8: 1501-1510.

Lewis, M. 2002. Spectral characterization of Australian arid zone plants. Canad. J. Remote Sensing. 28: 1-12.

Maas, E.V. 1986. Salt tolerance of plants. Appl. Agric. Res. 1: 12-26.

Marschner, H. 1986. Mineral Nutrition in Higher Plants. Acad. Press, London, pp. 674.

Mujeeb, R., U. Soomro, M. Zahoor and S. Gul. 2008. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) Cultivars. World J. Agric. Sci. 4 (3): 398-403.

Neumann, P. M. 1995. Inhibition of root growth by salinity stress: Toxicity or an adaptive biophysical response, Kluwer Academic Publishers, The Netherlands, pp. 299-304.

Noureen, Z., M. Ashraf and H. Mahmood. 2007. Inter-accessional variation for salt tolerance in pea (*Pisum sativum* L.) at germination and screening stage. Pak. J. Bot. 39(6): 2075-2085.

Ozturk, A., A. Unlukara, A. Ipek and B. Gurbuz. 2004. Effects of salt stress and water deficit on plant growth and essential oil content of lemon balm (*Melissa Officinalis* L.) Pak. J. Bot. 36(4): 787-792.

Rahman, M.H., A. Okubo, S. Sugiyama and H.F. Mayland. 2008. Physical, chemical and microbiological properties of an Andisol as related to land use and tillage practice. Soil and Tillage Res. 101(1-2): 10-19.

Rhoades, J. D. 1974. Drainage for salinity control, Drainage for Agriculture (Ed.) :J. Van Schilfrarde. ASA Mohograph no. 17, Amer. Soc. Agronomy, Madison, Wis., 433-467 pp.

Shannon, M. C., C. M. Grieve, S. M. Lesch and H. Draper. 2000. Analysis of salt tolerance in nine leafy vegetables irrigated with saline drainage water. J. Am. Soc. Hortic. Sci. 125: 658-664.

Steel, R. G. D. and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd Edition. McGraw-Hill Book Company. Pp: 86–167.

Tattini, M., P. Bertoni and S. Caselli. 1992. Genotypic responses of olive plants to sodium chloride. J. Plant Nutr. 15: 1467–1485.

Ueda, A., S. Kanechi, S. Uno and S. Inagaki. 2003. Photosynthetic limitations of a halophyte sea aster (*Aster tripolium*) under water stress and NaCl stress. J. Plant Res. 116: 65-70.

Welbaum, G. E., T. Tissaoui and K. J. Bradford. 1990. Water relations of seed development and germination in muskmelon (*Cucumis melo* L.). III. Sensitivity of germination to water potential and abscisic acid during development. Plant Physiol. 92: 1029–1037.

Wiesman, Z., D. Itzhak and N. B. Dom. 2004. Optimization of saline water level for sustainable Barnea olive and oil production in desert conditions. Sci. Hort. 100: 257–266.

Wiesner, M., T. Taye, A. Hoffmann, P. Wilfried, C. Buettner, I. Mewis and C. Ulrichs. 2007. Impact of the Pan-Tropical weed *Parthenium hysterophorus* L. on human health in Ethiopia. Tropentag. Witzenhausen.

Yurtseven, E., A. Unlukara, A. Top and A. Tek. 2001. Effect of salinity and irrigation interval on yield and vegetative growth of kolza (*Brassica napus oleifera*). First National Irrigation Congress, 8-11 November 2001, Antalya, Turkey.