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Short Communication

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Improving Salinity Tolerance in *Brassica* (*Brassica napus* var. Bsa and *Brassica campestris* var. Toria) by Exogenous Application of Proline and Glycine Betaine

Ameer Khan[a], Hira Fatima[b], Abdul Ghani[a], Muhammad Nadeem[c], Abida Aziz[b], Mujahid Hussain[a]* and Muhammad Ikram[a]

[a]Department of Botany, University of Sargodha, Sargodha, Pakistan
[b]Department of Botany, The University of Lahore, Sargodha, Pakistan
[c]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan

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Abstract. The pot culture experiment was conducted to determine the influence of proline and glycine betaine on *Brassica* under saline conditions. Different salinity levels (0, 65, 130 mM) were created according to the saturation percentage of the soil. Proline (0, 4, 8 mM) and glycine betaine (0, 5, 10 mM) were exogenously applied to find out their effects on growth and physiological changes produced in *Brassica* under salinity stress. Salinity stress reduced the growth of the plants and induced the physiological and biochemical changes. Different growth parameters of plants such as plant height, shoot, root fresh and dry weight was decreased with the increase of salinity stress. Salinity has also reduced the chlorophyll content, protein content and nitrate reductase activity of the *Brassica*. But the application of proline and glycine betaine was more effective to reduce the effect of salinity. Collected data from the present experiment indicated that adverse effects of salinity were counteracted by proline and glycine betaine. Overall, it was observed that exogenous application of both proline and glycine betaine has reduced the effect of salinity.

Keywords: *Brassica napus*, *Brassica compestris*, proline, glycine betaine

Introduction

Soil salinity along with a variety of environmental stresses is now a very serious problem all over the world due to its adverse effects on plant growth and physiology (Taie *et al.*, 2013). Salt stress is a great challenge for agriculture. Yield of the crop is reduced because crops fail to cope with salinity stress (Aymen and Cherif, 2013). There is 22 million hectares arable land in Pakistan. About 24% of crops of Pakistan are grown on rainfed land, whose area is about 4.6 million hectares (Muhammad and Muhammad, 2007). All over the world 35% agricultural production has been decreased due to salinity. Salinity affects 7% of the world's entire land area (Chaum *et al*., 2012). Salinization of arable land is increasing day by day and it is expected that after 25 years 30% of the total land area will face the problem of salt stress (Latef and Chaoxing, 2014; Kapoor *et al*., 2013). Crop growth and productivity are decreased by soil salinity (Cominelli *et al*., 2013).

Plants protect themselves from injurious and destructive effects of salt stress by producing different compatible osmoprotectant metabolites such as proline and glycine betaine (Chelli-Chaabouni *et al*., 2010). These osmolytes gather in the plant and protect tissue and cellular membranes of the plant (Anjum *et al*., 2012). It has been reported that foliar spray of proline and glycine betaine is valuable for plants in mitigating salt induced injuries (Ahmad *et al*., 2012; Hoque *et al*., 2007).

Proline and glycine betaine are also source of carbon and nitrogen. They stabilize the structures of membranes. Proline metabolism has a main role in storage and transfer of energy (Gilberti *et al*., 2014). The effects of salinity stress can be decreased by foliar application of the osmolytes i.e. proline and glycine betaine. In the plants stress tolerance quality is enhanced by the application of foliar spray and it is also a beneficial plan (Ali and Ashraf, 2011).

Brassica species are present in family Cruciferae. Members of the family Cruciferae are known as mustard plants. The petals of the plants belonging to this family are in a cross manner i.e. four petals are cross shaped. Canola (*Brassica napus* and *Brassica campestris* L.) is an important oil seed crop, its world average is 1,820 kg/ha (Chambo *et al*., 2014). 13% of the world's demand of oil is obtained from canola. Oil content of canola seeds is 40% (Snowdon *et al*., 2007). The experiment

*Author for correspondence;
E-mail: mujahid.hussain7877@gmail.com

was conducted to determine the effect of proline and glycine betain on brassica under saline conditions.

Materials and Methods

The pot culture experiment was conducted in Department of Botany, University of Sargodha, Sargodha Pakistan. Proline and glycine betaine were applied exogenously to improve salinity tolerance in plants. The experiment was laid out in completely randomized design (CRD) with three replications.

Physiological parameters. *Chlorophyll content*. Method proposed by Davies (1976) was used to compute the chlorophyll contents. The extraction from the 0.5 cm chopped leave pieces was done with 5 mL acetone (80%) and kept at 10 °C. The absorbance of the supernatant was measured at 645 and 663 nm on spectrophotometer. Chl a, Chl b and total chlorophyll were calculated by using the following formula:

Chl. a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000 × W
Chl. b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000 × W
Total Chl. = [20.2 (OD 645) + 8.02 (OD 663)] × V/100 × W
V = Volume of the extract
W = Weight of the sample

Total free amino acids. Method proposed by Hamilton and Van Slyke (1943) was used to compute the total free amino acids. Chopped segments of leaves were extracted with 0.2M phosphate buffer of 7.0 pH. 1 mL from extract, 10% pyridine and 2% ninhydrin solution were put in the test tube. Distilled water was used to make the volume upto 50 mL. The optical density of this coloured solution was seen at 570 nm on spectrophotometer (Hitatchi, 220, Japan). A standard curve was made with Leucine and then calculation for free amino acids was done by this formula:

$$\text{Total amino acids (mg/g fresh wt)} = \frac{\text{Graph reading of sample} \times \text{volume of sample} \times \text{dilution factor}}{\text{Weight of fresh tissue}} \times 100$$

Nitrate reductase activity (NRA). Method proposed by Sym (1984) was used to compute the nitrate reductase activity.

Procedure. Phosphate buffer with the molarity of 0.02 M was added in leaf sample. From this mixture 1 mL was taken out and 0.02 M KNO_3 solution was entered in it. The amount of KNO_3 used for this purpose was 1 mL and 1-naphthyl ethylene diamine dihydrochloride (0.02%) was added in the solution after vigorous shaking of 1-naphthyl ethylene diamine dihydro-chloride. With NO_2 diazocomplex, a pink colour was produced. Spectrophotometer was used to determine the absorbance at 542 nm.

Total soluble sugars. Method proposed by Yemm and Willis (1954) was used to compute the total soluble sugars.

Procedure. In the test tubes of 25 mL, plant extract (0.3 mL) was added. 6 mL of anthrone reagent was added in the test tubes. Then the test tubes were warmed in the boiling water bath for 10 min. These test tubes were cooled down by placing them in chilled water for 10 min and then incubated for 20 min by maintaining the temperature at 25 °C. Spectrophotometer was used to measure the optical density at 625 nm (Hitatchi, 220, Japan). Standard curve was developed for the calculation of soluble sugars.

Na and K analysis. *Digestion*. In the digestion tubes concentrated H_2SO_4 was added with 0.5 g of ground material (Wolf and Stahl, 1982). 35% hydrogen peroxide was added in the digestion tubes. After this they were heated at 350 °C in the digestion block. This process of heating was continued for 30 min. 0.5 mL of hydrogen peroxide was added in it. For making the digested material colourless 0.5 mL hydrogen peroxide was added and the tubes were placed again in the digestion block. This step was done again and again until the solution became colourless. The volume was kept 50 mL in volumetric flasks by adding distilled water.

Estimation of cations (Na^+ and K^+). Method proposed by Jenway (PFP 7) was used to determine sodium (Na^+) and potassium (K^+) with the help of flame photometer PFP7 (Yilmaz and Yavuz, 1999).

Calcium determination. Method proposed by salinity laboratory (Kunze and Dixon, 1986) was used to determine calcium.

Yield and yield components. Plant height was recorded using a meter rod. Data for yield and yield components were recorded at maturity.

Statistical analysis. Analysis of variance of the data from each attribute was computed using three factor factorial design (Steel *et al*., 1997).

Results and Discussion

As shown by Table 1 that salinity stress has significantly reduced the growth and production of brassica but the

exogenous application of proline and glycine betaine minimizes the effect of salinity. Salinity has decreased plant height (Fig. 1), shoot fresh weight (Fig. 2), shoot dry weight (Fig. 3), root fresh weight (Fig. 4), root dry weight (Fig. 5), Chl a (Fig. 6), Chl b (Fig. 7), total Chl (Fig. 8), total soluble sugars (Fig. 9), protein (Fig. 10), total free amino acids (Fig. 11), NRA (Fig. 12), potassium (Fig. 13), calcium (Fig. 14) and sodium (Fig. 15) increased under salt stress. As shown that 8 mM and 10 mM concentration of proline and glycine betaine were more effective to reduce the effect of salinity as compared to 4 mM and 5 mM concentrations of proline and glycine betaine. Sodium ions present in the growth mediums showed antagonistic effect on calcium and potassium ions.

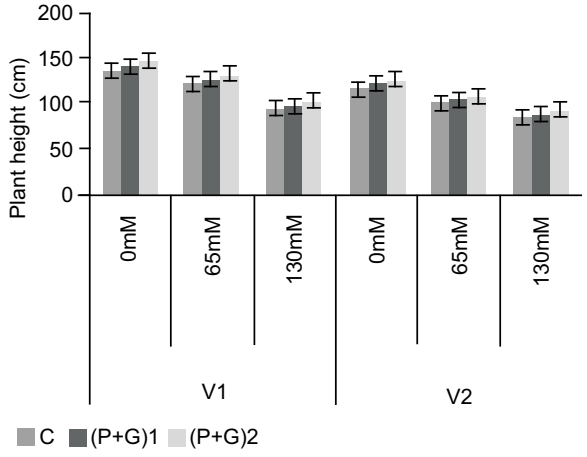

Fig. 1. Concentration of proline/glycine betaine.

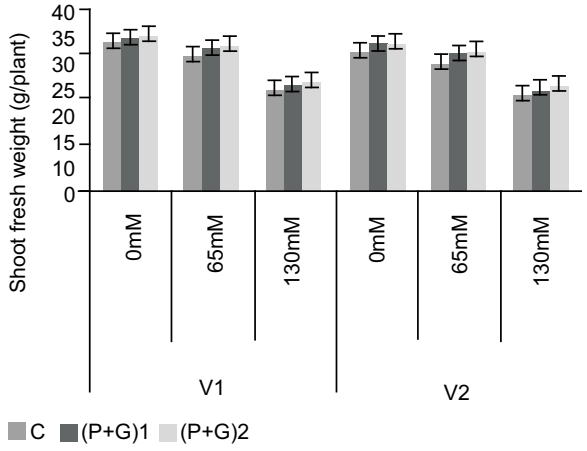

Fig. 2. Concentration of proline/glycine betaine.

Table 1. Salinity effects on *Brassica* growth and production

SOV	DF	Parameters														
		Plant height	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Chl a	Chl b	Total Chl	Sugars	Proteins	A.A	NRA	Na	Ca	K
Variety (V)	1	2361.92**	14.10**	20544.7**	297.47**	482.23**	20.49**	20.72**	142841**	1202.56**	8517.43**	538452**	7618.79**	4593750**	80.60**	4511001**
Treatment (T)	2	3667.83**	263.18**	131470**	1786.21**	5189.66**	2516.62**	6440.18**	668953**	10245.7**	99259.5**	6194365**	226283**	1.1E+08**	4918.25**	4.0E+07**
Chemicals (C)	2	188.26**	12.16**	1477.30**	36.41**	464.87**	32.59**	195.34**	157850**	137.93**	5292.14**	1349845**	13771.5**	1.4E+07**	379.26**	2998814**
V × T	2	115.95**	0.14NS	372.45**	6.71**	38.32**	0.00NS	0.00NS	109487**	274.53**	892.85**	279862**	2045.37**	656250**	0.00NS	377554**
V × C	2	1.93NS	0.26NS	6.01**	0.14NS	10.59**	0.00NS	0.00NS	110148**	5.51**	67.22**	3070.57**	123.20**	0.00NS	0.00NS	12138.5**
T × C	4	2.63*	0.32NS	9.36**	0.46NS	19.05**	1.91*	13.17**	137291**	1966.87**	112.45**	73593.8**	928.13**	143569**	13.89**	92754.2**
V × T × C	4	0.54NS	0.09NS	3.33*	0.34NS	5.42**	0.00NS	0.00NS	121428**	16.84**	93.59**	39303.2**	816.04**	0.00NS	0.00NS	2969.79**
Error	36															
Total	53															

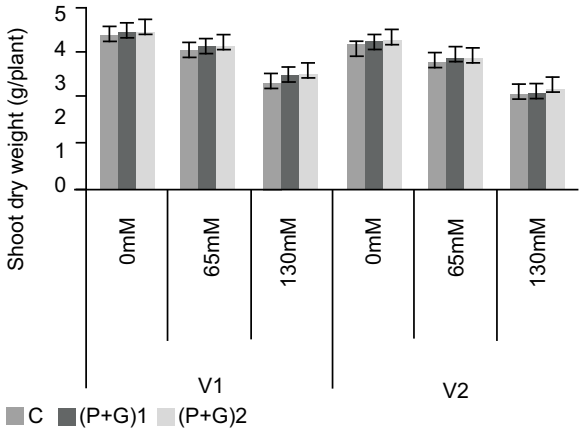

Fig. 3. Concentration of proline/glycine betaine.

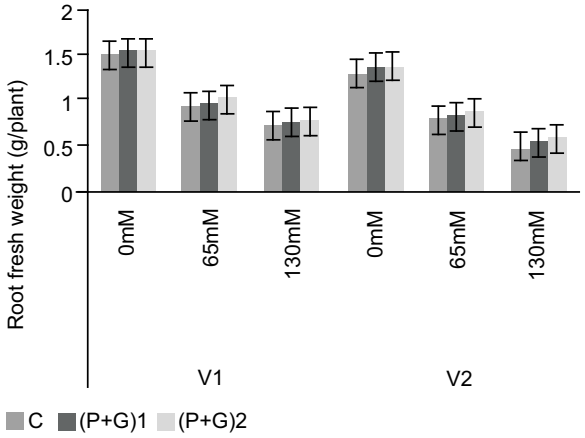

Fig. 4. Concentration of proline/glycine betaine.

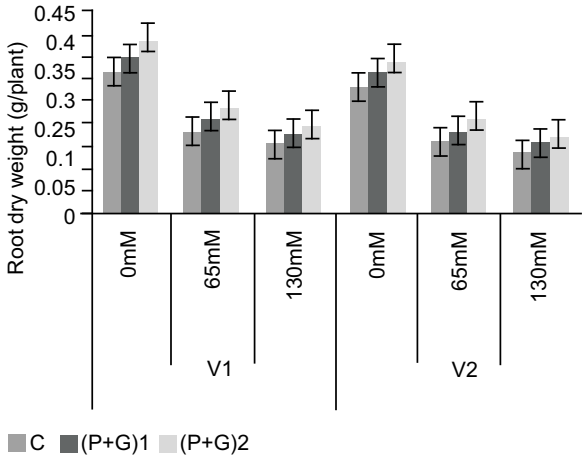

Fig. 5. Concentration of proline/glycine betaine.

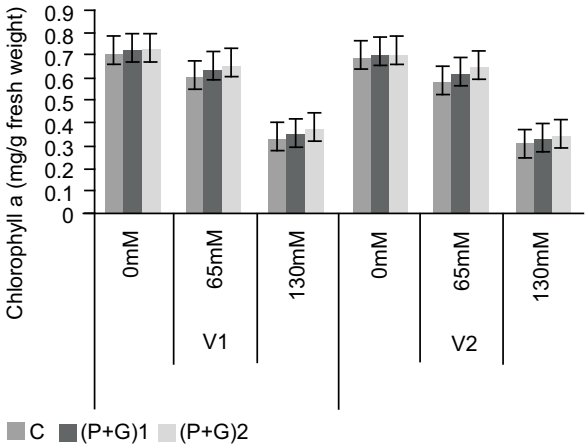

Fig. 6. Concentration of proline/glycine betaine.

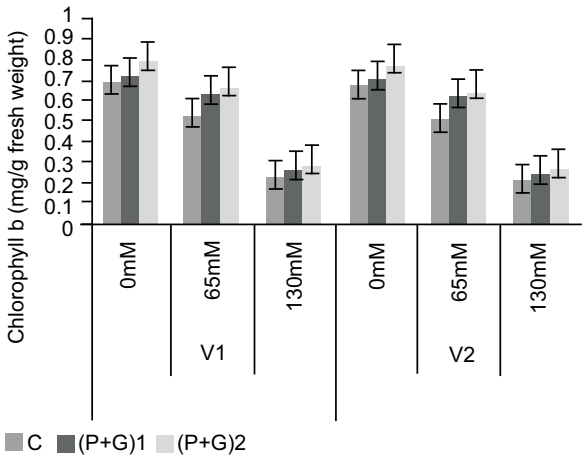

Fig. 7. Concentration of proline/glycine betaine.

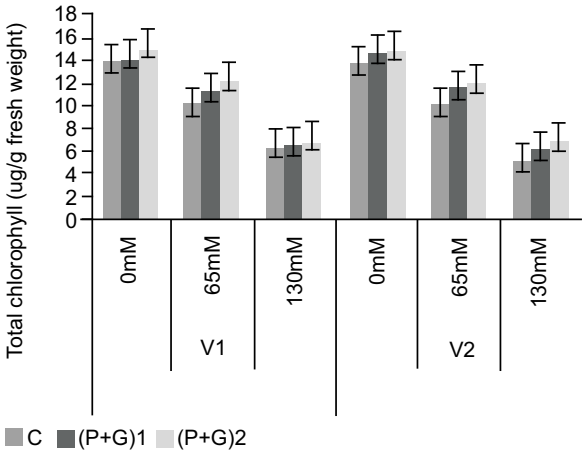

Fig. 8. Concentration of proline/glycine betaine.

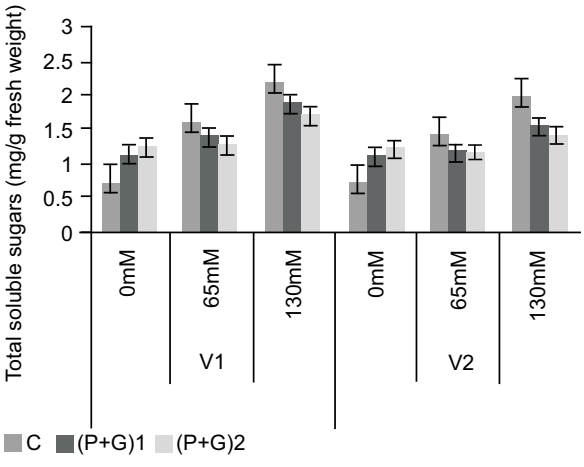

Fig. 9. Concentration of proline/glycine betaine.

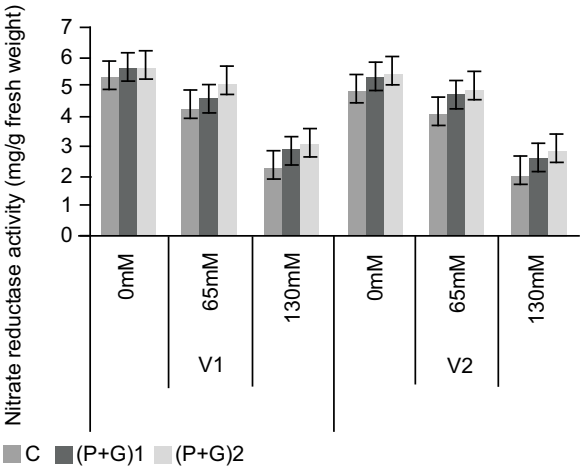

Fig. 12. Concentration of proline/glycine betaine.

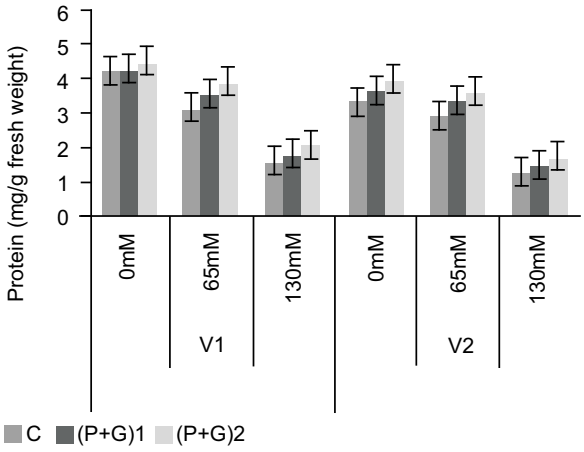

Fig. 10. Concentration of proline/glycine betaine.

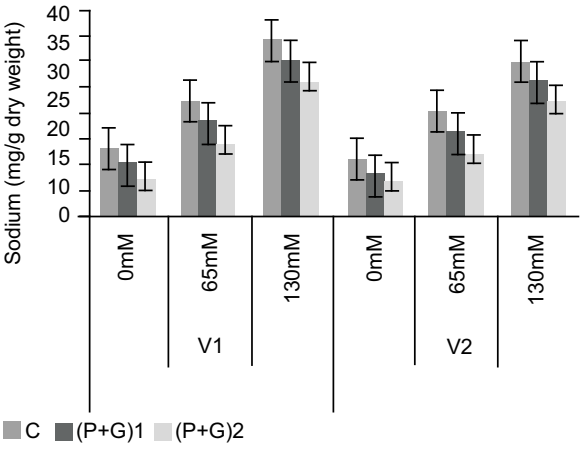

Fig. 13. Concentration of proline/glycine betaine.

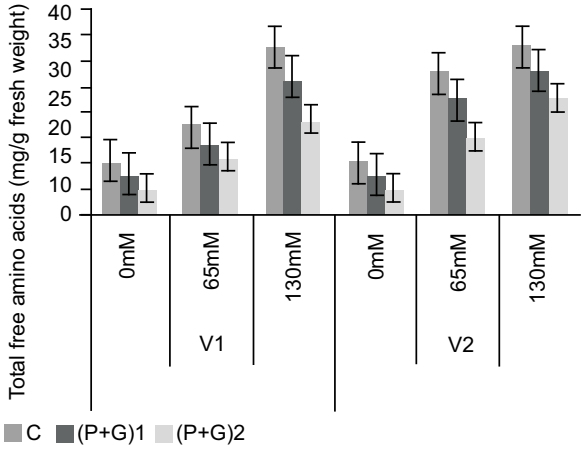

Fig. 11. Concentration of proline/glycine betaine.

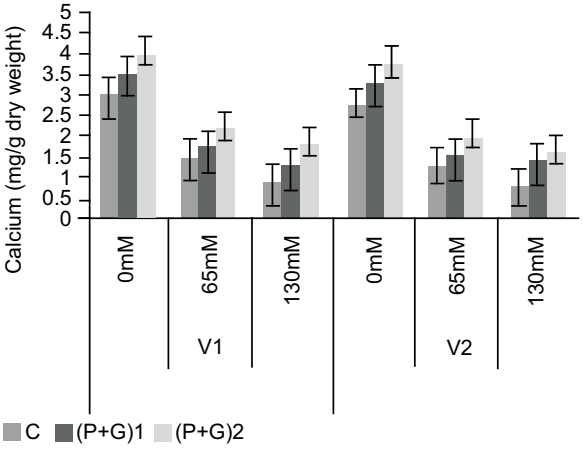

Fig. 14. Concentration of proline/glycine betaine.

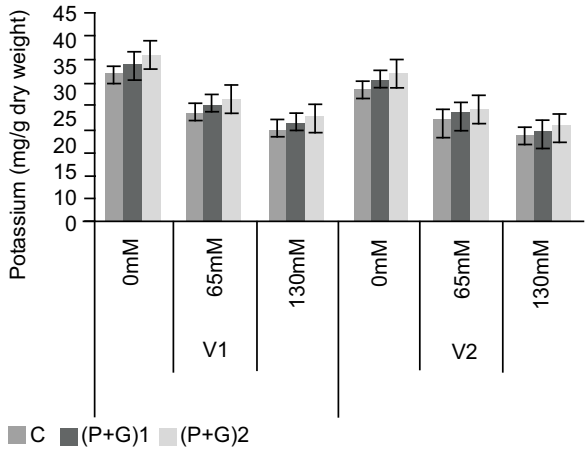

Fig. 15. Concentration of proline/glycine betaine.

Figure 1-15 shows the effect of exogenous application of proline and glycine betaine on plant height, shoot and root fresh and dry weight, shoot, Chl a, Chl b, total Chl, protein, total free amino acids, NRA, sodium, calcium, potassium of brassica under saline and non-saline conditions.

Salinity tolerance in brassica increased by exogenous application of proline and Glycine betaine. Glycine betaine content was observed to be increased under salt stress (Amandeep *et al*., 2014).

Glycine betaine was found effective to decrease salt stress damage when it was exogenously applied on canola (Athar *et al*., 2015). With glycine betaine, proline also play role in improving plant growth. The application of 1 and 5 mM proline improved the growth of brassica varieties (Posmyk and Janas, 2007). Salinity levels increases uptake of Na^+ in all plant parts including root, stem and leaf. Simultaneously it decreases the calcium and potassium uptake due to antagonistic effects. The addition of Na^+ in plant parts increased as the level of salinity was increased. The amount of proline, soluble carbohydrates and reduced sugar increased as salinity increased (Mostajeran and Gholaminejad, 2014).

Proline is a widespread compatible solute. There are many roles for proline in saving plants from harmful and damaging effects of salinity. It can stabilize the membranes and guard them from harmful ions which can destroy their structure (Khan *et al*., 2009). Proline and glycine betaine can be used to check the salt tolerance ability of different plant species (Ahmad *et al*., 2009).

Foliar spray of proline and glycine betaine enhances the development of both salt affected and non-stressed plants of canola varieties. Same results have been observed in maize (Nawaz and Ashraf, 2007) and wheat (Raza *et al*., 2007).

It is clear from the outcomes of current study that salinity stress reduced plant growth, plant height, dry biomass and fresh weight. Growth of all the plants increased by applications of proline and glycine betaine, either the plants were facing salinity or were grown under non saline conditions. Chl a, Chl b and total chlorophyll were also reduced under salt stress but their value increased when proline and glycine betaine were applied on the plants. The 4 and 5 mM levels of proline and glycine betaine are less effective to reduce salinity than 8 and 10 mM levels of proline and glycine betaine. Proline and glycine betaine decreased the harmful effects of salinity stress and enhanced the growth of plants. The encouraging effects are clear from the above results.

References

Ahmad, P., Hakeem, K.R., Kumar, A., Ashraf, M., Akram, N.A. 2012. Salt induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *African Journal of Biotechnology*, **11:** 2694-2703.

Ahmad, P., Jeleel, C.A., Azooz, M.M., Nabi, G. 2009. Generation of ROS and non-enzymatic antioxidants during abiotic stress in plants. *Botany Research International*, **2:** 11-20.

Ali, Q., Ashraf, M. 2011. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *Journal of Agronomy Crop Science*, **197:** 258-271.

Amandeep, R., Kapoor, N., Bhardwaj, R. 2014. Role of Brassinosteroids in osmolytes accumulation under salinity stress in *Zea mays* plants. *International Journal of Science and Research*, **3:** 2319-7064.

Anjum, S.A., Farooq, M., Xie, X., Liu, X.J., Ijaz, M.F. 2012. Antioxidant defense system and proline accumulation enables hot pepper to perform better under drought. *Scientia Horticulturae*, **140:** 66-73.

Ashraf, M., Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, **59:** 206-216.

Athar, H.U.R., Zafar, Z.U., Ashraf, M. 2015. Glycine

betaine Improved photosynthesis in canola under salt stress: evaluation of chlorophyll fluorescence parameters as potential indicators. *Journal of Agronomy and Crop Science*, **201:** 428-442.

Aymen, E.M., Cherif, H. 2013. Influence of seed priming on emergence and growth of coriander (*Coriandrum sativum* L.) seedlings grown under salt stress. *Acta Agricultural Slovent*, **101:** 41-47.

Chambo, E.D., Oliveira, N.T.D., Garcia, R.C., Duarte-Junior, J.B., Ruvolo-Takasusuki, M.C.C., Toledo, V.A. 2014. Pollination of rapeseed (*Brassica napus*) by Africanized honeybees (*Hymenoptera*: Apidae) on two sowing dates. *Anais da Academia Brasileira de Ciências*, **86:** 2087-2100.

Chaum, S., Singh, H.P., Samphumphuang, T., Kirdmanee, C. 2012. Calcium-alleviated salt tolerance in indica rice (*Oryza sativa* L. spp. *indica*): physiological and morphological changes. *Austratian Journal of Crop Sciences*, **6:** 176-182.

Chelli-Chaabounia, A., Mosbahb, A.B., Maalej, M., Gargouric, K., Gargouri-Bouzidd, R., Drira, N. 2010. *In vitro* salinity tolerance of two pistachio rootstocks: *Pistacia vera* L. and *P. atlantica* Desf. *Environmental and Experimental Botany*, **69:** 302-312.

Cominelli, E., Conti, L., Tonelli, C., Galbiati, M. 2013. Challenges and perspectives to improve crop drought and salinity tolerance**.** *New Biotechnology Journal*, **8:** 355-361.

Davies, B.H. 1976. *Chemistry and Biochemistry of Plant Pigments*. T. W. Goodwin (eds.), vol. **2,** pp. 38-165, 2nd edition, Academic Press, London, UK.

Gilberti, S., Funck, D., Forlani, G. 2014. D1-pyrroline-5-carboxylate reductase from *Arabidopsis thaliana*: stimulation or inhibition by chloride ions and feedback regulation by proline depend on whether NADPH or NADH acts as co-substrate. *New Phytologist*, **202:** 911-919.

Hamilton, P.B., VanSlyke, D.D. 1943. Amino acid determination with ninhydrin. *Journal of Biological Chemistry*, **150:** 231-233.

Hoque, M.A., Banu, M.N.A., Okuma, E., Amako, K., Nakamura, K., Shimoishi, Y., Murata, Y. 2007. Exogenous proline and glycinebetaine increase NaCl induced ascorbate glutathione cycle enzyme activities and proline improves salt tolerance more than glycinebetaine in tobacco bright yellow 2 suspension cultured cells. *Journal of Plant Physiology*, **164:** 1457-1468.

Kapoor, R., Evelin, H., Mathur, P., Giri, B. 2013. Arbuscular mycorrhiza Approaches for Abiotic Stress Tolerance in Crop Plants for Sustainable Agriculture. In: *Plant Acclimation to Environmental Stress*, N. Tuteja and S. S. Gill (eds.), pp. 359-401, Springer, New York, USA.

Khan, M.A., Shirazi, M.U., Khan, M.A., Mujtaba, S.M., Islam, E., Mumtaz, S., Shereen, A., Ansari, R.U., Ashraf, M.Y. 2009. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*, **41:** 633-638.

Kunze, G.W., Dixon, J. 1986. Pretreatment for mineralogical analysis. *Methods of Soil Analysis*: *Part-1*, 2nd edition, *Physical and Mineralogical Methods*, pp. 91-100, American Society of Agronomy, Wisconsin, USA.

Latef, A.A.H.A., Chaoxing, H. 2014. Does the inoculation with *Glomus mosseae* improve salt tolerance in pepper plants. *Journal of Plant Growth Regulation*, **33:** 644-653.

Mostajeran, A., Gholaminejad, A. 2014. Effect of salinity on sodium and potassium uptake and proline, carbohydrates contents of turmeric plant parts. *Journal of Current Chemical and Pharmaceutical Sciences*, **4:** 10-21.

Muhammad, S., Muhammad, T.S. 2007. Comparative use of organic substrates and mineral fertilizers in growing maize (*Zea mays* L.) for maintaining soil fertility in rainfed area of Pakistan. *Pakistan Journal of Botany*, **39:** 1953-1959.

Nawaz, K., Ashraf, M. 2007. Improvement in salt tolerance of maize by exogenous application of glycinebetaine: growth and water relations. *Pakistan Journal of Botany*, **39:** 1647-1653.

Posmyk, M.M., Janas, K.M. 2007. Effects of seed hydropriming in presence of exogenous proline on chilling injury limitation in *Vigna radiata* L. seedlings. *Acta Physiologiae Plantarum*, **29:** 509-517.

Raza, S.H., Athar, H.R., Ashraf, M., Hameed, A. 2007. GB-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environmental and Experimental Botany*, **60:** 368-378.

Snowdon, R., Luhs, W., Friedt, W. 2007. Oil seed rape. *Oil Seeds*, pp. 55-114, Springer Berlin Heidelberg, Germany.

Steel, R.G.D., Torrie, J.H., Dickey, D.A. 1997. *Principles and Procedures of Statistics. A Biometrical Approach*, 666 pp., 3rd edition, McGraw Hill Book Co., New York, USA.

Sym, G.J. 1984. Optimization of the *in vivo* assay condition for nitrate reductase in barley. *Journal of the Science of Food and Agriculture*, **35:** 550-556.

Taie, H., Abdelhamid, M.T., Dawood, M.G., Nassar, R.M. 2013. Presowing seed treatment with proline improves some physiological, biochemical and anatomical attributes of faba bean plants under sea water stress. *Journal of Applied Sciences*, **9:** 2853-2867.

Wolf, B., Stahl, O. 1982. AG CAR-A Galactic S DOR variable. *Astronomy and Astrophysics*, **112:** 111-115.

Yimm, E.W., Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, **57:** 508-514.

Yilmaz, H., Yavuz, O. 1999. Content of some trace metals in honey from south-eastern Anatolia. *Food Chemistry*, **65:** 475-476.

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Efficacy of Boron as Foliar Feeding on Yield and Quality Attributes of Maize (*Zea mays* L.)

Muhammad Tahir[ac], Waqas Shehzad[a], Muhammad Aqeel Sarwar[ab]*, Sajid Hussain[a] and Muhammad Imran[b]

[a]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
[b]Crop Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan
[c]College of Agriculture, Bahauddin Zakariya University, Bahadur Sub Campus, Layyah, Pakistan

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Abstract. Boron being an essential micronutrient plays a vital role in improving crop productivity and quality. Therefore a field study was conducted at agronomic research area, University of Agriculture, Faisalabad, Pakistan, during spring season 2014, to evaluate the effect of boron as foliar application on yield and quality of maize. The experiment was laid out in randomized complete block design with three repeats. There were nine treatments in the experiment including a control treatment, four treatments of foliar sprays of 0.5% borax and four treatments of water sprays (without borax) with different time intervals. Statistical analysis revealed that use of boron significantly affected maize yield and quality and the application of boron with three foliar sprays of 0.5% borax had significant positive effect on most of yield contributing parameters, giving plant height (217.00 cm), cob length (15.81 cm), cob diameter (6.10 cm), cob weight (147.00 g), number of grains per cob (481.00), 1000 grain weight (333 g), grain yield (5.26 t/ha), biological yield (14.75 t/ha) and harvest index (35.66%). However in case of quality, four foliar sprays of 0.5% borax displayed better results by producing higher seed protein (10.06%) and oil contents (4.72%), respectively. From the results of experiment it can be concluded that three foliar sprays of 0.5% boron triggers yield attributes while four foliar sprays of 0.5% boron encourages qualitatively to maize crop.

Keywords: maize, boron, foliar application, yield

Introduction

Maize (*Zea mays* L.) being the highest yielding cereal crop in the world after wheat and rice holds a prominent position in major crops. In Pakistan it is cultivated in all the provinces, with Punjab and Khyber Pakhtunkhwa being the major producers. It fits well in our cropping system due to higher yield potential, short duration, high net economic return and ability to grow in varying environments (Shahzad *et al*., 2012). Being a multi-purpose crop for food, feed and fodder; currently, except potato, maize is the most profitable and dependable crop in our agriculture system. At present 1.13 million hectares are utilized for maize cultivation in Pakistan with total production of 4.69 million tonnes and an average grain yield of 4.15 t/ha (GOP, 2014). Although the soil and climatic conditions of Pakistan are favorable for maize production, but per hectare yield is very low as compared to other maize growing countries of the world. Low yield of maize in Pakistan is due to many constraints but among them, poor plant nutrition (micronutrients), traditional sowing methods and lack of optimal crop stand are the factors of prime importance (Ahmad *et al*., 2011; Alias *et al*., 2008).

Various micronutrients including boron and their application methods have significant effect on growth and yield of different crops (Nadim *et al*., 2013; Ahmad *et al*., 2012). However, maize is the kind of crop especially sensitive to micronutrients deficiency and definite quantity of micronutrients is indispensable for its proper growth and development (Ziaeyan and Rajaie, 2009). Boron is one of those micronutrients which are rapidly being deficient in soils. It is needed in small amount but proved essential micronutrient for plant growth. Its deficiency symptoms and nutritional disorder characteristics appear when plant faces reduced supplies of boron (Leghari *et al*., 2016). Boron impacts transport of carbohydrates, cell division, cell wall strength and development, onset of fruits and seed development and hormonal production (Gunes *et al*., 2003). Its severe deficiency causes abnormal development of reproductive organs and ultimately results in reduction of plant yield **(**Rashid *et al*., 2004). Boron is able to mitigate the drought effects as explained by Davis *et al*. (2003) and its application improved the parameters of the main

*Author for correspondence; E-mail: maqeeluaf@gmail.com

yield components, thus increasing yield level and enriching the chemical composition of crops.

Boron can be used as soil and foliar applications on growing crop. Foliar application of boron is believed to retain significant carbohydrate phloem mobility to flowering meristematic cell from either senescing leaves/bark. Thus foliar spray of boron is not only a source to apply boron at a particular growth stage but also permits a rapidly-acting action to mitigate the problem soon after the deficiency diagnosis (Shahzad *et al*., 2012). Therefore, the present study was conducted to elucidate the effect of boron as foliar application on yield and quality of maize under Faisalabad conditions.

Materials and Methods

The experiment was conducted at the research area of Agronomy Department, University of Agriculture, Faisalabad, Pakistan, during spring 2014. The experimental area is located between 30.35-31.47° N latitude and 72.08-73° E longitude at an elevation of 150 m above the sea level. The experiment was carried in randomized complete block design with triplicate run and a net plot size of 5 × 4.20 m. Experimental treatments was performed as:

T_0 = Control (no water and boron spray)
T_1 = One water spray at 4-5 leaves stage (V4-V5 stage)
T_2 = One foliar spray of 0.5% borax ($Na_2B_4O_7 \cdot 10H_2O$) at 4-5 leaves stage
T_3 = Two water sprays (1st at 4-5 leaves stage and 2nd with one week interval)
T_4 = Two foliar sprays of 0.5% borax (1st at 4-5 leaves stage and 2nd with one week interval)
T_5 = Three water sprays (1st at 4-5 leaves stage, 2nd and 3rd with one week interval)
T_6 = Three foliar sprays of 0.5% borax (1st at 4-5 leaves stage, 2nd and 3rd with one week interval)
T_7 = Four water sprays (1st at 4-5 leaves stage, 2nd, 3rd and 4th with one week interval)
T_8 = Four foliar sprays of 0.5% borax (1st at 4-5 leaves stage, 2nd, 3rd and 4th with one week interval).

Composite soil samples were collected at a depth of 30 cm. then air dried, crushed, and tested for physical and chemical properties of soil. The research field had a clay loam soil, having soil pH (7.7), O.M (0.88%), E.C (2.4 d/Sm), N (0.21%), P (13.2 mg/kg), K (174.5 mg/kg) and B (0.2 mg/kg). Maize hybrid "Monsanto-6525" was used as test crop. Sowing was done on 10th February, 2011 with the help of hand drill at 70 cm spaced rows using seed rate of 25 kg/ha. Fertilizer was applied @ 250-125-125 kg/NPK/ha. Half of N and whole of P and K were applied at sowing in the form of urea, diammonium phosphate and sulphate of potassium, respectively, while remaining nitrogen was applied in splits. Boron fertilizer as borax (11% B) was applied per treatment and the volume of water used for each boron foliar spray was @ 250 L/ha. Crop was kept weed free by hoeing twice to avoid weed-crop competition. Insect pest were kept under the threshold level through chemical control. For this purpose Furadon was applied @ 20 kg/ha after first irrigation to control stem borer and shoot fly. All agronomic practices except those under study were kept normal and uniform for all the treatments. The crop was harvested manually 115 days after sowing at its physiological maturity on 15th of June 2014. Ten plants were selected at random at harvest from each plot for sampling and observations regarding plant height at maturity (cm), cob length (cm), cob diameter (cm), cob weight (g), number of grains per cob were noted and then averaged for recording mean of these parameters. Similarly, from the seed lot of every plot, five samples, each of 1000 grains were randomly taken, recorded their weight and then mean 1000 achene weight was computed. The grain yield and biological yield were taken on plot basis for each treatment, averaged and then converted to kg/ha. While harvest index was computed as the ratio of achene yield to biological yield and calculated as follows:

Harvest index (%) = Achene yield/biological yield × 100

Data collected was statistically analyzed using Fisher's analysis of variance technique and treatment's means were compared by LSD at 0.05 probability (Steel *et al*., 1997).

Results and Discussion

Maize yield attributes. Data regarding yield attributes of maize crop as affected by boron foliar applications are presented in Table 1-2. Significant increase in plant height was observed by boron supplement ($P \leq 0.05$). Maximum plant height (217.00 cm) was measured in T_6 (three foliar sprays of 0.5% borax) followed by T_4 (two foliar sprays of 0.5% borax) and T_8 (four foliar sprays of 0.5% borax) with plant height 214.00 cm and

212.33 cm, respectively, which were statistically significant as compared to T_1 (control) measuring plant height (197.67 cm). The improvement in plant height due to application of boron might be, because it increased the internodal length of plant by increasing number of cells as it is known that the boron is associated with the development of cell wall and cell differentiation and hence, helps in root elongation and shoot growth of plant (Shehzad and Maqsood, 2015). Similar findings have also been described by Tombo *et al*. (2008) who stated that plant height was positively and significantly affected by boron applications.

Comparison of treatment means ($P \leq 0.05$) showed significant effects of boron feeding on cob length. Largest cob length (15.81 cm) was recorded in T_6 (three foliar sprays of 0.5% borax) which was statistically at par with T_4 (two foliar sprays of 0.5% borax) having cob length 15.77 cm, while the treatments T_8 (four foliar sprays of 0.5% borax) and T_2 (one foliar spray of 0.5% borax) devising cob length (15.40 cm and 15.35 cm, respectively) were followed by T_4. However, the smallest length of cob (14.21 cm) was recorded in T_0 (control), where no water and boron spray was applied. The increase in cob size by boron application was because of its role in cell division, expansion and other physiological processes like, inhibiting the unnecessary conversion of sugars into starch which is essential for cob development. These results are in strong line with Tahir *et al*. (2012) who reported similar findings for cob development.

The statistics regarding the cob length displayed ($P \leq 0.05$), larger cob diameter in all boron foliar applied treatments. Maximum cob diameter (6.10 cm) was resulted in T_6 (three foliar sprays of 0.5% borax) tracked by T_4 (two foliar sprays of 0.5% borax), T_8 (four foliar sprays of 0.5% borax) and T_2 (one foliar spray of 0.5% borax) having cob diameter (6.04 cm); (6.00 cm) and (5.95 cm), respectively with no significant difference

Table 1. Mean square values for boron as foliar application on yield attributes of maize (*Zea mays* L.)

Source of variation	Df	Mean square								
		PH	CL	CD	CW	GNPC	TGW	GY	BY	HI
Replication (R)	2	28.58	0.007	0.07	0.074	3.370	114	0.00021	0.002	0.027
Treatments (T)	8	1036.88**	0.997	0.96	650.7**	854.6**	456.99**	0.0728	0.217	0.575
Error (R × T)	16	423.24	0.002	0.03	84.59	6.87	49.43	0.0009	0.0005	0.047
Total	26									

** = means significance at $P \leq 0.05$ level of probability.

Table 2. Effect of boron as foliar application on yield attributes of maize (*Zea mays* L.)

Treatments	Parameters								
	PH	CL	CD	CW (g)	GNPC	TGW	GY	BY	HI (%)
	(cm)						(kg/ha)		
T_0 = Control	197.67d	14.21g	5.50e	132.00f	435.00f	295.33e	4.82f	13.95g	34.55e
T_1 = One water spray	201.45cd	14.40f	5.63de	134.00ef	439.00f	304.67de	4.87f	14.08f	34.59e
T_2 = One spray of 0.5% borax	208.67abc	15.35b	5.95abc	141.67bc	466.33c	320.00bc	5.15c	14.56c	35.37abc
T_3 = Two water sprays	201.78cd	14.61e	5.70d	136.33def	448.00e	306.67de	4.95e	14.25e	34.74de
T_4 = Two sprays of 0.5% borax	214.00ab	15.77a	6.04ab	146.00a	477.00ab	328.00ab	5.21ab	14.63b	35.61a
T_5 = Three water sprays	202.67cd	14.90d	5.80cd	138.33cd	452.00de	309.33cd	5.00de	14.28de	35.01cd
T_6 = Three sprays of 0.5% borax	217.00a	15.81a	6.10a	147.00a	481.00a	333.00a	5.26a	14.75a	35.66 a
T_7 = Four water sprays	205.67bcd	15.00c	5.90bc	139.00cd	455.00d	312.00cd	5.05d	14.30d	35.21bc
T_8 = Four sprays of 0.5% borax	212.33ab	15.40b	6.00ab	144.00ab	475.00b	324.83ab	5.18bc	14.58c	35.53ab
LSD ($P \leq 0.05$)	8.9024	0.0691	0.1996	3.9799	4.5369	12.169	0.0522	0.0391	0.3761

PH = plant height; CL = cob length; CD = cob diameter; CW = cob weight; GNPC = grains number per cob; TGW = thousand grain weight; GY = grain yield; BY = biological yield; HI = harvest index; any two means not sharing a letter in common differ significantly at $P \leq 0.05$.

(P≤0.05), whereas the smallest cob diameter (5.50 cm) was noted from T_0 (control). Almost similar date fashion was recorded for cob weight depicting that treatments T_6 (three foliar sprays of 0.5% borax), T_4 (two foliar sprays of 0.5% borax) and T_8 (four foliar sprays of 0.5% borax) measured statistically same cob weight (147.00 g; 146.00 g; 144.00 g, respectively) but different from that of T_0 (control) with cob weight (132 g). The improvement in cob diameter might be due enhanced cell division and cell expansion, whereas increase in cob weight was because of increased cob length and diameter of maize that was attained by the foliar application of boron. The results showed a similarity to the work done by Pasha *et al*. (2002).

The individual comparison of treatments means (P≤0.05) elucidated that highest number of grains per cob (481.00) was noted in T_6 (three foliar sprays of 0.5% borax) being statistically similar with T_4 (two foliar sprays of 0.5% borax) that devised (477.00) grains number per cob, while the lowest (435.00) was noted in T_0 (control). The increase in number of grains per cob might be due to reason that boron application improves the grain setting by improving the grain filling process and reducing the male sterility, often observed in boron deficient conditions. These results are reassuring to the conclusions made by Sultana *et al*. (2015) and Ahmad *et al*. (2000).

1000-grain weight statistics (P≤0.05) evidenced that the treatment, T_6 (three foliar sprays of 0.5% borax) fashioned the maximum 1000-grain weight (333.00 g). It was trailed by T_4 (two foliar sprays of 0.5% borax) that was statistically at par with T_8 (four foliar sprays of 0.5% borax) by giving (328.00 g) and (324.83 g), respectively while minimum 1000-grain weight (295.33 g) was found in T_0 (control). The increase in 1000-grain weight by foliar application of boron was because, the presence of boron in plant, regulates the translocation of assimilates to grain, as boron sustains the proper functioning of enzymes and integrity of plasma membrane that drops starch contents in leaves and promotes photosynthates translocation towards seeds, finally enhancing 1000-achene weight (Arif *et al*., 2006). These results are similar with the findings of Tahir *et al*. (2009) who reported that 1000-grain weight was increased by boron application. Similarly, Khan *et al*. (2006) and Hussain and Yasin (2004) recorded significant increase in 1000-grain weight with foliar application of micronutrients especially boron.

The efficiency of foliar application of boron is ultimately determined by the level of grain yield per hectare, which in turn is a function of cumulative behavior of all the yield components. Comparison of treatments means (P≤0.05) revealed that the statistically supreme grain yield (5.26 t/ha) was observed in T_6 (three foliar sprays of 0.5% borax) which was at par with T_4 (two foliar sprays of 0.5% borax) producing grain yield 5.21 t/ha, whereas T_0 (control) provided least grain yield of 4.82 t/ha. Improved grain yield with boron nutrition was due to its imperative role in pollen germination, proper seed setting, sugar metabolism and balance between net photosynthetic rate and respiration (Khan *et al*., 2006). These findings are in accordance with that of Nadim *et al*. (2011).

Boron foliar application also showed significant difference (P≤0.05) in case of biological yield as the highest total biomass (14.75 t/ha) was obtained from those plots where T_6 (three foliar sprays of 0.5% borax) was applied, followed by T_4 (two foliar sprays of 0.5% borax) producing (14.63 t/ha) biological yield. However, T_8 (four foliar sprays of 0.5% borax) and T_2 (one foliar sprays of 0.5% borax) were statistically at par with each other, while lowest biological yield (13.95 t/ha) was recorded in T_0 (control). The improvement in biological yield might be due to enhanced plant height, cob length, cob diameter, 1000-grain weight and grain yield by increasing the cell division, cell elongation, pollen tube germination and other physiological processes (Tombo *et al*., 2008). The results are supported by Ziaeyan and Rajaie (2009) as they found that foliar application of boron increased biological yield in maize.

The data pertaining to harvest index revealed maximum harvest index (35.66 %) in T_6 (three foliar sprays of 0.5% borax) though, it was statistically similar with T_4, T_8 and T_2 (two, four and one foliar sprays of 0.5% borax) by documenting (35.61%, 35.53 and 35.37%) harvest index, respectively. While minimum (34.55%) was recorded where no water and boron spray (T_0) was applied (P≤0.05). The maximum harvest index in treatment T_6 (three foliar sprays of 0.5% borax) was due to the reason that grain and biological yields were maximum in this treatment that directly affected the harvest index. The improvement in harvest index as a result of boron application might be due to the enhanced photosynthetic and metabolic activity which leads to an increase in various plant pathways responsible for stimulation of plant growth, accumulation of biomass

and resultantly the harvest index. These results are similar with the outcomes of Tombo *et al*. (2008) and Alam *et al*. (2000).

Maize quality attributes. Quality attributes data revealed significant ($P \leq 0.05$) effects of different boron applications. In case of seed protein contents (Fig. 1) highest (10.06%) was obtained from those plots which were treated with T_8 (four foliar sprays of 0.5% borax) trailed by T_6 (three foliar sprays of 0.5% borax) and T_4 (two foliar sprays of 0.5% borax) that fashioned seed protein (9.80%, 9.53%, respectively). However, lowest protein contents (8.13%) was recorded from T_0 (control) plots. The increase in protein contents with foliar application of boron was due to the reason that as it enhanced the nitrogen uptake rate, and nitrogen being the essential part of amino group is the building block of proteins.

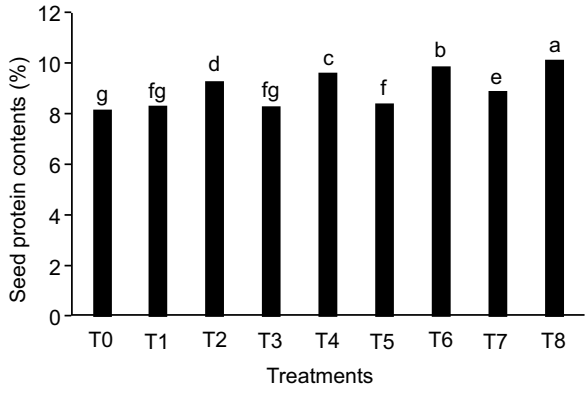

Fig. 1. Effect of boron as foliar application on seed protein contents of maize.

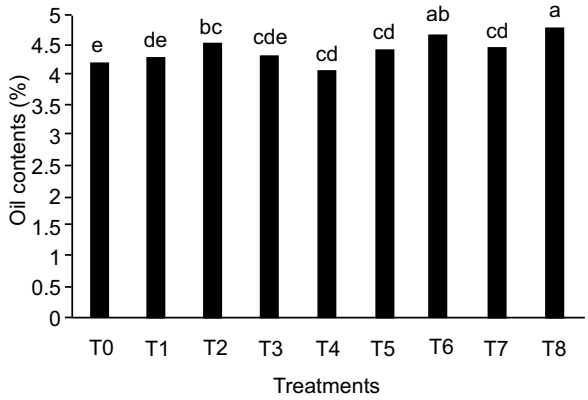

Fig. 2. Effect of boron as foliar application on seed oil contents of maize.

These results are in line with the findings of Malhi *et al*. (2006). Similar results had also been reported by Dwivedi *et al*. (2002) as he described significantly increased protein contents with the foliar application of boron.

Seed oil contents of maize presented in Fig. 2 revealed that the maximum seed oil content (4.72%) was recorded in maize treated with T_8 (four foliar sprays of 0.5% borax) which was statistically at par with T_6 (three foliar sprays of 0.5% borax) having seed oil content 4.65%. While the minimum seed oil content (4.17%) was recorded in T_0 (control). These results supported the work of Ahmad *et al*. (2000) as they stated that foliar application of boron improved seed oil contents.

Conclusion

On the basis of results it was concluded that maize crop should preferably be fertilized @ three foliar sprays of 0.5% borax; 1st at 4-5 leaves stage, 2nd and 3rd with one week interval, along with recommended dose of other fertilizer nutrients to realize higher and supportable production.

References

Ahmad, A., Tahir, M., Ehsan Ullah, M., Naeem, M., Ayub, M., Rehman, H., Talha, M. 2012. Effect of silicon and boron foliar application on yield and quality of rice. *Pakistan Journal of Life and Social Sciences*, **10:** 161-165.

Ahmad, A., Khan, I. 2000. Effect of boron fertilization on oil accumulation, acetyl-CoA concentration and acetyl-CoA carboxylase activity in the developing seeds of rapeseed (*Brassica campestris* L.). *Australian Journal of Agricultural Research*, **5:** 1023-1029.

Ahmad, S.Q., Ghaffar, M., Ahmad, F. 2011. Genetic diversity analysis for yield and other parameters in maize (*Zea mays* L.) genotypes. *Asian Journal of Agricultural Sciences*, **3:** 385-388.

Alam, S.M., Iqbal, Z., Latif, A. 2000. Effect of boron application with or without zinc on the yield of wheat. *Pakistan Journal of Soil Science*, **18:** 95-98.

Alias, M.A., Bukhsh, H.A., Ahmad, R., Cheema, Z.A., Ghafoor, A. 2008. Production potential of three maize hybrids as influenced by varying plant density. *Pakistan Journal of Agricultural Sciences*, **45:** 413-417.

Arif, M., Chohan, M.A., Ali, S., Gul, R., Khan, S. 2006.

Response of wheat to foliar application of nutrients. *Journal of Agriculture and Biological Sciences*, **1:** 30-34.

Davis, J.M., Sanders, D.C., Nelson, P.V. 2003. Boron improves growth, yield, quality and nutrient content of tomato. *Journal of The American Society for Horticultural Science*, **128:** 441-446.

Dwivedi, S.K., Singh, R.S., Dwivedi, K.N. 2002. Effect of sulphur and zinc nutrition on yield and quality of maize in typic ustochrept soil of Kanpur. *Journal of Indian Society of Soil Science*, **50:** 70-74.

GOP, 2014. *Economic Survey of Pakistan* 2014-2015. Finance and Economic Affairs Division, Government of Pakistan, Islamabad, Pakistan, pp. 15.

Gunnes, A., Alpaslan, M., Inal, A., Adak, M.S., Eraslan, F., Cicek, N. 2003. Effects of boron fertilization on the yield and some yield components of bread and durum wheat. *Turkish Journal of Agriculture and Forestry*, **27:** 329-335.

Hussain, F., Yasin, M. 2004. *Soil Fertility Monitoring and Management in Rice-Wheat System*. Annual Report, LRRP, NARC, Islamabad, Pakistan, pp. 1-33.

Khan, R., Gurmani, A.H., Gurmani, A.R., Zia, M.S. 2006. Effect of boron application on rice yield under wheat rice system. *International Journal of Agriculture and Biology*, **8:** 805-808.

Leghari, A.H., Laghari, G.M., Ansari, M.A., Mirjat, M.A., Laghari, U.A., Leghari, S.J., Laghari, A.H., Abbasi, Z.A. 2016. Effect of NPK and boron on growth and yield of wheat variety TJ-83 at Tandojam soil. *Advances in Environmental Biology*, **10:** 209-216.

Malhi, S.S., Gan, Y., Raney, J.P. 2006. Yield, seed quality and boron uptake of Brassica oil seed crops in response to boron fertilization. *Agronomy Journal*, **99:** 570-577.

Nadim, U.A., Awan, I.U., Baloch, M.S., Khan, N., Naveed, K. 2013. Micronutrient use efficiency in wheat as affected by different application methods. *Pakistan Journal of Botany*, **45:** 887-892.

Nadim, M.A., Awan, I.U., Baloch, M.S., Khan, E.A., Naveed, K., Khan, M.A., Zubair, M., Hussain, N. 2011. Effect of micronutrients on growth and yield of wheat. *Pakistan Journal of Agricultural Sciences*, **48:** 191-196.

Pasha, B.U., Ali, A., Saleem, M., Niazi, B.H. 2002. Role of boron for potassium/sodium ratio in sunflower under saline conditions. NARC, Islamabad, Pakistan, **25:** 69-78.

Rashid, A., Yaseen, M., Ashraf, M., Mann, R.A. 2004. Boron deficiency in calcareous soil reduces rice yield and impairs grain quality. *International Rice Research Notes*, **29:** 58-60.

Shahzad, K., Shehzad, M.A., Iqbal, M., Arif, M. 2012. Response of maize (*Zea mays* L.) genotypes to soil and foliar application of boron. *Asian Journal of Pharmaceutical and Biological Research*, **2:** 65-72.

Shehzad, M.A., Maqsood, M. 2015. Integrated nitrogen and boron fertilization improves the productivity and oil quality of sunflower grown in a calcareous soil. *Turkish Journal of Field Crops*, **20:** 213-222.

Steel, R., Torrie, J., Dickey, D. 1997. *Principals and Procedures of Statistics. A Biometrical Approach*, McGraw Hill Book Co. Inc., pp. 1409-1414, 3rd edition, New York, USA.

Sultana, S., Muhmood, A., Shah, S.S.H., Saleem, I., Niaz, A., Ahmed, Z.A., Wakeel, A. 2015. Boron uptake, yield and quality of carrot (*Daucus carota* L.) in response to boron application. *International Journal of Plant and Soil Science*, **8:** 1-5.

Tahir, M., Ali, A., Khalid, F., Naeem, M., Fiaz, N., Waseem, M. 2012. Effect of foliar applied boron application on growth, yield and quality of maize (*Zea mays* L.). *Pakistan Journal of Scientific and Industrial Research*, **55:** 117-121.

Tahir, M., Tanveer, A., Shah, T.H., Fiaz, N., Wasaya, A. 2009. Yield response of wheat (*Triticum aestivum* L.) to boron application at different growth stages. *Pakistan Journal of Life and Social Sciences*, **7:** 39-42.

Tombo, Y., Tombo, N., Cig, F., Erman, M., Celen, A.E. 2008. The effect of boron application on nutrient composition, yield and some yield components of barley (*Hordeum vulgare* L.). *African Journal of Biotechnology*, **7:** 3255-3260.

Ziaeyan, A.H., Rajaie, M. 2009. Combined effect of zinc and boron on yield and nutrients accumulation in corn. *International Journal of Plant Production*, **3:** 35-44.

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A Brief Description of 'Inqalab Mung' Mungbean (*Vigna radiata* L. Wilczek) Variety Released for the Agro-Climatic Conditions of Khyber Pakhtunkhwa, Pakistan

Muhammad Mansoor[a]*, Amanullah[a], Noman Latif[a], Nazir Hussain[a] and Abdul Majeed[b]
[a]Arid Zone Research Center (PARC), Dera Ismail Khan, KPK, Pakistan
[b]Agricultural Research Institute, Dera Ismail Khan, KPK, Pakistan

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Abstract. 'Inqalab Mung' (DM-3) was developed through cross between VC1482 C and NM-92 at Agricultural Research Institute, Dera Ismail Khan, Pakistan. Various experiments for production technology and yield performance of Inqalab Mung were conducted from 2005 to 2008 in different seasons and locations. Result showed that 40 kg/ha seed rate with 30 cm row spacing, fertilizer dose @ 20:50 kg/ha $N:P_2O_5$ and inoculation with rhizobium strain Vm M1 were optimal for its maximum yield. Inqalab Mung outclassed among all candidate lines included in NUYT-2007 and 2008 with average yields of 961.5 kg/ha and the highest grain yield of 3620 kg/ha. Inqalab Mung has 28-36% high grain yield potential compared to the standard variety NM 98 and parent NM 92 along with resistance to charcoal rot, cercospora leaf spot and yellow mosaic virus (YMV). Provincial Seed Council (PSC), Khyber Pakhtunkhwa, approved DM-3 as 'Inqalab Mung' for general cultivation in KPK in 2014.

Keywords: mungbean (*Vigna radiata* L. Wilczek), Inqalab Mung, grain yield, bold seeded

Introduction

Mungbean (*Vigna radiata* L. Wilczek), is an indigenous legume and one of the most important pulse crops. Mungbean is rich in digestible proteins (24%) and utilized in the cereal-based diets (Khattak *et al*., 2006). It contains vitamin A (94 mg), iron (7.3 mg), zinc (3 mg), calcium (124 mg) and folate (549 mg) per 100 g dry seed. Usually it is used in split form (Dhal) and in other different food products (Rasul *et al*., 2012). Fallow period window of 70-90 days during April to June in rice wheat cropping system is very suitable to plant mungbean. Mungbean is low input requiring, short duration, high value and restorative crop (Achakzai *et al*., 2012). Being leguminous crop it fixes nitrogen thereby improving soil fertility (Khan *et al*., 2008). Fitting mungbean in cereal cropping system can increase farmers' income, improve soil productivity and saving irrigational water (Hussain *et al*., 2012). Mungbean cultivars so far release in Pakistan have comparatively longer growth duration (90-110 days), indeterminate growth habit (Jahan and Golam, 2012), low yielding (400 kg/ha), small seed, susceptible to yellow mosaic virus (YMV) and insects (Rehman *et al*., 2009). Developing cultivars having short growth duration (55 to 65 days), high yield potential (up to 2000 kg/ha), synchronized maturity, resistance to MYMV, Cercospora leaf spot and having bold seeds (Aslam *et al*., 2010). Agricultural Research Institute, Dera Ismail Khan released 'Inqalab Mung' a variety developed from cross between AVRDC line and NM-92, that gives better yield (up to 2.5 t/ha) and is early maturing, bold seeded and having resistant against Mungbean Yellow Mosac Virus (MYMV) disease. The variety has been recommended for general cultivation in both spring and summer seasons in KPK province. It is the first ever variety approved for general cultivation in both seasons. Moreover, it has got full adaptability to recent climate changes prevailing in the area.

The present study therefore, presents the detailed developmental and evolution process of this new high yielding bold seeded mungbean variety.

Materials and Methods

AVRDC genotype VC 1482C having high yield potential but un-acclimatized to the agro-climatic conditions of Pakistan (highly susceptible to MYMV) was crossed with NM-92 (local mungbean cv.) having high resistant to MYMV adopting breeding procedures of Khattak *et al*. (2003b). F_1 generation of the cross was planted during summer (May-July) 1999 and the recombinants were harvested individually. F_2-F_5 generations were raised as plant to row progenies for selecting high

*Author for correspondence; E-mail: joyadkpk@gmail.com

yielding recombinants having resistance to MYMV during kharif 1999 to 2003. Kabuli Mung, a highly susceptible MYMV line was used as spreader and planted after each five rows to intensify MYMV disease. MYMV disease rating (0-9) was done as per method used by Sadiq *et al*. (2006). After getting enough seed, the line was tested in preliminary yield trial (PYT) at institute to check the performance of promising line with improved check varieties. Replicated yield trials major varietal trial (MVT) and advance yield trial (AYT) were conducted using randomized complete block design (RCBD) with plant-to-plant (10 cm), row-to-row spacing (30 cm), number of rows (6) and row length (4 m) (Ahmad *et al*., 2004). The trials data were analysed according to Steel and Torrie (1980).

Results and Discussion

Yield performance. ***Preliminary yield trial.*** Twenty one lines were evaluated in PYT during 2005. Inqalab Mung (DM-3) gave better yield (1130 kg/ha) against check variety NM-92 (1038 kg/ha) (Table 1).

Major yield trial (MYT). In MYT, Inqalab Mung (DM-3) out yielded all other lines including check with grain yield 1273 kg/ha (Table 2).

Advance yield trial (AYT). In AYT, Inqalab Mung (DM-3) again gave highest yield (1056 kg/ha) in the trial (Table 3).

National uniform yield trial in 2007 and 2008. In mungbean NUYT conducted during 2007, candidate variety Inqalab Mung (DM-3) was tested at fifteen locations all over the country and gave best yield as compared to both check varieties NM-06 and NM-92. Similarly in NYUT-2008, candidate variety (DM-3) was tested at thirteen locations and it performed better than check variety NM-06 (Table 4-6).

Disease resistance. The candidate variety Inqalab Mung (DM-3) was tested at CDRI, NARC, Islamabad for disease reaction, it was found resistant to most common diseases in Pakistan i.e. charcoal rot, bacterial leaf spot and leaf crinkle virus while moderately resistant yellow mosaic virus (Idahosa *et al*., 2010) (Table 7).

Table 1. Performance of DM-3 (Inqalab Mung) in preliminary yield trial-2005 (spring)

Entries	Plant height (cm)	Days to 50% flowering	Day to 90% maturity	No. of pods/plant	1000 grain weight (g)	Grain yield (kg/ha)
DM1	58.33 efg	42.33 ij	86.67 gh	59.00 hi	40.00 i	726.7 ij
DM2	64.33 bc	43.33 hi	87.33 g	80.67 c	50.33 bc	949 bcd
DM3	55.00 hij	43.00 hij	84.33 h	97.00 a	52.33 a	1130 a
DM4	53.33 ijkl	42.00 j	88.33 efg	70.00 de	47.00 d	850 efg
DM5	54.00 ijk	45.67 fg	90.00 def	54.00 kl	39.00 i	662.7 jk
DM6	64.33 bc	50.00 a	94.33 a	60.67 ghi	42.00 h	746 hij
DM7	50.67 l	48.00 bc	91.67 bcd	68.33 de	46.33 de	850.3 efg
DM8	69.33 a	47.00 cde	91.33 bcd	66.67 ef	45.00 ef	841.3 efg
DM9	52.00 jkl	46.33 efg	91.00 cd	63.67 fg	44.33 fg	780.7 f-i
DM10	61.00 de	47.00 cde	91.67 bcd	80.00 c	49.00 c	905 cde
DM11	67.00 ab	48.00 b	92.67 abc	70.33 de	47.00 d	863.7 def
DM12	57.33 fgh	50.67 a	93.67 ab	55.00 jkl	42.00 h	735 hij
DM13	64.67 bc	44.00 h	88.33 efg	52.00 l	39.67 i	600 k
DM14	60.33 ef	42.33 ij	87.33 g	54.33 kl	40.00 i	601.3k
DM15	52.00 jkl	43.33 hi	87.33 g	57.33 ijk	41.67 h	772 ghi
DM16	63.67 cd	43.00 hij	87.67 fg	71.67 d	49.00 c	864 def
DM17	56.33 ghi	47.67 bcd	90.00 def	62.33 gh	43.00 gh	791.7 f-i
DM18	51.00 kl	48.00 bc	90.33 cde	63.67 fg	44.67 f	820.7 e-h
DM19	60.67 de	46.67 def	90.67 cde	58.33 ij	42.00 h	720.3 ij
NM-98	58.33 efg	45.33 g	91.67 bcd	81.67 c	51.00 ab	976.7 bc
NM-92	59.00 efg	47.67 bcd	94.66 a	87.33 b	50.67 b	1038 b
CV	3.21	1.71	1.62	3.41	2.21	6.75%

Table 2. Performance of DM-3 (Inqalab Mung) in major yield trial-2005 (Kharif)

Entries	Plant height (cm)	Days to 50% flowering	Day to 90% maturity	No. of pods/plant	1000 grain weight (g)	Grain yield (kg/ha)
DM2	65.67 a	44.33 ab	87.67a	80.33 b	50.00 b	1086 bc
DM3	56.00 c	43.67 ab	84.33 b	88.00 a	53.67 a	1273 a
DM4	55.00 c	46.33ab	88.00 a	75.67 c	48.33 b	1044 c
DM7	53.33 c	45.67 a	88.33 a	72.00 d	46.33 c	918.3 d
DM10	61.33 b	43.00 ab	87.67 a	65.33 e	46.33 c	795.3 e
DM11	66.67 a	44.33 b	87.00 ab	67.67 e	45.00 c	836 de
DM-16	64.67 a	44.00 ab	88.65 a	58.00 f	45.67 c	670.3 f
NM-98	60.35 b	44.00 ab	89.00 a	81.00 b	49.67 b	1107 bc
NM-92	61.33 b	43.67 ab	89.66 a	87.33 a	50.00 b	1173 b
CV	2.79 %	4.07 %	2.18 %	2.24 %	2.27 %	5.59 %

Table 3. Performance of DM-3 (Inqalab Mung) in advanced yield trial-2006

Entries	Plant height (cm)	Days to 50% flowering	Day to 90% maturity	No. of pods/plant	1000 grain weight (g)	Grain yield (kg/ha)
DM2	65.67 a	43.67 NS	84.33 b	88.00 a	53.67 a	1051 a
DM3	56.00 c	44.33	87.67 ab	80.33 b	50.00 b	1056 a
DM4	55.00 c	46.33	88.00 ab	75.67 c	48.33 bc	707 b
DM7	53.33 c	45.67	88.33 a	72.00 d	46.33 c	585 c
NM-98	60.33 b	44.00	89.00 a	81.00 b	49.67 b	1053 a
NM-92	61.33 b	43.67	89.67 a	87.33 a	50.00 b	750 b
CV	2.56 %	3.42 %	2.38 %	2.28 %	2.57 %	7.31%

Table 4. Yield data of Inqalab Mung as compared to two checks at various locations in National uniform yield trial-2007

Location	DM-3 Grain yield (kg/ha)	Check (NM-06) Grain yield (kg/ha)	Check (NM-92) Grain yield (kg/ha)
NIAB, Faisalabad	1456	1324	1394
NIFA, Peshawar	763	555	728
AZRI, Bhakkar	197	160	202
NIA, Tandojam	1268	1107	1081
BARI, Chakwal	1130	1030	969
ARI, Quetta	362	838	336
ARI, Bahawalpur	851	653	615
QARI, Larkana	1272	1213	751
ARI, Mingora	3620	2969	2708
PRI, Faisalabad	786	1048	674
Kalurkot	1112	973	1112
Average	1092	1003	896

Other characters of 'Inqalab Mung'. Varieties released by Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad though are high yielding and disease resistant yet cannot acclimatize well in KPK owing to different agro climatic conditions in KPK than in Punjab (Khattak *et al*., 2003b). This newly evolved variety 'Inqalab Mung' performed very well throughout Pakistan as depicted in National uniform yield trials 2007 and 2008. Having bold seed size, decreased plant height, stiff stem and short duration as compared to improved varieties prevailing in the country i.e., NM 98, NM-06 and Dera Mung. Besides general preference of farmers, seed size is the main contributing factor towards grain yield (Khattak *et al*., 2003a; 2003b) in mungbean because it fetches higher price compared to small grained varieties (Ali *et al*., 1997). High harvest index % of 'Inqalab Mung' proves to its superior physiological efficiency in partitioning the photosynthates for grain formation leading thereby to distinct increase in the grain yield. Breeding mungbean genotypes, having improved determinate growth habit that can only be achieved through conversion of more photosynthates to flowers, pods and ultimately to grain formation at the start of reproductive growth (Khattak *et al*., 2001). Due to short stature and stiff stem 'Inqalab Mung' is lodging resistant. Despite

Table 5. Yield data of DM-3 (Inqalab Mung) and various entries included in mung (National uniform yield trial-2007) along with contributor's location

Entry	Contributor's location	Average grain yield (kg/ha)
NCM-209	NARC, Islamabad	787
BRM-303	ARI, Bahawalpur	977
97001	PRI, Faisalabad	963
C1/95-3-45	NIA, Tandojam	842
BRM-307	ARI, Bahawalpur	876
NCM 252-7	NARC, Islamabad	883
DM-3	ARI, DI Khan.	1092**
NM-4	NIAB, Faisalabad	844
2 CMG – 501	BARI, Chakwal	876
DM-4	ARI, DI Khan	1038
NCM 257-2	NARC, Islamabad	786
3 CMG -507	BARI, Chakwal	952
NM-06	Check	1003
NM-92	Check	896
97003	PRI, Faisalabad	859

Table 6. Yield data of DM-3 (Inqalab Mung) and various entries included in mung (National uniform yield trial-2008) along with contributor's location

Entry	Contributor's location	Average grain yield (kg/ha)
NM-06	Check	711
NCM-209	NARC, Islamabad	654
BRM-303	ARI, Bahawalpur	766
2 CMG – 516	BARI, Chakwal	758
BRM-307	ARI, Bahawalpur	755
97001	PRI, Faisalabad	725
NM-6	NARC, Islamabad	751
98004	PRI, Faisalabad	806
C1/95-3-45	NIA, Tandojam	762
DM-3	ARI, DI Khan.	831**
NM-5	NIAB, Faisalabad	797
NCM 252-7	NARC, Islamabad	719
DM-4	ARI, DI Khan	819

Table 7. Disease reaction of DM-3 (Inqalab Mung) and in National uniform yield trials 2007 and 2008

Disease	Response
Yellow mosaic virus	Moderately resistant
Charcoal rot	Resistant
Bacterial leaf spot	Resistant
Leaf crinkle virus	Resistant

Source = consolidated disease data of National uniform yield trials.

resistant to MYMV, these two distinct characters of 'Inqalab Mung' are giving edge to the variety for preference over earlier released varieties. The description of 'Inqalab Mung' is given in Table 8.

Management techniques. ***Weed control.*** Water extracts of sorghum, eucalyptus and *Acacia nilotica* were used as a natural weed control approaches in comparison with hand weeding and Stomp 330 EC (Pre-emergence herbicide). The extract of *Acacia nilotica* pods outclassed the other treatments in weeds control and increasing grain yield followed by Hand weeding twice + Stomp 330 EC treatment. The allelo-chemicals existed in the water extract of *Acacia nilotica*'s pods were found to be dual acting agents i.e., controlling obnoxious weeds as well as enhancing mungbean yield. It was observed that extract was instrumental in damaging weeds flora and was also capable of leaching down in lower quantities to the root zone of mungbean thereby promoting growth of the crop (Mansoor *et al*., 2004). The results advocated the need for the use of allelo-chemicals for control of weeds, which was economical and environmental friendly (Singh *et al*., 2006).

***The optimal time of planting*.** For ensuring maximum yield, it is concluded that mungbean sown during spring

Table 8. Description of mungbean variety (Inqalab Mung)

Characters	Ranges
Days to maturity	75-85
Number of primary branches	4-5
Number of secondary branches	5-7
No. of leaves	40-45
Leaf length	13-15 cm
Leaf width	10-12 cm
Days to 50% flowering	32-35
Flowering duration	18-20
Pod length/pod width	8 – 9 cm / 6-7 mm
Pod shape	Semi flat
Mature pod colour	Brownish black
Cluster/plant	10-12
Pod/cluster	7 (average)
Pod/plant	60 (average)
Seed/pod	10 – 12
Seed colour	Dark green
Seed shape	Oval
Coat pattern	Plane
Seed testa texture	Smooth
Seed length	4-5 mm
Seed width	3-3.5 mm
1000 grain weight	52.00 g (average)

on 1st March and Kharif on 1st May were more appropriate from agronomic and ecological perspectives. This was due to the fact that it has increased net return compared to other planting dates, by boosting grain yield and its associated components (maximum number of pods/plant, 1000-grain weight and number of grain/pod). The next significant planting date was 15th of May (Sadiq *et al*., 2006). Mungbean growers can get maximum return if cv. 'Inqalab Mung' is sown during spring and planting is completed in the month of March, moreover, the farmers may also be able to plant second crop on the same field in early June which may not only enhance income of resource poor farmers but will also increase soil fertility due to its nitrogen fixation capability. Rehman *et al*. (2009) also concluded same results for M-1 (Mungbean cv.) for Peshawar valley but with different dates of planting. This alteration may be due to different varieties used in their experiment.

The suitable strain of Rhizobium for effective nodulation. Among rhizobium strains, Vm M1 was more effective in producing more number of effective nodules on 'Inqalab Mung' (Ahmed *et al*., 2006). Vice versa results were true in the plots which were un-inoculated (Achakzai *et al*. 2012).

Phosphatic fertilizer dose. Among phosphatic fertilizer doses the dose of 20:50 $N:P_2O_5$ kg/ha was more economical than 20:70 $N:P_2O_5$ kg/ha in terms of net return.

The proper seed rate for standardizing plant population. It is concluded from the experimental findings that seed rate of 40 kg/ha with row spacing of 30 cm, followed by seed rate of 40 kg/ha with row spacing of 20cm has resulted in better plant stand establishment and productivity of Cv. 'Inqalab Mung' (Ahmad *et al*., 2004).

Conclusion

'Inqalab Mung' variety is suitable for edible purpose and contains 20% proteins, 306 Kcal energy, vitamin-A 5%, iron 6.6%, Zinc 3.4% and amino acids 11%. 'Inqalab Mung' is a high yielding, bold seeded, disease resistant and dual nature variety fit for spring and kharif seasons. The variety is erect with main stem length 45 cm having indeterminate plant type with non-shattering habit. Seed is bold, oval shaped and dark green in colour. 'Inqalab Mung' variety is resistant to common diseases found in Pakistan.

References

Achakzai, A.K.K., Habibullah, Shah, B.H., Wahid, M.A. 2012. Effect of nitrogen fertilizer on the growth of mungbean (*Vigna radiata* L. Wilczek) grown in Quetta. *Pakistan Journal of Botany*, **44:** 981-987.

Achakzai, A.K.K. 2012. Effect of various levels of nitrogen fertilizer on some vegetative growth attributes of pea (*Pisum sativum* L.) cultivars. *Pakistan Journal of Botany*, **44:** 655-659.

Ahmed, Z.I., Anjum, M.S., Rauf, C.A. 2006. Effect of Rhizobium inoculation on growth and nodule formation of green gram. *International Journal of Agriculture and Biology*, **8:** 235-237.

Ahmad, R., Mahmood, I., Kamal, J., Bukhari, S.A.H. 2004. Growth and yield response of three mungbean (*Vigna radiata* L.), cultivars to varying seeding rates. *International Journal of Agriculture and Biology*, **6:** 538-540.

Aslam, M., Hussain, N., Zubair, M., Hussain, S.B., Baloch, M.S. 2010. Integration of organic and inorganic sources of phosphorus for increased productivity of mungbean (*Vigna radiata* L). *Pakistan Journal of Agriculture Science*, **47:** 111-114.

Hussain, A., Ali, A., Noorka, I.R. 2012. Effect of phosphorus with and without rhizobium inoculation on nitrogen and phosphorus concentration and uptake by mungbean (*Vigna radiata* L.). *Journal of Agriculture Research*, **50:** 49-52.

Idahosa, D.O., Alika, J.E., Omoregie, A.U. 2010. Genetic variability, heritability and expected genetic advance as indices for yield and yield components selection in cowpea (*Vigna unguiculata* L.) Walp. *Journal of Academia Arena*, **2:** 22-26.

Jahan, N., Golam Adam, A.M.M. 2012. Growth and yield performance of BARI mung-5 under different time of sowing. *Journal of Bangladesh Academy of Sciences*, **36:** 227-231.

Khan, F.S., Ahmed, Z.I., Ansar, M., Shah, H. 2008. Response of mung bean genotypes to rhizobium inoculum and varying levels of nitrogen fertilizer. *Pakistan Journal of Agriculture Research*, **21:** 33-44.

Khattak, G.S.S., Ashraf, M., Saeed, I., Alam, B. 2006. A new high yielding mung bean (*Vigna radiata* L. wilczek) variety "Ramzan" for the agro climatic conditions of NWFP. *Pakistan Journal of Botany*, **38:** 301-310.

Khattak, G.S.S, Ashraf, M., Elahi, T., Abbas, G. 2003a.

Selection for large seed size at the seedling stage in mungbean (*Vigna radiata* L. Wilczek). *Breeding Sciences*, **53:** 141-143.

Khattak, G.S.S., Zamir, R., Muhammad, T., Shah, S.A. 2003b. Breeding mungbean (*Vigna radiata* L. Wilczek) genotypes for the agro climatic conditions of NWFP. *Pakistan Journal of Botany*, **35:** 763-770.

Khattak, G.S.S., Haq, M.A., Ashraf, M., Tahir, G.R., Marwat, E.U.K. 2001. Detection of epistasis, and estimation of additive and dominance components of genetic variation for synchrony in pod maturity in mungbean (*Vigna radiata* L.) Wilczek. *Field Crops Research*, **72:** 211-219.

Mansoor, M., Ahmad, K., Himayatullah, Yaqoob, M. 2004. Development of effective and economical weed management strategy for mungbean. *Pakistan Journal of Weed Sciences*, **10:** 151-154.

Rasul, F., Cheema, M.A., Sattar, A., Saleem, M.F., Wahid, M.A. 2012. Evaluating the performance of three mungbean varieties grown under varying inter-row spacing. *Journal of Animal & Plant Sciences*, **22:** 1030-1035.

Rehman, A., Khalil, S.K., Nigar, S., Rehman, S., Haq, I., Akhtar, S., Khan, A.Z., Shah, S.R. 2009. Phenology, plant height and yield of mungbean varieties in response to planting date. *Sarhad Journal of Agriculture*, **25:** 147-152.

Sadiq, M.S., Saleem, M., Haider, S., Abbas, G. 2006. NIAB Mung. A high yielding and disease resistant mung bean variety. *Journal of Agriculture Research*, **44:** 99-103.

Singh, J., Mathur, N., Bohra, S., Bohra, A., Vyas, A. 2006. Comparative performance of mung bean (*Vigna radiata* L.) varieties under rainfed condition in Indian Thar desrert. *American Eurasian Journal of Agriculture and Environment Science*, **1:** 48-50.

Steel, R.G.D., Torrie, J.H. 1980. *Principles and Procedures of Statistics. A Biometrical Approach*, Mc Graw Hill Book Co., New York, USA.

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Assessment of Vegetation-Edaphic Correlation of Wetland Complex of Soon Valley, Pakistan using Multivariate Techniques

Arooba Zia, Sheikh Saeed Ahmad* and Hafsa Bashir
Department of Environmental Sciences, Fatima Jinnah Women University, Mall Road, Rawalpindi, Pakistan

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Abstract. Vegetation-edaphic correlation was studied to abserve ecological relationship of plants. Because of the interrelationship of environmental variables various ordination techniques i.e., Two-way Indicator Species Analysis (TWINSPAN), Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA)) were employed to reveal the inherent pattern on visual inspection. Total 36 herbaceous species belonging to 16 families were identified in the area of Ucchali wetland complex, Soon Vally, Khusab, Pakistan, with *Cynodon dactylon* as dominant species owing to its high tolerance to prevailing environmental conditions. Results of CCA exposed elevation, organic matter and pH to be the most influencing factors in growth and distribution of flora of the area. Different analyses such as biplots, data attribute plots and pie symbols plots were accomplished for the species of the study area. Because of the induced change, various non-native and invasive species were also recorded in the area which might contribute to further altering the range ecology of the area. Apart from promoting sustainable use of natural resources in Ucchali Wetlands Complex (UWC); ecological integrity of the Ramsar sites must be conserved and improved through ecological interventions related to illicit cutting of forest, unsustainable utilization of water resources, municipal pollution, agricultural intensification, etc.

Keywords: TWINSPAN, DCA, CCA, multivariate techniques, vegetation, wetland complex

Introduction

The association between vegetation and environment is a very significant topic of plant ecology. A huge number of environmental variables are correlated as a result of an overriding influence; it is often complicated to conclude which factors are in fact causing vegetation patterns (Tavili *et al*., 2010). In the field of ecology, study of vegetation in relation to different environmental factors with the use of ordination technique and classification method has become well acknowledged and to study the complex nature of plant communities various multivariate techniques are available (Ali and Malik, 2010). A multivariate analysis integrating ecological, physical and socio-economic characteristics to investigate flora of the area has been well researched and numerous approaches and techniques exist to study their association. For the classification of flora according to ecological liking a computer based software application i.e. TWINSPAN exists (Ahmad *et al*., 2014a; 2014b; 2014c; Hill, 1979), while for the phytosociological ordination an indirect analysis technique i.e. DCA is widely used owing to its inclusive results (Urooj *et al*., 2015; Kent and Coker, 1996). CCA is a more modern day technique to study the correlation between flora and environmental variables in addition to its comprehensive results in the form of graphs and pie charts (Ahmad, 2011; Ter Braak and Smilaur, 2002). The study on multivariate and statistical analysis is not the only method because various researches from international and national level have already been successfully conducted for classification of flora (Urooj *et al*., 2015; Ahmad and Quratulain, 2011; Ahmad, 2010; Xiaoni *et al*., 2007). Studies related to ethno medical survey (Ghani *et al*., 2014), estimation of nutrients in medicinal plants (Ghani *et al*., 2012) and evaluation of biomass and carrying capacity (Saleem and Mirza, 2012) has already been conducted in Soon Valley of Pakistan. However the vegetation associated with environmental variables has not been quantitatively correlated in the region. Hence, this study aims to examine the relationship between species distribution with the changing environmental factors and gradients by using ordination technique.

Ucchali wetlands complex located in a cup shaped valley called Soon Valley in district Khushab of Pakistan. The complex has three Ramsar sites surrounded by mountains which are Ucchali, Khabeki and Jahlar. It extends from Khabeki in the east to Sakesar in the west and covers approximately an area of 745 km^2 (Fig.1). The area is sub-humid, sub-tropical and has suffered

*Author for correspondence; E-mail: drsaeed@fjwu.edu.pk

drought in the past. The area is the habitat to globally threatened waterfowl species. Owing to the increased agricultural practices the area is suffering decrease in its native species. These native species define the ecology of the region. Understanding vegetation and their relationship with environmental variables will serve as important information to land managers in managing, reclaiming and developing ecosystems. It will also provide a conceptual basis for the description of resource potential and ecological integrity of the area.

Materials and Methods

Vegetation sampling. Terrestrial vegetation from the UWC was sampled in the month of September and March, when most of the species were expected to be growing. For the study 150 quadrates were laid by stratified random sampling approach (Kent and Coker, 1996). The quadrate size of 1x1 m^2 was chosen for herbaceous and shrubby vegetation, since most plants had stunted growth. Domin cover scale with modifications by Bailey and Poulton (1968) was used to interpret visually estimated species cover value (Mueller-Dombois and Ellenberg, 1974). Metadata for each quadrate i.e., species attributes along with latitude, longitude and elevation of each quadrate were also recorded using Garmin eTrex GPS. Plant specimens which were unidentified in field were collected and identified.

Soil sampling. Soil samples 6-10 cm deep from each of the fifty quadrats was collected in plastic bag with the help of spade. The coded sample bags were approx 1 kg for obtaining relevant data on physical and chemical characteristics.

Physicochemical analysis of soil. Soil physicochemical properties play a very significant role in spatial distribution of vegetation community (Tavili *et al*., 2010). For this purpose soil was air dried after mixing it thoroughly and then dried samples were sieved to remove particles (rock, debris, gravel) larger than 2 mm. These subsamples were then retained for moisture, pH, texture, moisture, EC, OM and macronutrients (N, P and K) and micronutrient (Zn, Cu, Fe and Mn) analysis. Soil analysis was conducted in the laboratory of FJWU.

EC and pH were determined by Crison MM40+ portable meter. Walkley (1947) titrimetric method was used for determination of organic matter in soil. Soil texture was analyzed with the help of Octagon Digital Sieve Shaker instrument (Brady, 1990) and Allen (1974) method was employed for finding out the moisture in soil. Phosphorus was analyzed by Olsen's method using spectrophotometer (Nathan and Gelderman, 2012). Potassium and micronutrients in soil were determined by using atomic absorption spectrophotometer (Ehi-Eromosel *et al*., 2012). Nitrogen in soil was analyzed by first digesting the soil and then titration of digested solution against sulphuric acid prior to addition of an indicator (Urooj *et al*., 2016).

Ordination analysis. Ordination method was employed in this study for multivariate approach to analyze the vegetation clusters and affects of multiple environmental variables on these vegetation clusters simultaneously. The data sets pertaining to herbaceous and shrubby vegetation and edaphic factors were subjected to three type of multivariate analysis i.e., TWINSPAN (Two-way Indicator Species Analysis), DCA (Detrended Correspondence Analysis) and CCA (Canonical Correspondence Analysis).

Two way indicator species analysis (TWINSPAN). The first step in multivariate was to use the TWINSPAN technique to define distribution pattern of vegetation groups on the dataset having total of 150 stands. TWINSPAN is the most accepted hierarchical divisive clustering technique in community ecology (Leps and Smilauer, 2003). It constructs a two way table called dendrogram which expresses the relationship of sample and species (Hill, 1979). TWINSPAN using PCORD-5 application was used in this study to classify the species and sample data simultaneously along the UWC.

Detrended correspondence analysis (DCA). DCA is an indirect ordination technique used to mark out the similarities and differences between the vegetation compositions of quadrate samples (Hill and Gauch, 1980). It was used to correlate the change in vegetation pattern along the length of the underlying environmental gradients. It was used to ordinate clusters of species and to validate the accuracy of TWINSPAN results by species cluster formation.

Canonical correspondence analysis (CCA). CCA is a direct ordination technique and was used to analyze the relationship between environmental variables and identified vegetation species with the help of CANOCO 4.5 software (Xiaoni *et al*., 2007). The environmental variables for CCA analysis included pH, moisture, EC, OM, macronutrients (N, P and K) and micronutrients (Fe, Mn, Cu and Zn) of soil. The results of the ordination analysis were finally portrayed in the form of plots and charts for visual interpretation.

Results and Discussion

Species diversity. Native and wild species are usually considered as undesirable or sometimes harmful plants that intervene with the cultivated crops by taking away nutrients from soil. In fact these plants are vital component of biodiversity and play significant role in contributing to species diversity. Native species possess intrinsic value and some of them contain medicinal and nutritional value. These plants are very valuable and their loss may sometimes escalate to affect the entire ecosystem (Ruby *et al*., 2011). Total 37 plant species belonging to 16 families were identified around the area of the UWC (Table 1).

Many of the herbaceous and shrubby vegetation found in the study area serve as medicine and food source for local community (Ghani *et al*., 2014; Arshad, 2011) surveyed ethno medicinal plants of Soon valley. They reported that communities are dependent on them for their common day ailments and other value added products. Various medicinal plants of the region have also been categorized as rare included and endangered species therefore, serious efforts need to be put in for their sustainable and long term conservation. *Solanum nigrum* (black nightshade) of family Solanaceae is a herb or perennial shrub found in the area and useful for digestive disorders, corrosive ulcers and chronic skin diseases. *Chenopodium album* (fat hen, bathu) also

Table 1. List of species with families

Species	Families	Habitat	Species	Families	Habitat
Achyranthes aspera L.	Amaranthaceae	Tropical regions	*Fagonia olivieri* L.	Zygophyllaceae	Sandy soils over limestone or gravels
Alhagi maurorum	Fabaceae	Temperate and tropical regions	*Imperata c ylindrical* L.	Poaceae	Tropical and subtropical climates
Amaranthus viridis L.	Amaranthaceae	Heavy organic to very sandy soils	*Juncus* sp. L.	Juncaceae	Temperate regions
Brachiaria ramose (L.)	Stapf. Poaceae	Tropical regions	*Justacia adhatoda* L.	Acanthaceae	Moist places
Bromus pectinatus L.	Poaceae	Temperate regions	*Malva parviflora* L.	Malvaceae	All soil types
Buxus papillosa (C. K. Schn.)	Buxaceae	Tropical and sub tropical regions and are frost tolerant	*Parthenium hysterophorus* L.	Asteraceae	Semi-arid, subtropical, tropical and warmer temperate regions
Carissa opaca (Stapf.)	Apocynaceae	Tropical and sub tropical regions	*Peganum harmala* L.	Nitrariaceae	Dry deserted areas
Chenopodium album L.	Amaranthaceae	Soils rich in nitrogen especially wasteland	*Phyla nodiflora* L.	Verbenaceae	Marshy soil
Chrysopogon serrulatus (Hoch.)	Poaceae	Tropical and sub tropical regions	*Prosopis julifora* (Sw.)DC	Fabaceae	Sandy, rocky, poor and saline soils
Conyza bonariensis L.	Asteraceae	Tropics and Subtropics	*Rhynchosia minima* L.	Fabaceae	Pantropical grasslands
Cynodon dactylon (L.) Pers.	Poaceae	All soil types	*Setaria pumila* (Poir.)	Poaceae	Warm temperate areas
Datura innoxia	Solanaceae	Abondened and wasteland	*Solanum nigrum* L.	Solanaceae	Wooded areas
Desmostachya bipinnata (L.) Pers.	Poaceae	Temperate and tropical regions	*Solanum surattense* L.	Solanaceae	Coastal plains
Dichanthium annulatum	Poaceae	Moutain slopes and disturbed ground	*Suaeda fruticosa* L.	Amaranthaceae	arid and semi-arid salt marshes
Digitaria ciliaris (Retz.) Koel.	Poaceae	Tropical regions	*Typha domingensis* (Pers.)	Typhaceae	Temperate and tropical regions
Dodonaea viscose L. (Jacq.)	Sapindaceae	Cosmopolitan distribution	*Withania somnifera* L.	Solanaceae	Open places and distributed areas
Echinochloa colonum L.	Poaceae	Tropics	*Xanthium strumarium* L.	Asteraceae	Farmland, old lands, roadsides, wastelands, riverbanks and overgrazed pasturelands
Eragrostris papposa	Poaceae	Rocky places	*Ziziphus nummularia* (N. Burman)	Rhamnaceae	Deserted areas

found in the region belongs to family Chenopodiaceae and has been widely reported to be used for the treatment of urinary problems. *Withania somnifera* (ashwagandha) commonly cures weakness and is a blood purifier belonging to the family Solanaceae. *Peganum harmala* (harmal) heals stomach disorder while *Achyranthes aspera* (puthkanda) possess anti inflammatory properties. (Ghani *et al.*, 2014; 2012)

Ecologists have adapted multivariate techniques to analyze the site characteristics responsible for prevailing species of an area. The distribution of species and complex community structure is due to range of intermingling factors (Ahmad *et al*., 2014a; 2014b; 2014c). In arid and semi arid region, soil texture, pH, moisture, organic matter, macronutrients and micronutrients play significant role in growth, distribution and abundance of species (Zare *et al*., 2011).

Vegetation classification. Soil found in UWC upon analysis had loamy texture. Soil texture has profound influence on many soil properties and it affects the suitability of soil for many uses. This soil feature governs how nutrients, water and air move in soil (Brady and Weil, 1996).Vegetation of UWC from the 150 quadrates led to indentify 37 species of the area, which are presented in dendrogram in Fig.1 with the help of TWINSPAN method. The dendrogram formulates species into groups and communities on the basis of species close association and also prominently show up the dominant species. Hence, dendrogram was used to classify the vegetation in groups and sub groups. Each major group contained certain communities. These communities were named primarily after the dominant species in each community.

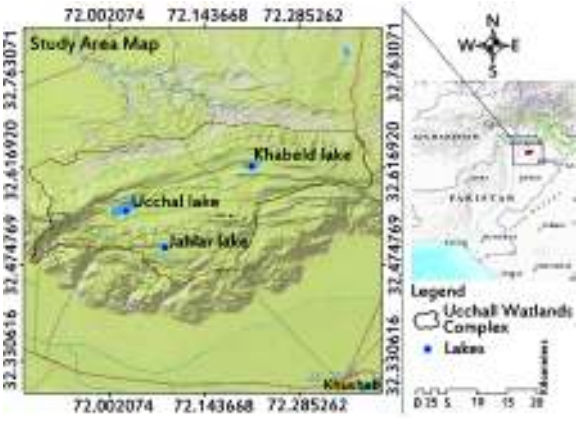

Fig. 1. Study area map of Ucchali wetlands complex.

DCA was also used as an indirect ordination to find main common gradient among species and to classify them in groups and communities. The close cluster of species represented their close association and common interest regarding life form and habitat. Likewise distances among points in the graph are taken as the degree of similarity or difference. Additionally these species clusters were also almost coherent with the results of TWINSPAN dendrogram. Some of the species had unique characteristics and were separate from the communities. These outlier species were *Digitaria ciliaris, Suaeda fruticosa* and *Ziziphus nummularia* (Fig. 2).

Community I, Alhagi-Typha is called after its dominant species *Alhagi maurorum* and *Typha domingensis,* belonging to Group I had total of eleven species. These eleven species included *Achyranthes aspera*, *Alhagi maurorum*, *Buxus papillosa*, *Withania somnifera, Xanthium strumarium*, *Typha domingensis*, *Buxus papillosa*, *Malva parviflora*, *Parthenium hysterophorus*, *Fagonia olivieri* and *Prosopis julifora.*

While **Community II**, under the same group I had four number of species i.e., *Amaranthus viridis*, *Imperata cylindrical*, *Chenopodium album* and *Solanum nigrum.* In addition this community was called as *Imperata-Solanum*.

Although **Community III** had *Bromus pectinatus*, *Datura innoxia*, *Chrysopogon serrulatus*, *Setaria pumila* and *Solanum surattense*. But owing to the abundance of *Chrysopogon serrulatus* and *Setaria pumila* in this community it was labeled as *Chrysopogon-Setaria*.

Commnunity IV had dominant species *Dichanthium annulatum* and *Carissa opaca* hence, called the name *Dichanthium - carissa*.

Community V named as *Juncus - Phyla* after its species *Juncus* sp. and *Phyla nodiflora,* while **Community VI** called *Justacia -Eragrostris* enclosed four other species (Fig.2). *Suaeda fruticosa* and *Ziziphus nummularia* also belonged to the same group I but were classified as outliers since they didn't form community with other species of the group.

group II communities enclosed total six species.

Commnunity VII had dominant species *Brachiaria ramose* and *Desmostachya bipinnata*. Species members of *Brachiaria-Desmostachya* included *Brachiaria ramose, Desmostachya bipinnata, Echinochloa colonum, Cynodon dactylon* and *Conyza bonariensis. Digitaria*

ciliaris, the most abundant species in the area belonged to the same group but being an outlier remained detached from the community (Fig. 3).

Dominance curve was plotted for species of UWC against their rank abundance and log of sum values (Fig. 4). In the studied area the most dominant species was *Cynodon dactylon* and then following it were *Brachiaria ramose* and *Desmostachya bipinnata*. On the other hand the least abundant species in the area was *Buxus papillosa.*

Vegetation-soil interaction. CCA being a direct ordination technique plots the species response towards their specific edaphic factors. Variables such as pH, EC, OM, macronutrients (N, P and K) and micronutrients

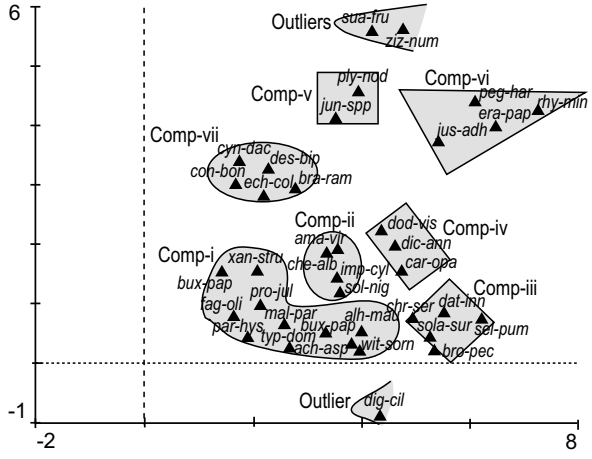

Fig. 2. Detrended correspondence analysis (DCA) with ucchali wetland complex (UWC).

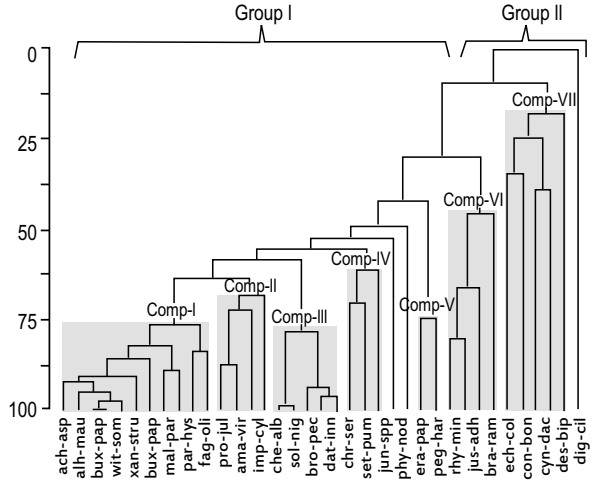

Fig. 3. Two way cluster analysis of UWC.

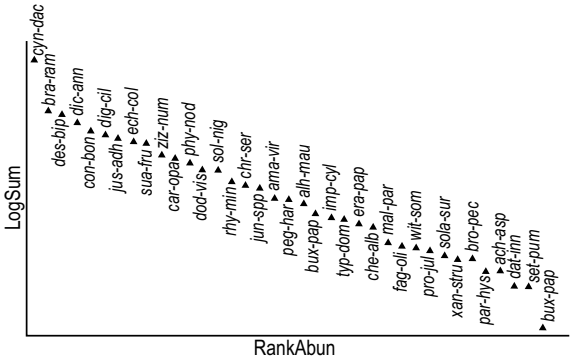

Fig. 4. Dominance curve for species of UWC

(Zn, Fe, Cu and Mn) were used to detect their promoting or limiting influence on growth of identified vegetation in the area.CCA biplot in Fig. 5 correlated the species against the edaphic factors of that particular region. The black triangles symbolize species, while red arrows correspond to the environmental variables. The angle that variables make with the line of axis illustrates its degree of correlation with that axis. The length of the arrow is proportional to its magnitude of influence on the species i.e., longer the arrow greater the influence and vice versa. Moreover, the species closer to the arrow signify its greater influence with that factor compared to the species that are plotted far away. Organic matter and elevation had the longest arrow than any other variable in the biplot hence it suggests its strongest effect on the species of UWC as compared to other edaphic factors. Phosphorus had more influenced on

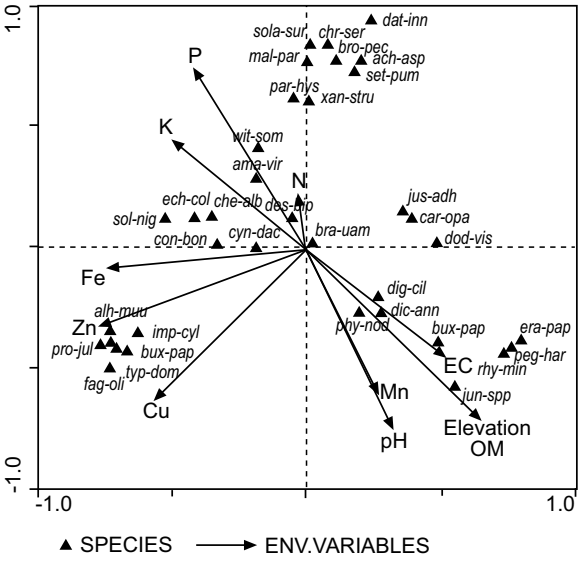

Fig. 5. Biplot UWC

the species: *Amaranthus viridis* and *Withania somnifera.* On the other hand *Dichanthium annulatum, Phyla nodiflora* and *Juncus* spp. were greatly influenced by organic matter in soil and elevation of the area. While EC affected on *Buxus papillosa* and *Digitaria ciliaris.* Similarly, Zn had shown major affect on the growth of *Alhagi maurorum, Prosopis julifora, Suaeda fruticosa, Imperata cylindrical, Buxus papillosa, Fagonia olivieri* and *Typha domingensis*. *Cynodon dactylon* the most abundant species in the UWC was remarkably affected by Fe in soil. N had the shortest arrow of all and seemed to impact *Brachiaria ramose* species. The species plotted far away were free of the impact of these plotted environmental variables whereas species in the close proximity responded well to its nearest variable.

Loess fitted model was used to draw data attribute plots of least and most abundant species of the area. This helped better to understand the specific species response with collective environmental variables. Each arrow of environmental variable shows its increasing value and angles between the arrows show the correlation between them. To assess this association with environmental variable *Cynodon dactylon* and *Buxus papillosa* was chosen since *Cynodon dactylon* was the most abundant species found in the area and *Buxus papillosa* was found to be less abundant comparatively to the other vegetation recorded in the UWC. In Fig. 6 *Cynodon dactylon* has uniform contour lines and no negative value which signifies its consistent distribution having positively influenced by the edaphic factors of the area. In contrast data attribute plot for *Buxus papillosa* in Fig. 7 illustrate its correlation with the environmental variables of the area. Fig. 7 depicts that this least abundant species have weak correlation as indicated by low values and random contour lines.

Pie symbol plots are used to represent environmental variables of the area quantitatively into fractions of classes based upon the number of samples. Each fraction of class is represented by separate colour code and has unique value range. The space between the pie symbols illustrates the relative abundance of species in sample plots. While the slices and their width represents the relative frequency and abundance of species in that class range.

Soil pH is a master variable that affects soil physical, chemical and biological properties. Degree of acidity or alkalinity affects which trees, shrubs or herbs will dominate the landscape under natural conditions and

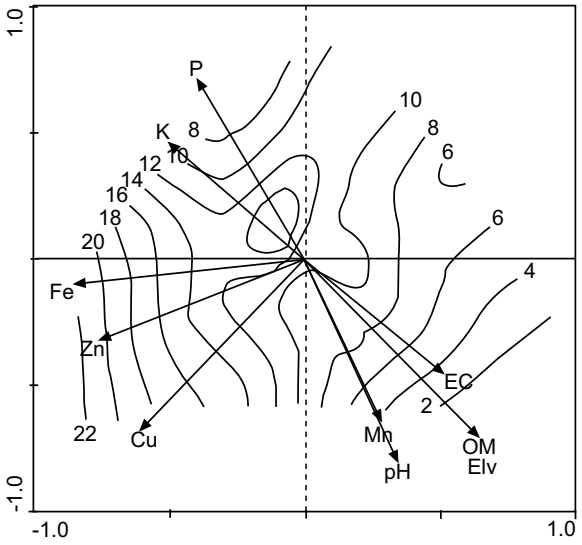

Fig. 6. Data attribute plot for *Cynodon dactylon* along with soil parameters

also controls which cultivated crops can grow well at that site (Brady, 1990). pH range of 5.5 to 6.5 provide the most satisfactory plant nutrients level overall (Brady and Weil, 1996) . The pH in the complex varied from 6.76 to 9.38 with the median value of 8.3 and had basic and alkaline properties due to prevailing dry arid climatic conditions. Most of the species favored the class pH-1 (i.e. 6.7-7.03) and this class range supported species

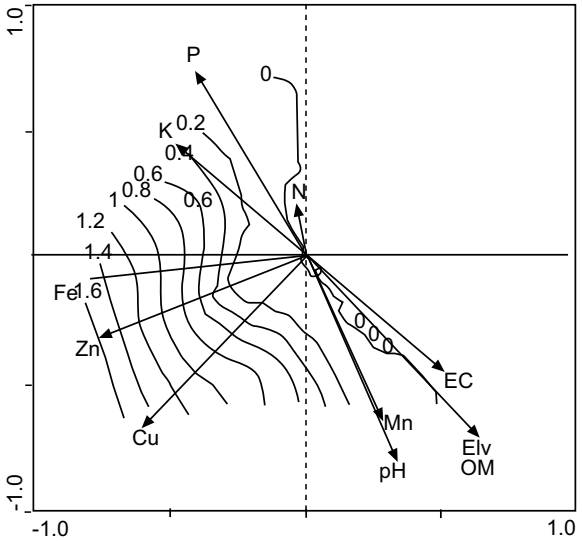

Fig. 7. Data attribute plot for *Buxus papillosa*along with soil parameters: Data attribute plot for *Buxus papillosa*along with soil parameters

diversity and richness. However certain species only grow in single pH range; for example *Solanum surattense, Datura innoxia* growth prevailed only in class pH-1 and *Buxus papillosa* only grew at pH-4 (Fig. 8a).

Soil EC measure amount of salts present in soil and is an indicator of soil health. The quantity of food provided to plant is controlled by soil EC (Kitchen *et al*., 1999). However, in arid and semi arid climates naturally excess salts are present (Zare *et al*., 2011). Soil of the region had EC value range of 7.43 to 9.85 dS/m. Species diversity is directly related with soluble salts in soil which correspond to soil electrical conductivity (Nakem *et al*., 2006). Four distinct classes for EC ranged from 7.43 to 9.85. These classes consisted of 38, 40, 35 and 37 number of species which illustrate the favourable EC range for species growth in the region to be EC-2 (Fig. 8b).

Organic matter (OM) is another important soil parameter that measures soil fertility. Organic matter by binding mineral particles holds and supply water and nutrients to plants. It defines growth, distribution, abundance and richness of species in an area (Rezaei, 2003). It has been reported that a well drained productive soil

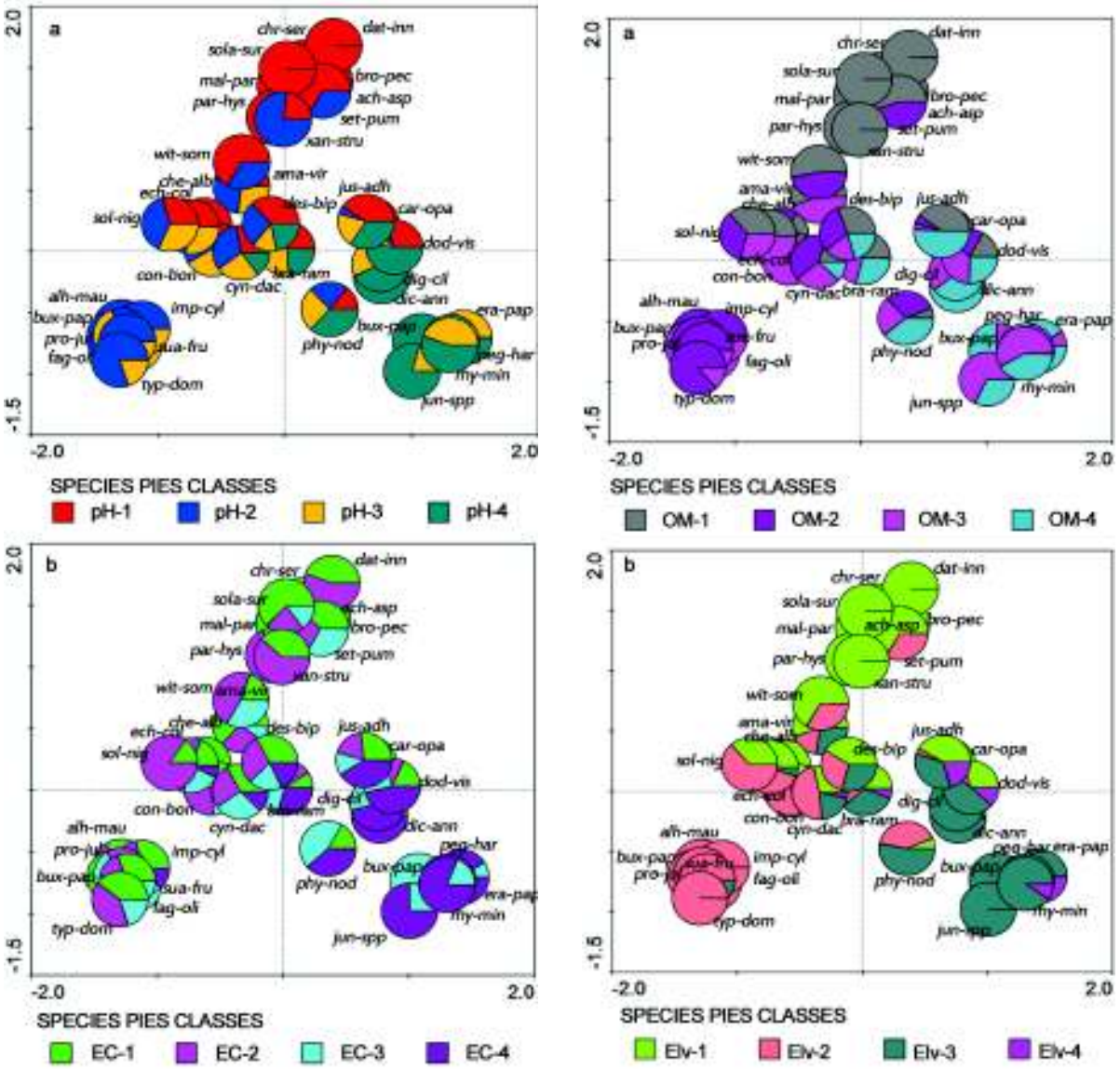

Fig. 8. Pie symbol scatter plot for (a) pH (b)EC (c) OM (d) elevation

constitute of 1 to 6% organic matter (Brady and Weil, 1996). The Fig. 8c explains the tolerance of species in terms of their survival to environmental gradient i.e., organic matter in the following case. This environmental gradient is classified into four distinctive classes. Soil in the vicinity of UWC had significant amount of organic matter ranging from minimum 0.57 to up to maximum 1.95%. The samples were distributed in these classes as 39, 37, 40 and 34. The OM-3 proved to be best class for species richness and its value range was 0.97-1.65. However, the *Xanthium strumarium, Solanum nigrum and Datura innoxia* could only persist in the range of OM-1.

Distinct altitudes have varying environmental conditions which ultimately lead to changing species distribution. Species such as (Fig. 8d) *Juncus* spp. and *Buxus papillosa* preferred higher elevation for its persistence compared to species *Xanthium strumarium*, *Datura innoxia* and *Chrysopogon serrulatus* which preferred lower elevations.

Nitrogen, phosphorus and potassium (N, P and K) deficiencies create complications in plants. Rowe *et al*. (2016) related the soil macronutrients availability with plants productivity in natural ecosystem. High concentration of macronutrients affects plant positively by increasing species abundance and distribution (Passioura, 2002). Most saline soils have low N and P content with high salt concentration and high species abundance (Ramakrishan and Kumar, 1976). Four classes were made on the basis of nitrogen in soil of selected quadrates. These classes had N value in ranges from 0.028-0.93 and had an average value of 0.04. Figure 9a showed that most of the species diversity could be found in N-1. The pie symbols in Fig. 9b represent species and these species were classified according to the available P in soil sample of the studied area. This was done to formulate the best range of class to favour the diverse number of species. The class values varied from 5.34 to 12.86 mg/kg with an average of 9.04. Class P-1 occupied 39 species, P-2 had 39, P-3 included 37 and P-4 contained 35 species. However, *Datura innoxia* showed growth only in P-4. While *Rhynchosia minima* only preferred P-1 class. Figure 9c represents the scatter plot for available K which divided into four ranges of classes. The range had maximum value of 95 to 377 mg/kg. These four classes contained 39, 41, 33, 37 species respectively. However, *Datura innoxia* favoured K-3.

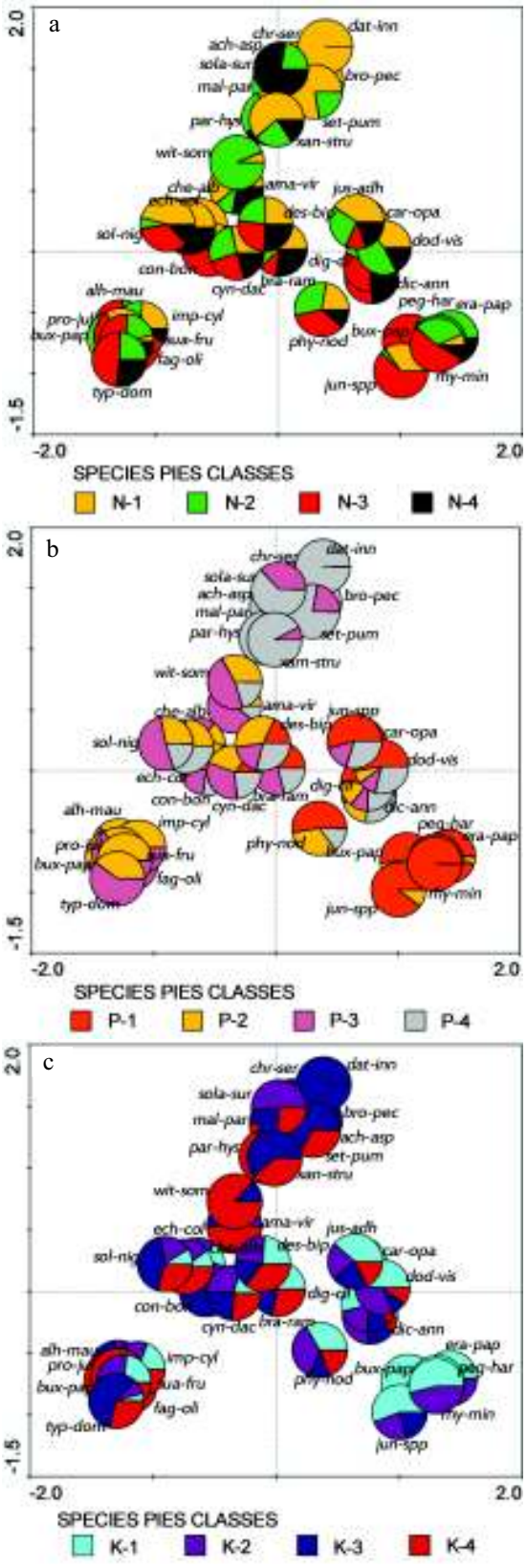

Fig 9. Pie symbol scatter plot for (a) K (b) N (c) P

The Zn value range of area could be divided into four distinctive classes. Each class represented a unique range; this was helpful in finding out the Zn range which favored species diversity. The value ranged from 0.1 to 0.99 with an average value of 0.44. However, some species preferred growth only in certain range of class. *Datura innoxia* cannot grow in any other class except for Zn-2 (Fig. 10a).

Graph in Fig. 10b had soil Cu value varying from 0.44-0.98 and had average of 0.73 with 63 distinct values. The calculated Cu of UWC could be divided into four distinct classes having separate Cu range to gather the best range that ensure diversity. It can be seen that some species only showed growth in a specific Cu range.

Having 63 distinct values the Fe calculated from the soil samples of the region had values starting from 1.02 to 4.9 with an average of 2.98. This range was classified to make four separate classes i.e. Fe-1, Fe-2, Fe-3 and Fe-4. Each class favoured specific species and number of species in these four classes were 38, 37, 39 and 36. However, Fig. 10c point up that there are few species that only grew in a particular Fe range. *Imperata cylindrical* showed growth only in Fe-1.

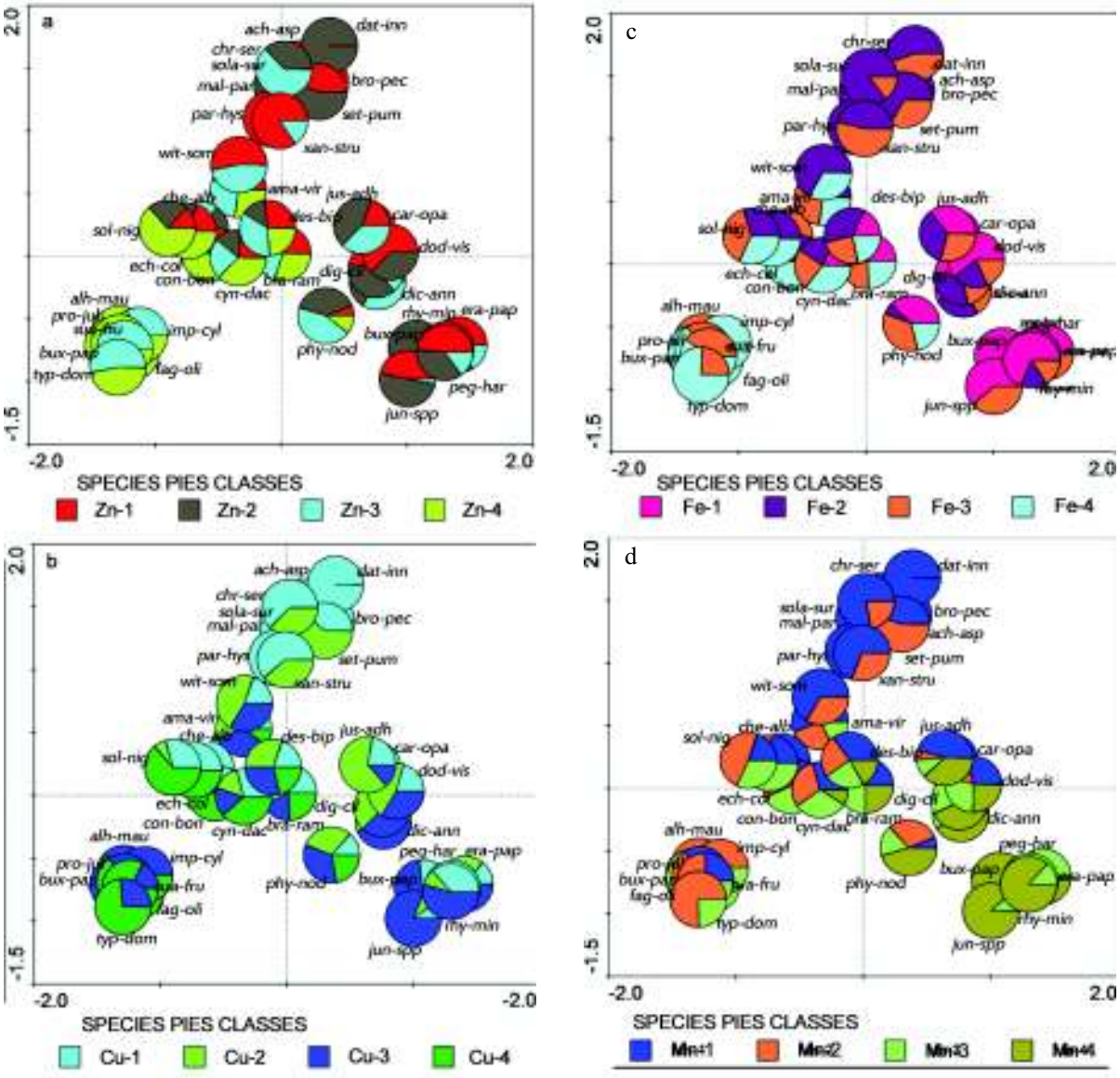

Fig 10. Pie symbol scatter plot for (a) Zn (b) Cu (c) Fe (d) Mn

Identified plant species Mn value ranged from 1.44 to 1.99. These classes had 43, 35, 37 and 35 members in them, respectively. Most of the species preferred to grow in first class i.e., Mn-1. Despite this *Rhynchosia minima, Juncus* spp., *Eragrostris papposa* and *Peganum harmala*only persisted in Mn-3 and Mn-4 i.e. Mn range of 1.71 to 1.99; as shown in Fig. 10d.

Despite all this, various non-native and invasive species were also recorded from the UWC. *Dodonea viscose* and *Prosopis* spp. found in the area are introduced species and grown as the result of overgrazing and land degradation (Arshad, 2011). *Dodonea viscose* is a small shrub of tropical, subtropical and warm temperate regions. It grows in open areas and is resistant to salinity, pollution and drought conditions (Selvam, 2007). *Prosopis* spp. is now considered problematic and difficult to identify because of its freely hybridizing nature with other species of the region (Zachariades *et al*., 2011). These species grow because of induced change in an area and alter the range ecology. The unique ecosystem of the complex may further get deteriorated by these invasive species since they replace the indigenous flora and climax species of the region. The UWC has three Ramsar sites and supports wide variety of important migratory birds and globally threatened wildlife, and change in ecology may affect this biodiversity in the longer term. As a result existing habitat and the dependent wildlife cannot readily adapt to the changing conditions and ultimately results in dispersal of local species from the area.

Conclusion

This study successfully provides an ecological interpretation of distribution of plant species and their communities along the edaphic factors in UWC. The vegetation composition of the area is defined by arid climatic conditions, high soil moisture and low nitrogen content. Elevation, organic matter and pH were more significantly correlated and were identified as major factors driving the floristic patterns. However, is continuous conservation of species diversity is required and medicinal flora must be valued in the area for effective management. GIS based census study of flora and fauna must be done in order to recognize and identify the important wildlife corridors in the vicinity of the complex, so as to prioritize the area in terms of its protection, promotion and maintenance of ecological processes.

References

Ahmad, S.S., Nawaz, M., Wahid, A., Malik, B., Urooj, R. 2014a. Scaling of multivariate ground flora data of gatwala forest park (GFP), Faisalabad Pakistan. *World Applied Sciences Journal*, **29:** 1492-1496.

Ahmad, S.S., Murtaza, R., Shabir, R., Ahmed M.N., Shah, T.A. 2014b. Environmental diversification and spatial variations in Riparian vegetation: A case study of Korang river, Islamabad. *Pakistan Journal of Botany*, **46:** 1203-1210.

Ahmad, S.S., Erum, S. Khan, S.M. and Nawaz, M. 2014c. An Appraisal of ecological distribution of herbaceous flora at Changa manga park Lahore, Pakistan. *Pakistan Journal of Botany*, **46:** 19-25.

Ahmad, S.S., Quratulain, A. 2011. Exploring the vegetation dynamics and community assemblage in Ayubia National Park, Rawalpindi, Pakistan, using CCA. *Biodiversity Journal*, **2:** 115- 120.

Ahmad, S.S. 2011. Canonical correspondence analysis of the relationships roadside vegetation to Its edaphic factors: A case study of Lahore-Islamabad motorway (M-2). *Pakistan Journal of Botany*, **43:** 1673-1677.

Ahmad, S.S. 2010. Detrended correspondence analysis of vegetation along motorway (M-2), Pakistan. *Pakistan Journal of Botany*, **42:** 2473-2477.

Ali, S.M., Malik, R.N. 2010. Spatial patterns of vegetation with underlying soil properties prevailing along drain side areas in Islamabad City. *Pakistan Journal of Botany*, **42:** 2397-2410.

Allen, S.E. 1974. *Chemical Analysis of Ecological Materials*. 565pp. Blackwell Scientific Publications, Oxford, London, UK.

Arshad, M. 2011. *Management Plan Ucchali Wetlands Complex*: *A Part of Salt Range Wetlands Complex*. PWP Unpublished Report to WWF, Islamabad. 6-89pp.

Bailey, A.W., Poulton, C.E. 1968. Plant communities and environmental interrelationship in a portion of the Tillamook burn, northwestern oregon. *Ecology*, **49:** 1-13.

Brady, N. C., Weil, R.R. 1996. *The Nature and Properties of Soils*.740pp., 11th edition, Prentice-Hall Inc, NJ, USA.

Brady, N.C. 1990. *The Nature and Properties of Soil*, 621 pp. MacMillan Publishing Company, New York, USA.

Ehi-Eromosel, C.O., Adaramodu, A.A., Anake, W.U., Ajanaku, C.O., Edobor-Osoh, A. 2012. Comparison of three methods of digestion for trace metal analysis in surface dust collected from an E-waste recycling site. *Nature and Science*, **10:** 1-6.

Ghani, A., Mustafa, I., Ali, Z., Ishtiaq, M., Ahmed, I., Hasan, N. 2014. Ethno-medicinal survey of plants of Soon valley, Khushab district, Punjab, Pakistan. *Journal of Medicinal Plants Research*, **8:** 1031-1034.

Ghani, A., Ali, Z., Ishtiaq, M., Maqbool, M., Parveen, S.2012. Estimation of macro and micro nutrients in some important medicinal plants of Soon valley, district Khushab, Pakistan. *African Journal of Biotechnology*, **11:** 14386-14391

Hill, M.O., Gauch Jr, H. G.1980. Detrended correspondence analysis: An improved ordination technique. *Vegetation*, **42:** 47-58.

Hill, M. O.1979. *TWINSPAN: A FORTRAN Program for Arranging Multivariate Data in an Ordered Two-Way Table by Classification of the Individuals and Attributes*. Section of Ecology and Systematics, iv+49 pp., Ithacan, Cornell University, New York, USA.

Kent, M., Coker, P. 1994. *Vegetation Description and Analysis. A Practical Approach*. 363 pp. Jahan Wiley & sons, London, UK.

Kitchen, N. R., Sudduth, K. A., Drummond, S. T. 1999. Soil electrical conductivity as a crop productivity measure for claypan soils. *Journal of Production Agriculture*, **12:** 607-617.

Leps, J., Smilauer, P. 2003. *Multivariate Analysis of Ecological Data Using CANOCO*, pp, 1-360 2nd edition. Cambridge University Press. UK.

Mueller-Dombois, D., Ellenberg, H. 1974. *Aims and Methods of Vegetation Ecology*. 547PP., Wiley New York, NY, USA.

Nathan, M.V., Gelderman, R. 2012. Recommended chemical soil test procedure. Available Online: http://www.agvise.com/wpcontent/uploads/2014/06/Recommended-chemical-soil-testprocedures-2012. pdf. Retrieved on December 28th, 2013.

Nkem, J. N., Virginia, R. A., Barrett, J. E., Wall, D. H., Li, G. 2006. Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. *Polar Biology*, **29:** 643-651.

Passioura, J. B. 2002. Soil conditions and plant growth. plant, *Cell and Environment*, **25:** 311-318.

Ramakrishnan, P. S., Kumar, R.1976. Adaptive responses of an alkaline soil population of *Cynodon dactylon* (L.) persistence to NPK nutrition. *The Journal of Ecology*, 187-193.

Rezaei, S.A. 2003. The use of soil quality index in site capability assessment for extensive grazing. *Ph.D Thesis*, University of Western Austrailia, Perth, Australia. 227pp.

Rowe, E.C., Toberman, H., Adams, J. L., Lawlor, A. J., Thacker, S. A., Patel, M., Tipping, E. 2016. Productivity in a dominant herbaceous species is largely unrelated to soil macronutrient stocks. *Science of the Total Environment*, **572:** 1636-1641

Ruby, T., Rana, S. A., Rana, N., Inayat, T. P. 2011. Weeds as viable habitat for arthropod species in croplands of central Punjab. *Pakistan Journal of Agricultural Sciences*, **48:** 145-152.

Saleem, A., Mirza, Z.S. 2012. Estimation of biomass and carrying capacity of scrub rangelands in Ucchali wetland complex, Soon valley. *Biological Society of Pakistan*, **58:** 93-100.

Selvam, V. E. 2007. *Trees and Shrubs of the Maldives*. 238 pp., 1st edition, RAP Publication, FAO Regional office for Asia and the Pacific, Bangkok, Thailand

Tavili, A., Rostampour, M., Zare Chahoukia, M.A., Farzadmehr, J. 2010. CCA application for vegetation-environment relationships evaluation in arid environments (Southern khorasan rangelands). *Desert*, **14:** 101-111.

Ter Braak, C., Smilauer, P. 2002. *CANOCO Reference Manual on CanoDraw for Windows User's Guide*: software for canonical Community ordination (version 4.5). Micro-computer Power, *Ithaca*, USA.

Urooj, R., Ahmad, S. S., Ahmad, M. N., Ahmad, H., Nawaz, M. 2016. Ordination study of vegetation analysis around wetland area: a case study of Mangla dam, Azad Kashmir, Pakistan. *Pakistan Journal of Botany*, **48:** 115-119.

Urooj, R. Ahmad, S. S., Ahmad, M. N., Khan, S. 2015. Ordinal classification of vegetation along Mangla dam, Mirpur, AJK. *Pakistan Journal of Botany*, **47:** 1423-1428.

Walkley, A. 1947. A critical examination of a rapid method for determining organic carbon in soils – effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sciences*, **63:** 251-264.

Xiaoni, J., Jimin, C., Hui'e, W. 2007. Application present situation of DCA, CCA, and DCCA ordination of grassland vegetation communities in China. *Chinese Agricultural Science Bulletin*, **12:** 085.

Zachariades,C., Hoffmann, J.H., Roberts, A.P.2011. Biological control of mosquito (*Prosopis* species) (Fabaceae) in South africa. *African Entomology* **19:** 402-415.

Zare, S., Jafari, M., Tavili, A., Abbasi, H., Rostampour, M. 2011. Relationship between environmental factors and plant distribution in arid and semiarid area (case study: Shahriyar rangelands, Iran). *American-Eurasian Journal of Agriculture & Environmental Science*, **10:** 97-105.

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Bioactivity Studies on Two Wild Edible Mushrooms Extracts

Funmilayo Bosede Borokini*[a], Mary Tolulope Olaleye[b] and Labunmi Lajide[c]
[a]Department of Chemistry, University of Medical Science, Laje, Ondo City - 0340001, Ondo State, Nigeria
[b]Department of Biochemistry, Federal University of Technology, Akure, PMB 704, Akure, Ondo State, Nigeria
[c]Department of Chemistry, Federal University of Technology, Akure, PMB 704, Akure, Ondo State, Nigeria

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Abstract. The aqueous and alcoholic extracts of *Lentinus squarrosulus* and *Termitomyces robustus* were analysed for phytochemicals, antioxidant and antimicrobial activities. Polyphenolic profile of the ethanol extracts revealed the presence of phenolic acids and flavonoids at different concentrations. Total phenol and total flavonoid contents demonstrated concentration dependent increase and positive correlation with the antioxidant activities in the corresponding extracts in the range of TFC (r = 0.794-0.993; 0.904-1.000) and TPC (r = 0.317-0.999; 0.621-0.995) for *L. squarrosulus* and *T. robustus* extracts, respectively. Inhibition concentrations at 50% (IC_{50}) for each extract in terms of its reducing and scavenging ability ranged from 0.54 to 15.04 mg/mL for total antioxidant property (TAP), ferric reducing antioxidant property (FRAP), DPPH, OH and NO radicals. Mushrooms extracts exhibited significant antifungal activities against *Aspergillus fumigatus* and *Candida albican* compared to bonlate but weak antibacterial activities against *Bacillus substilis*, *Escherichia coli* and *Salmonella typhi* compared to streptomycin sulphate. The mushrooms possessed appreciable antioxidant and antifungal properties for promoting good health.

Keywords: phytochemicals, antioxidant, antimicrobial activity, mushrooms

Introduction

Antioxidants play an important role in maintaining human health due to their ability to scavenge free radicals in the bodies. Reactive free radicals either from endogenous sources, through normal physiological and metabolic processes or exogenous sources by exposure to pollutants are harmful and capable of oxidizing biomolecules, resulting in cell death and tissue damage (Barros *et al*., 2008; Ames *et al*., 1993). Flavonoids and other phenolic compounds of plant origin are non-enzymatic antioxidants that have been reported as scavengers of free radicals (Rice-Evans *et al*., 1997). Coumarin is useful in pharmaceutical for its physiological, bacteriostatic and anti-tumor activity, though its hepatotoxicity in animal models has been reported (Jain and Himanshu, 2012). Flavonoids and coumarin exhibit a common benzopyrone structure and have been reported to possess various beneficial properties (Cook and Samman, 1996). The place of natural antioxidants in form of food nutrients or phytochemicals from plant origin cannot be underestimated in maintaining human health due to their ability to scavenge free radicals in the body; thereby supplementing the defense mechanisms of antioxidant enzymes such as superoxide dismutase, glutathione and catalase to fight harmful substances and prevent cell damages (Halliwell, 1996). Phytochemicals have been evinced as effective antioxidants that can help in the fight against the prevailing degenerative and chronic diseases.

Many researchers reported that mushrooms are rich sources of natural phytochemicals that can make them find different applications as functional foods and excellent sources of nutraceuticals (Abdullah *et al*., 2015; Obodai *et al*., 2014). Some have been reported to function as antioxidants and potential fermentation agents to enhance feed nutrition (Abdullah *et al*., 2015) and primordial and anthropogenic radio-nuclides composition study in some edible mushrooms samples in Nigeria revealed that the effective doses from selected primordial radionuclides were within acceptable limit (Faweya *et al*., 2015; IAEA, 1994) making them safe for consumption. Nevertheless, mushrooms are yet to take the proper place in human diet and nutrition as many varieties are yet to be exploited and are still in the wild despite having been used as food and medicine since ancient times. Phenolic compounds, protein hydrolyzates and some amino acids, present in different foods, were evinced to possess antioxidant properties (Yun-Zhong *et al*., 2002). Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups (Arbaayah and Umi, 2013). Studies have established that macro fungi like mushrooms as well as fruits and vegetables are very important

*Author for correspondence; E-mail: borokinif59@yahoo.com

in diets to offer adequate security. Some common edible mushrooms have currently been found to possess physiologically beneficial bioactive substances with antioxidant activity, which is well correlated with their total phenolic content (Barros *et al*., 2007) and can promote good health.

Antibiotic resistance has also become a global concern in recent years. Despite the huge diversity of natural and synthetic antimicrobial compounds that have been isolated or synthesized against pathogenic microorganisms, infectious diseases remain one of the major threats to human health and bacterial resistance to many antibiotics has been increasing. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of novel sources for the potential antibacterial and antifungal activity (Nedelkoska *et al*., 2013; Colombo and Bosisio, 1996). In search of novel therapeutic alternatives, natural resources have been exploited in the previous years and among them mushrooms could be an alternative source of new antimicrobials.

Many fungal and plant based studies have found compounds with various health promoting properties, ranging from anti-carcinogenic, anti-inflammatory, and immunosuppressive (Hearst *et al*., 2010; Rao *et al*., 2009), antimicrobial, antioxidant, antitumor, cholesterol lowering and immunostimulatory effects as reported by many researchers (Kosani *et al*., 2013; Barros *et al*., 2007). Due to their rich deposit of bioactive compounds (Yamac and Bilgili, 2006) in many species of mushrooms; they produce a wide range of secondary meta bolites with high therapeutic effect and contain minerals, vitamins (Priya and Srinivasan, 2013), especially B-complex, micronutrients such as selenium or chromium, β-glucans, lipids, proteins, and all essential amino acids as well as other organic acids (Iwalokun *et al*., 2007). Most of the mushrooms consumed in Nigeria are picked from the wild by rural dwellers when environmental conditions favour their sporocarp formation (Aremu *et al*., 2009) and sold at high price in local markets; there is no record of any significant cultivation. Obodai *et al*. (2014) reported that either wild or cultivated samples of mushrooms are excellent low caloric diets, nutritionally rich for improving quality of life.

Many studies on nutritional and minerals contents of different common species have been carried out (Jonathan *et al*., 2011) but little or no work has been published on the antioxidant and antimicrobial activities of many wild edible species in many parts of Nigeria. Also there are several wild edible species of mushrooms which are yet to be exploited. The present work therefore, has been focussed at evaluating the phenolic compounds, antioxidant and antimicrobial activities of *Termitomyces robustus* and *Lentinus squarrosulus* which are two common edible wild mushrooms in the western part of Nigeria.

Materials and Methods

Preparations of materials. The two species *Termitomyces robustus* and *Lentinus squarrosulus* (Fig. 1-2) obtained from local markets in Nigeria, were scraped and thoroughly cleaned with water to remove sand (both the pileus and stipes), cut into smaller pieces, oven dried at 60 °C, then ground and sieved to give 40 mm

Fig. 1. *T. robustus* (Purchased at Koko market, Owo).

Fig. 2. *L. squarrosulus* (Purchased at Koko market, Owo).

mesh size powder. The microorganisms (bacterial; *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* and fungi; *Aspergillus fumigatus*, *Fusarium solani* and *Candida albican* were obtained from Federal University of Technology, Akure in Ondo State, Nigeria, identified and subsequently maintained as stock strains. Simple susceptibility screening test using agar well diffusion method was employed and each microorganism was suspended in sterile saline and diluted to 10^{-6} colony forming unit (CFU).

Preparation of bioactive extracts. Bioactive extracts of each powdered mushroom were obtained by weighing 20 g into cleaned and dried reagent bottle and 400 mL each of distilled water, methanol and ethanol were separately added and subjected to cold maceration process for 24 h to obtain the aqueous extract and 72 h to obtain the alcohol extracts. The mixtures were filtered using cheese cloth and then through Whatman No.1 filter paper. The filtrates were then concentrated using rotary evaporator and freeze drier (Iweala and Okeke, 2005).

Determination of antibacterial activity. The antibacterial activity of aqueous, methanolic and ethanolic extracts of the mushrooms against *B. subtilis, E. coli* and *S. typhi bacteria* was evaluated by using agar well diffusion method (Srinivasan *et al*., 2001). Plate count agar (PCA) plates were inoculated with 100 µL of standardized inoculum ($1.5x10^8$ CFU/mL) of each selected bacterium and spread with sterile swabs. Wells of 8 mm size diameter were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. About 0.5 mL volume of each of the extracts was poured into a well of inoculated plates. Streptomycin sulphate (10 µg/mL) was used as a positive control which was introduced into a well instead of extract. The solvents; deionized water, methanol or ethanol were used as a negative control which was introduced into a well instead of the extracts. The plates thus prepared were left at room temperature for 10 min allowing the diffusion of the extract into the agar. After incubation for 24 h at 37 °C, the plates were observed. If antibacterial activity was present on the plates, the zone of inhibition was measured and expressed in mm.

Determination of antifungal activity. The antifungal activity of mushroom extracts was evaluated against food associated fungi by using poisoned food technique. Potato dextrose agar (PDA), was weighed (39 g) and dispersed in a litre of deionised water sterilized at 121 °C for 15 min, allowed to cool (45 °C) before pouring 20 mL into separated dishes. The fungi; *Aspergillus fumigatus*, *Fusarium solani* and *Candida albican* were inoculated on potato dextrose agar (PDA) plates and incubated for 25 °C for 72 h, to obtain young actively growing colonies of moulds and 0.2 mL of each of the extract was mixed with 20 mL of cooled (45 °C) molten PDA medium and allowed to solidify at room temperature for 30 min. Thereafter 10 µL of fungal spores in distilled water was added at the centre of the solidified PDA plates. PDA plates with 10 µg/mL of bonlate were used as positive control. PDA plates with the solvents; deionized water, methanol or ethanol were used as negative control (McCutcheon *et al*., 1994). The inoculated plates were incubated at 25 °C and colony mean diameter was measured and recorded after 3 days. Percentage mycelial growth inhibition (% MGI) was calculated as given below:

$$\% \text{ MGI} = \frac{\text{Diameter of fungal colony in control} - \text{diameter of fungal colony in extract}}{\text{diameter of fungal colony in control}} \times 100$$

Quantification of phenolic compounds in ethanolic extracts by HPLC-DAD. Chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm × 250 mm) in reverse phase, packed with 5 µm diameter particles. The mobile phase was water containing 1% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 10 min and changed to obtain 20, 30, 50, 60, 70, 20 and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Silva *et al*. (2014) with slight modifications. *L. squarrosulus* and *T. robustus* extracts and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, *L. squarrosulus* and *T. robustus* extracts were analysed at a concentration of 15 mg/mL. The flow rate was 0.6 mL/min, injection volume 50 µL and the wavelength were 270 for gallic acid, 278 nm for coumarin, 327 nm for chlorogenic acid and caffeic acid, and 365 nm for quercetin, quercitrin, kaempferol and rutin. Stock solutions of standards references were prepared in the high-performance liquid chromatography (HPLC) mobile phase at a concentration range of 0.025-0.250 mg/mL for quercetin, quercitrin, kaempferol, coumarin and rutin; and 0.030-0.300 mg/mL for gallic, caffeic and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time

with those of reference standards and by DAD spectra (200-500 nm). Calibration curve for gallic acid: Y = 13480x + 1257.5 (r = 0.9998); coumarin: Y = 11983x + 1196.9 (r = 0.9997); chlorogenic acid: Y = 11786x + 1267.1 (r = 0.9991); caffeic acid: Y = 13048x + 1345.6 (r = 0.9995); rutin: Y = 12478x + 1194.9 (r = 0.9997), quercitrin: Y = 13641x + 1178.4 (r = 0.9997), kaempferol: Y = 11458x + 1269.4 (r = 0.9998) and quercetin: Y = 12783x + 1195.8 (r = 0.9996). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Silva *et al*., 2014).

Determination of total phenol contents. The total phenol content (TPC) of the samples was determined by mixing 0.5 mL of each extract with 2.5 mL 10% Folin-Cioalteu's reagent (v/v) and 2.0 mL of 7.5% Na_2CO_3. The reaction mixture was subsequently incubated at 45 °C for 40 min, and the absorbance measured at 760 nm in the spectrophotometer. All tests were performed three times. Gallic acid was used as a standard phenolic compound. The amount of total phenolic compound in the extract was determined as µg of gallic acid equivalent (GAE) per g dry weight (Singleton *et al*., 1999).

Determination of total flavonoid. The flavonoid content (TFC) of the extract was determined using a colorimetric assay developed by Zhishen *et al*. (1999). A known volume (0.5 mL) of each extract was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% w/v $NaNO_2$ was added to the flask. After 5 min, 0.6 mL of 10% w/v $AlCl_3$ was added and after 6 min, 2 mL of 1M NaOH was added to the mixture followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content expressed as mg rutin equivalent/g.

Determination of 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging ability. The free radical scavenging ability of the extract against DPPH free radical was evaluated by Ursini *et al*. (1994). Extracts of 1-5 mg/mL each was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

$$\% \text{ of Inhibition} = \frac{\text{Ao-A1}}{\text{Ao}} \times 100$$

where:

Ao = absorbance of the trolox and A1 = absorbance of the sample.

Determination of OH radical scavenging activity. Exactly 1-5 mg/mL of each extract of the samples were mixed with 1 mL of reaction mixture (100 µM $FeCl_3$, 104 µM ethylenediamine tetra acetic acid, 1.5 M H_2O_2, 2.5 M deoxyribose and 100 µM ascorbic acid in 10 mM KH_2PO_4-KOH, pH 7.4) and incubated for 1h at 37 °C. Thereafter, 1 mL of 0.5% thiobarbituric acid in 0.025 M NaOH and 1 mL of 2.8% trichloroacetic acid was added to the mixture and heated for 30 min at 80 °C before reading the absorbance at 532 nm against an appropriate blank solution (Heo and Lim, 2004). All tests were performed three times. Ascorbic acid was used as a positive control. Percent inhibition of OH was calculated by the following expression:

$$\% \text{ of Inhibition} = \frac{\text{Ao-A1}}{\text{Ao}} \times 100$$

where:

Ao = absorbance of the ascorbic acid and A1 = absorbance of the sample.

Determination of NO scavenging activity. Briefly, 5 mM sodium nitroprusside in phosphate-saline was mixed with different concentrations of the extracts: 1-5 mg/mL, before incubation at 25 °C for 150 min. Thereafter, the reaction mixture was added to Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride), before measuring the absorbance at 546 nm (Jagetia *et al*., 2004). Ascorbic acid was used as control. The nitric oxide radicals scavenging activity of the fractions was calculated according to the following equation:

$$\% \text{ of Inhibition} = \frac{\text{Ao-A1}}{\text{Ao}} \times 100$$

where:

Ao = absorbance of ascorbic acid and A1 = absorbance in the presence of the fractions and ascorbic acid.

Determination of ferric reducing antioxidant property (FRAP). The reducing property of the extract was

determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Pulido *et al*. (2000). Each of the extracts (1-5 mg/mL) was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate (III) (- $K_3[Fe(CN)_6]$). The mixture was incubated at 50 °C for 20 min, thereafter 2.5 mL, 10 % trichloroacetic acid was also added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was then mixed with equal volume of water and 1 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm, the higher the absorbance, the higher the reducing power.

Determination of total antioxidant activity (TAP). The assay is based on the reduction of Mo (VI)-Mo (V) by the extracts and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH as described by Prieto *et al*. (1999). Exactly 0.2 mL of the different extracts (1-5 mg/mL) was combined with 3 mL of each reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_2MoO_4$). The tubes were incubated at 95 °C for 90 min and the absorbance measured at 695 nm against the blank after the mixtures have cooled to room temperature. The antioxidant activity was expressed as gallic acid equivalent.

Statistical analysis. Values are presented as the mean ± SD of three replicates. ANOVA and LSD and Pearson correlation analyses were performed using the commercial software SPSS 16.0.

Results and Discussion

Phenolic compounds. Investigation of polyphenolic profile of ethanol extracts of *Lentinus squarrosulus* and *Termitomyces robustus* showed the presence of phenolic acids, flavonoids and coumarin (Table 1) which have been proofed through researches to possess various antioxidant properties. Flavonoids content was found to be higher in concentration than phenolic acids as indicated by the ratio of flavonoid to phenolic acids of 1.29 and 5.12 for *L. squarrosulus* and *T. robustus*, respectively in this study.

HPLC fingerprinting of extracts revealed the presence of the gallic acid (t_R = 11.67 min; peak 1), chlorogenic acid (t_R = 21.45 min; peak 2), caffeic acid (t_R = 24.08 min; peak 3), coumarin (t_R = 30.21; peak 4), rutin (t_R = 38.19 min; peak 5), quercitrin (t_R = 46.57 min; peak 6), quercetin (t_R = 49.97 min; peak 7) and kaempferol (t_R = 52.11 min; peak 8) (Fig. 3-4, Table 1). The limit of detection (LOD) and limit of quantification (LOQ) for the standards curves were: gallic acid (LOD = 0.015 and LOQ = 0.049 g/mL), chlorogenic acid (LOD = 0.008 and LOQ = 0.027 g/mL), caffeic acid (LOD = 0.023 and LOQ = 0.076 g/mL), coumarin (LOD = 0.021 and LOQ = 0.065 g/mL), rutin (LOD = 0.009 and LOQ = 0.031 g/mL), quercitrin (LOD = 0.027 and LOQ = 0.089 g/mL), quercetin (LOD = 0.011 and LOQ = 0.037 g/mL) and kaempferol (LOD = 0.030 and LOQ = 0.098 g/mL).

Table 1. Phenolic profile of ethanol extracts of mushrooms

Compounds	*L. squarrosulus*	*T. robustus*
	(mg/g)	
Gallic acid	$0.93^a \pm 0.01$	$1.02^a \pm 0.03$
Chlorogenic acid	$1.15^a \pm 0.01$	$1.49^b \pm 0.01$
Caffeic acid	$9.87^b \pm 0.03$	$0.53^c \pm 0.01$
Coumarin	$7.04^c \pm 0.02$	$3.37^d \pm 0.02$
Rutin	$0.61^d \pm 0.01$	$1.38^a \pm 0.01$
Quercitrin	$3.52^d \pm 0.02$	$6.12^e \pm 0.01$
Quercetin	$4.11^f \pm 0.03$	$3.80^d \pm 0.03$
Kaempferol	$7.19^c \pm 0.02$	$4.25^f \pm 0.01$

Values represent means of triplicate readings ± S.D. Values with the same superscript along the row are not significantly different (p = 0.05).

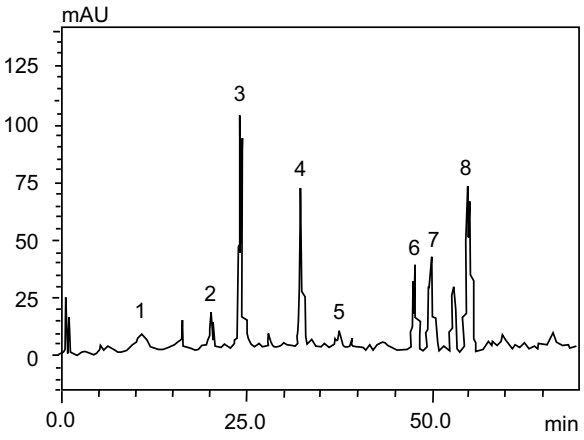

Fig. 3. Reverse-phase high performance liquid chromatography with Diode-Array Detection (HPLC-DAD) profile of *Lentinus squarrosulus* ethanol extract. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

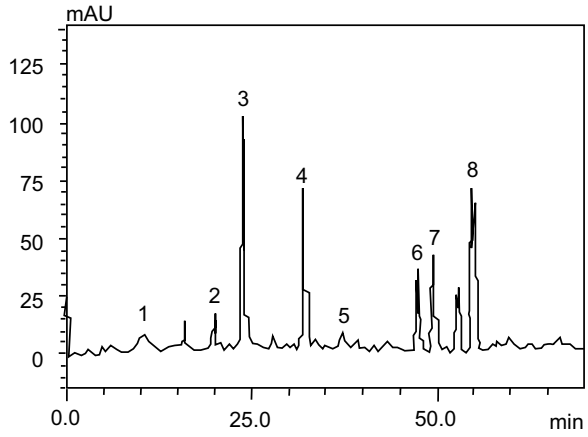

Fig. 4. HPLC-DAD profile of *Termitomyces robustus* ethanol extract.. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8).

Total phenol and total flavonoid contents of mushrooms. Total phenol contents (TPC) are reported in mg/g of DW as gallic acid equivalents by reference to standard curve ($y = 2.327$, $r^2 = 0.9849$) and total flavonoid contents (TFC) as rutin equivalent in mg/g by reference to standard curve ($y = 8.250x$, $r^2 = 0.998$). For the aqueous and methanol extracts, *T. robustus* gave significantly higher values of total phenol ($p = 0.05$) with the range 29.00 ± 0.41 to 61.60 ± 0.33 and 67.675 ± 0.72 to 259.96 ± 1.21, respectively than *L. squarrosulus* which range from 20.41 ± 0.16 to 55.859 ± 0.11 and 21.48 ± 0.23 to 85.94 ± 0.16, but higher concentrations were observed in ethanol extracts of *L. squarrosulus* ; 22.344 ± 0.31 to 107.421 ± 0.28 than *T. robustus* 16.76 ± 0.28 to 74.34 ± 0.31. The gallic acid used as the standard gave significantly higher values; 275.97 ± 0.63 to 1655.06 ± 3.42 than both mushrooms. Higher flavonoid contents were obtained in all the extracts of *T. robustus* (54.55 ± 0.09 to 248.4848 ± 0.72; 4.85±0.51 to 31.45 ± 0.44; and 0.97 ± 0.03 to 4.97 ± 0.11) than *L. squarrosulus* (60.61 ± 1.01 to 245.4545 ± 2.34; 2.42 ± 0.04 to 13.23 ± 0.16 and 0.36 ± 0.02 to 2.97 ± 0.04), respectively except at lower concentrations of the aqueous extract. TFC of rutin ranged from 34.85 ± 0.83 to 181.82 ± 0.89. The variance observed in the extractive abilities of the solvents used in this study for TFC and TPC could be due to the fact that phenolic compounds in the fungi belong to different classes of phenols, which are selectively soluble in different solvents (Prior and Cao, 1999) and are associated with other non-phenolic molecules. Extraction of phenolic compounds is influenced by their chemical nature, extraction method, sample size, time and storage conditions as well as the presence of interfering substances. Aqueous extracts gave highest total phenol contents, followed by methanol. Polarity seems to be the major influence, though inconsistent with the trend observed for TFC. Antiseptic and anti-inflammatory functions of phenols in mushrooms are documented. The total phenolic concentration of the wild mushrooms (wild champignon: (Champignon) *Agaricus* sp., *Boletus* sp., and *Macrolepiota* sp.) was found to be in the range of 4.87 to 13.74 mg CAE/g DW (Alvarez-Parrilla *et al*., 2007). Total flavonoid contents in the mushrooms extract varied from 1.40 to 29.80 mg QE/g of DW of extracts in the ethanolic extracts of oyster mushrooms (*P. pulmonarius, P. ostreatus, P. djamor* var. *djamor* and *P. djamor* var. *roseus*) and the split gill mushroom (*Schizophyllum commune*) (Arbaayah and Umi, 2013).

DPPH radical scavenging properties of the mushrooms extracts. All the extracts of the two mushrooms demonstrated H-donor activities (Fig. 5a-c). Higher DPPH radical scavenging activity was detected in alcohol extracts than the aqueous extracts, contrary to the trend lines for TFC. It however agreed with the trend obtained for total TPC with few exceptions. The scavenging ability seems to increase with decrease in polarity of the solvent but correlation between TFC and TPC with corresponding DPPH scavenging capacities were strong and positive (Tables 2-3). IC_{50} values which represented the concentration of extracts capable of inhibiting 50% of radical solution are presented in Table 4. The higher the scavenging ability of extract the lower the inhibition concentrations at 50% (IC_{50}). The IC_{50} for DPPH free radicals by a macro fungi; *S. commune* ethanol extracts which was 2.75 mg/mL as reported by Arbaayah and Umi (2013) was less than the range 3.39-8.78 mg/mL as calculated from the equation of the graph in this study. The aqueous, methanol and ethanol extracts of *T. robusts* showed the higher scavenging ability than *L. squarrosulus*. The alcohol extracts especially showed strong effectiveness in inhibiting DPPH though less effective than trolox.

Scavenging properties of OH of the mushrooms extracts. Scavenging of OH activity was determined by measuring the inhibition of degradation of deoxyribose by the free radicals generated by the Fenton reaction (Umamaheswari and Chatterjee, 2000). Aqueous extracts

Table 2. Pearson's correlation coefficient between total phenol content (TPC) and antioxidant assays

TPC	Samples		Pearson's correlation				
			DPPH	OH	NO	FRAP	TAP
Aqueous	r value	*T. robustus*	0.925*	0.887*	0.621	0.958*	0.995**
	p value		0.025	0.045	0.264	0.010	0.000
	r value	*L. squarrosulus*	0.889*	0.996**	0.844	0.977**	0.972**
	p value		0.043	0.000	0.072	0.004	0.006
Methanol	r value	*T. robustus*	0.993**	0.938*	0.817	0.977**	0.952*
	p value		0.001	0.018	0.091	0.004	0.013
	r value	*L. squarrosulus*	0.983**	0.968**	0.317	0.995**	0.855
	p value		0.003	0.007	0.603	0.000	0.065
Ethanol	r value	*T. robustus*	0.973**	0.993**	0.989**	0.982**	0.966**
	p value		0.005	0.001	0.001	0.003	0.007
	r value	*L. squarrosulus*	0.990**	0.985**	0.991**	0.998**	0.999**
	p value		0.001	0.002	0.001	0.000	0.000
Control	r value	-	0.985**	0.983**	0.965**	0.991**	0.992**
	p value		0.002	0.003	0.008	0.001	0.001

FRAP = ferric reducing antioxidant property; TAP = total antioxidant property.

Table 3. Pearson's correlation coefficient between total flavonoid content and antioxidant assays

TPC	Samples		Pearson's correlation				
			DPPH	OH	NO	FRAP	TAP
Aqueous	r value	*T.robustus*	0.936*	0.973**	0.904*	0.930*	0.915*
	p value		0.019	0.005	0.035	0.022	0.029
	r value	*L.squarrosulus*	0.940*	0.985**	0.926*	01.000**	0.973**
	p value		0.018	0.002	0.024	0.000	0.005
Methanol	r value	*T.robustus*	0.968**	0.994**	0.919*	0.990**	0.967**
	p value		0.007	0.001	0.028	0.001	0.007
	r value	*L.squarrosulus*	0.988**	0.969**	0.217	0.979**	0.795
	p value		0.002	0.007	0.726	0.004	0.108
Ethanol	r value	*T.robustus*	0.967**	0.984**	0.995**	0.954*	0.983**
	p value		0.007	0.002	0.000	0.012	0.003
	r value	*L.squarrosulus*	0.988**	0.975**	0.988**	0.999**	0.993**
	p value		0.002	0.005	0.002	0.000	0.001
Control	r value	-	0.987**	0.965**	0.955*	0.968**	0.988**
	p value		0.002	0.008	0.011	0.007	0.002

FRAP = ferric reducing antioxidant property; TAP = total antioxidant property.

of both mushrooms strongly inhibited the production of hydroxyl radicals compared with the standard (ascorbic acid) (Fig. 6a). Aqueous extracts showed significantly higher scavenging abilities than the alcohol, and both mushrooms demonstrated increase in percentage inhibition with increasing extracts concentration (Fig. 6a-c) in agreement with the study on *Aesculus indica* leaves reported earlier (Guno, 2009) and had strong positive correlations with TFC and TPC (Tables 2-3). The least anti- OH property was observed in the methanol extracts of *L. squarrosulus* with IC_{50} value of 15.04 mg/mL calculated from the equation of the graph (Table 2).

Nitric oxide (NO) radical scavenging properties of the mushrooms extracts. Nitric oxide is important as a regulatory and signaling molecule, but it is also implicated in inflammation, cancer and other pathological conditions (Dharmendra *et al*., 2012) in addition to the reactive oxygen species. NO is known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital

role in neuromodulation or as a neurotransmitter in the CNS (Gulati *et al*., 2006), high levels of these radicals are toxic to tissue and contribute to the vascular collapse, various carcinoma and ulcerative colitis (Rajan *et al*., 2011). There was no regular trend observed in the scavenging ability for the extracts, except that aqueous and methanol extracts displayed significantly higher inhibition than the ethanol extracts (Fig. 7a-c). The least IC_{50} values was observed in methanol extract of *T. robusts*, an indication of more potency against NO (Table 4). The extracts of both mushrooms exhibited appreciable scavenging properties compared with ascorbic acid and there was a general positive correlation with TFC and TPC (Tables 2-3).

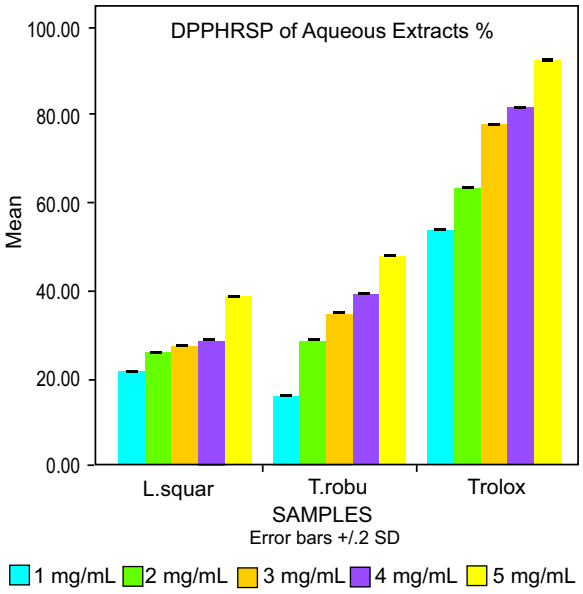

Fig. 5a. DPPH radical scavenging property in (aq).

Ferric reducing antioxidant properties of the mushrooms extracts. The reductive capabilities of different extracts of the mushrooms increased with increasing concentrations of the extracts (Fig. 8a-c). The aqueous extracts of both mushrooms showed higher activity than the alcohol extracts but much lower than the ascorbic acid at all concentrations. The extracts acted as electron donor to reduce the ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) (Arbaayah and Umi, 2013). The correlation of TFC and TPC with reducing capacities of corresponding extracts of *T. robustus* and *L. squarrosulus* were strongly positive (Tables 2-3). This agrees with the observation that the reducing power of a compound is known to be associated with the presence of certain antioxidant agents and reductones such as ascorbic acid (Jayaprakasha *et al.*, 2007; Duh *et al*., 1999). The activities of mushrooms in this study compared favourably well with 62771.43 and 58528.57 µmol/g reported for methanol extracts of *Boletus erythropus var. erythropus* and *Suillus luteus* (Keleş *et al.,* 2011), respectively.

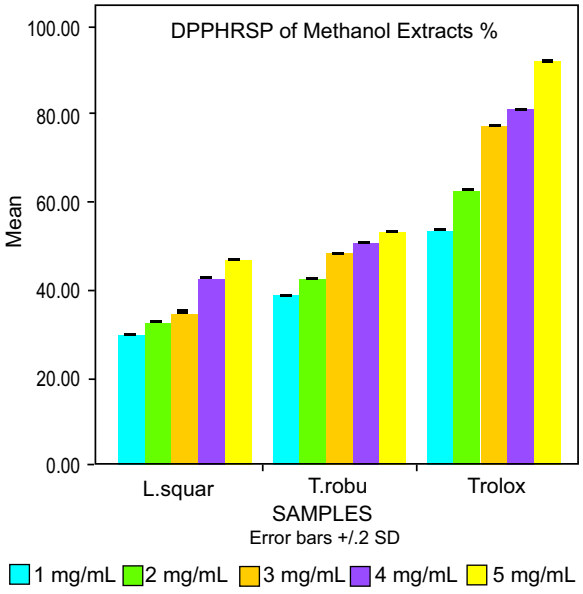

Fig. 5b. DPPH radical scavenging property in (MEOH).

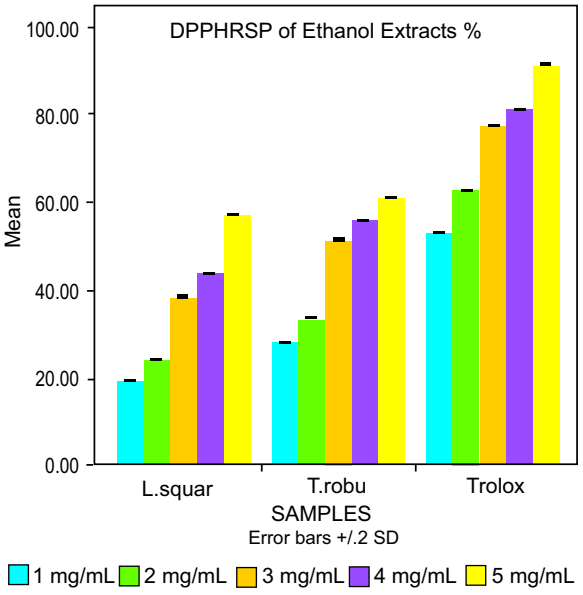

Fig. 5c. DPPH radical scavenging property in (ETOH).

Table 4. IC_{50} values for DPPH, OH and NO free radicals

Solvents	Samples	(IC_{50})				
		DPPH	OH	NO	FRAP	TAP
Aqueous	*T. robustus*	5.27	0.54	4.61	1.28	1.18
	L. squarrosulus	8.78	1.76	4.72	1.62	1.58
Methanol	*T. robustus*	3.93	8.72	3.80	2.41	2.66
	L. squarrosulus	5.86	15.04	8.54	5.16	4.71
Ethanol	*T. robustus*	3.39	5.24	3.93	8.48	1.94
	L. squarrosulus	4.35	5.74	4.57	3.04	4.03
Control	-	0.51	1.18	0.56	0.21	1.93
		Trolox	Ascorbic acid	Ascorbic acid	Ascorbic acid	Gallic acid

FRAP = ferric reducing antioxidant property; TAP = total antioxidant property.

Total antioxidant properties of the mushrooms extracts. The assay assessed the reducing power of the extracts based on their abilities to reduce Mo (VI) to Mo (V) and the results presented in Fig. 9a-c as gallic acid equivalent in mg/g.

Aqueous extracts exhibited higher reducing power than the methanol and ethanol extracts but all demonstrated concentration dependent increase in their reducing properties with positively strong correlations with corresponding TFC and TPC in the range TFC (r = 0.915 -0.983); TPC (r = 0.953- 0.995) for *T. robustus* and TFC (r = 0.795-0.993); TPC (r = 0.855 -0.999) for *L. squarrosulus* at 0.01- 0.05 significant levels. Some of the extracts showed stronger reducing ability than gallic acid that was used as reference standard. The result of this study revealed that both mushrooms extracts generally showed effective H donor activity, reducing power and free radical scavenging activity. Supplementation of these mushrooms in food will serve as natural antioxidants and can be used as a remedy to fight with oxidative stress. Inhibitory action of extracts could be enhanced by full recovery of polyphenols using suitable solvents because the affinity of polyphenolic complex is not the same for all types of solvents used (Koffi *et al*., 2010). Factors that influence the extraction of phenolic compounds in plant materials include chemical nature, extraction method, sample size, time and storage conditions as well as the presence of interfering substances (Prior and Cao, 1999).

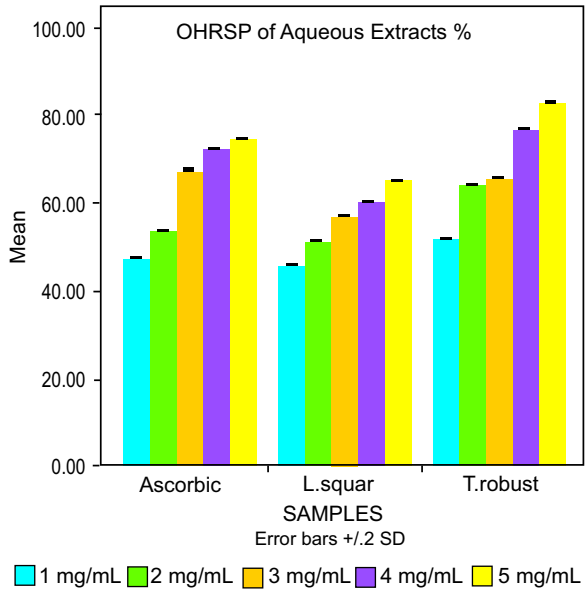

Fig. 6a. OH radical scavenging property in (Aq).

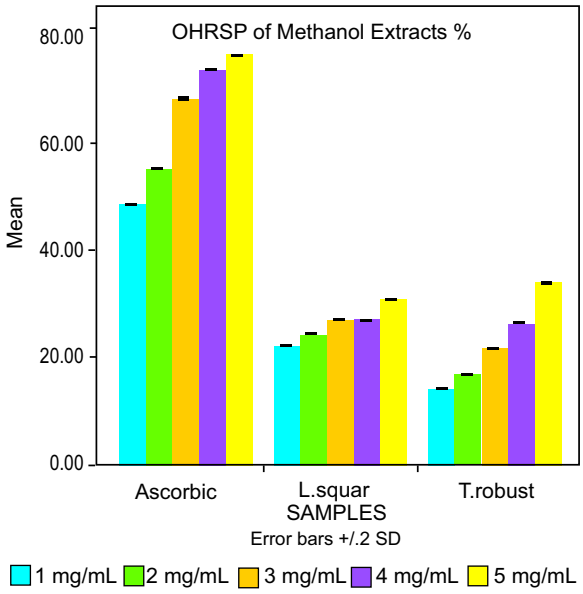

Fig. 6b. OH radical scavenging property in (MEOH).

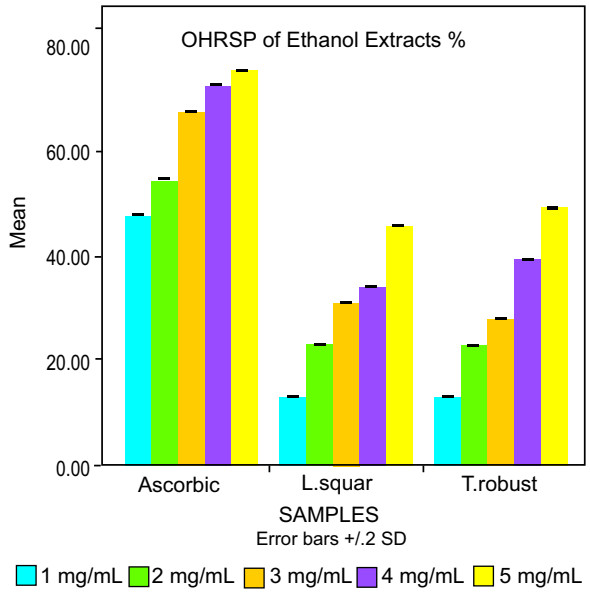

Fig. 6c. OH radical scavenging property in (ETOH).

Antimicrobial activities of mushrooms. The antibacterial activities of aqueous and alcoholic extracts of the mushrooms at concentration 0.05 g/mL are described in Fig. 10a. The ethanol extract of *L. squarrosulus* shows activity against *E. coli*; and the activity was fair as compared with streptomycin sulphate. Aqueous and methanol extracts of *L. squarrosulus* showed some inhibitory effect on *S. typhii*; and methanol extract was more effective and ethanol extract had no effect while none of the extracts of *T. robustus* showed any activity against *S. typhii* and *E. coli*. This is at variance with the results obtained by Hamowia and salfat (1994) in their study. *B. subtilis* was susceptible to aqueous and ethanol extracts of *L. squarrosulus* as well as aqueous extract of *T. robustus*, *L. squarrosulus* extracts demonstrated well marked antimicrobial property than *T. robustus* and could be potential source of antimicrobial agent.

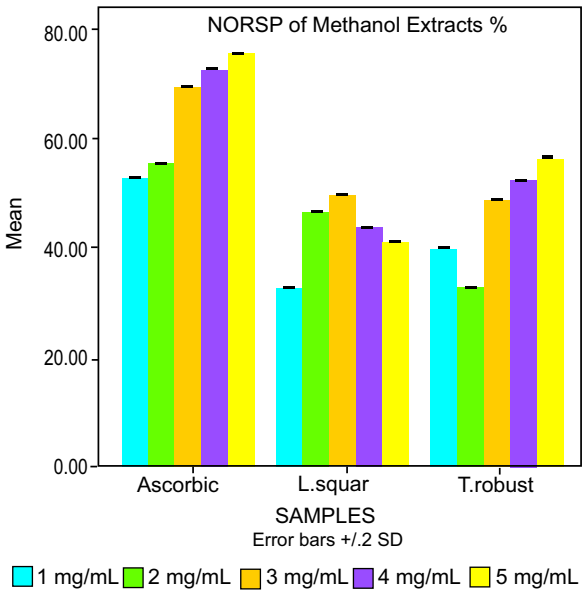

Fig. 7b. NO radical scavenging property in (MEOH).

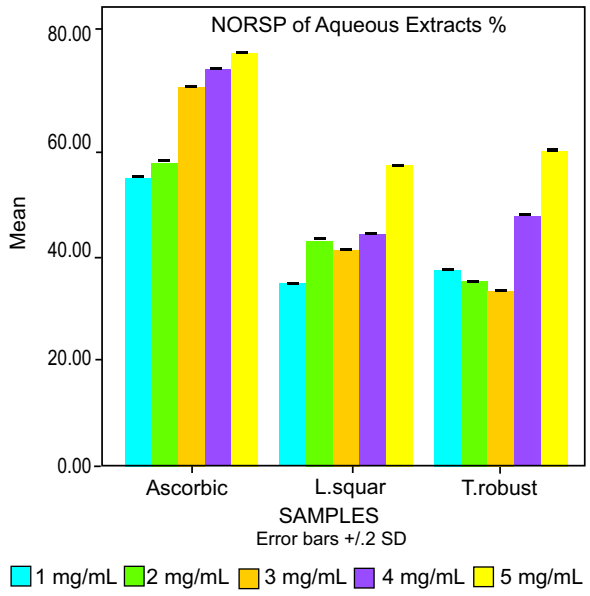

Fig. 7a. NO radical scavenging property in (Aq).

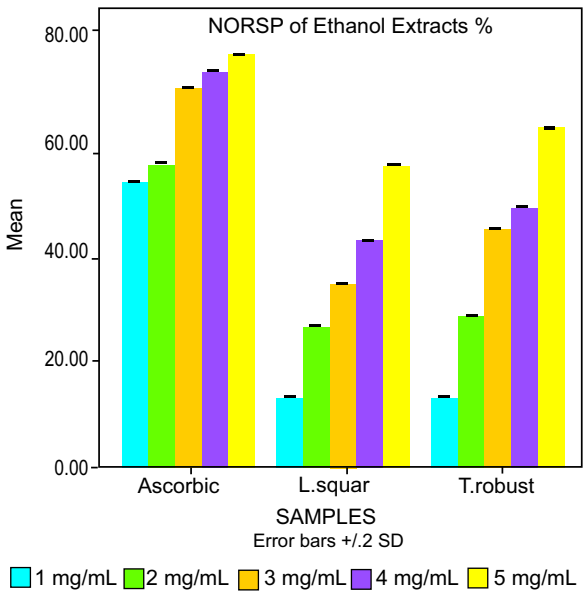

Fig. 7c. NO radical scavenging property in (ETOH).

Only *B. subtilis* showed some susceptibility to *T. robustus*. A possible explanation for this is that gram negative organisms are resistant to extracts of *T. robustus* and may be due to the composition of their cell wall. The antifungal activities of the mushrooms extracts against the selected pathogens are presented in Fig. 10b. These present the percentage mycelia growth inhibition of the samples extracts against filamentous fungi, *A. fumigatus*, *F. solamii* and *C. albican*. The results revealed that methanol extracts of both mushrooms were inactive against *A. fumigatus*, but the organism showed good susceptibility to aqueous and ethanol extracts. Aqueous, methanol and ethanol extracts of the two mushrooms showed strong inhibition against *F. solarmi* when compared with bonlate, which was used as positive control.

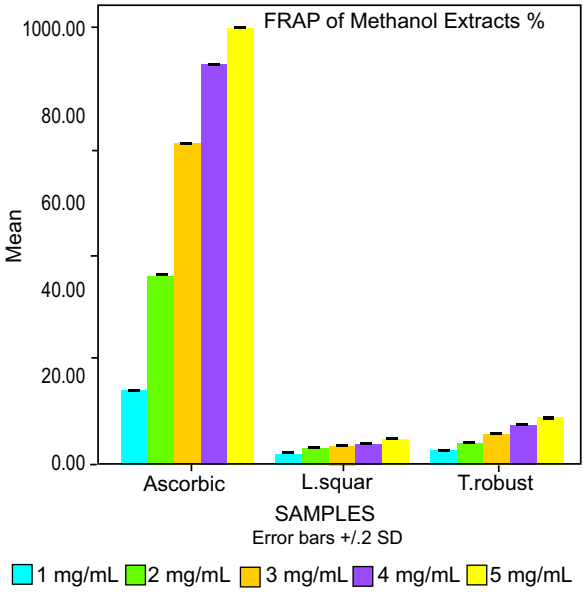

Fig. 8a. Ferric reducing property in (Aq).

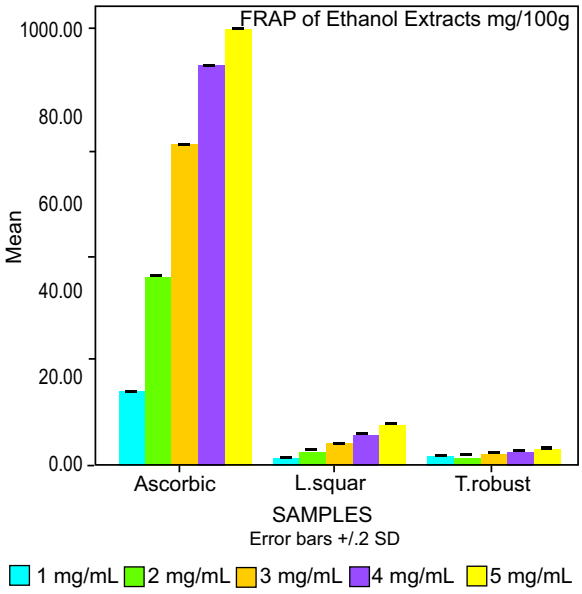

Fig. 8c. Ferric reducing property in (ETOH).

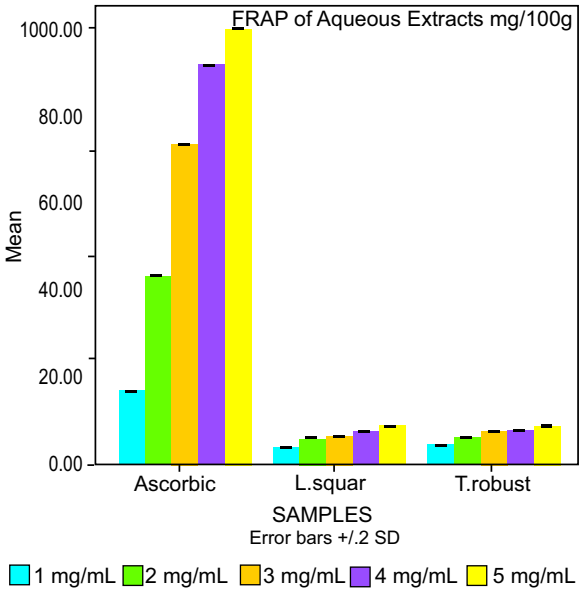

Fig. 8b. Ferric reducing property in (MEOH).

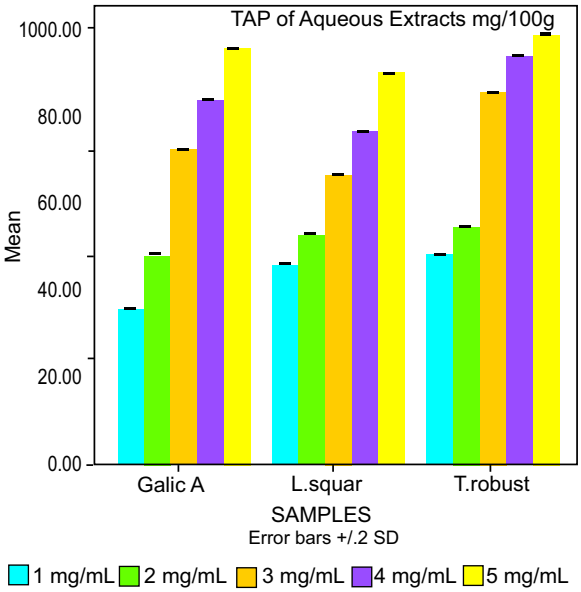

Fig. 9a. Total antioxidant property in (Aq).

While the aqueous extracts of the two mushrooms showed very weak activities against *C. albican*, the alcohol extracts demonstrated strong potency against the fungi. The high susceptibility displayed by the fungi to the extracts of the samples suggests that they contain bioactive agents that may be developed as antifungal drugs to treat infections caused by these organisms.

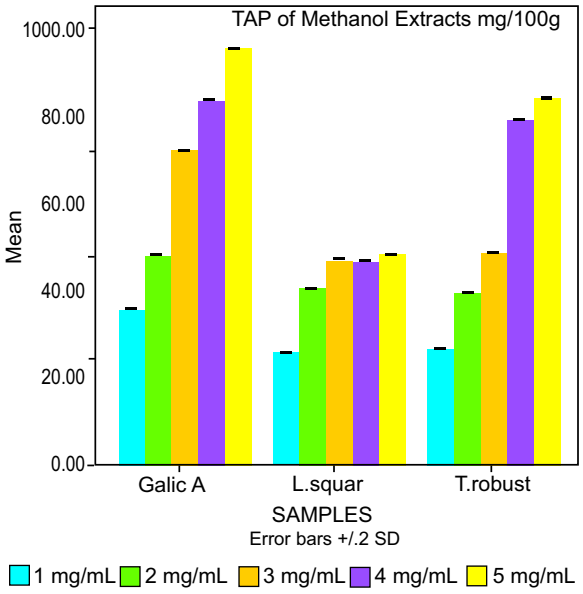

Fig. 9b. Total antioxidant property in (MEOH).

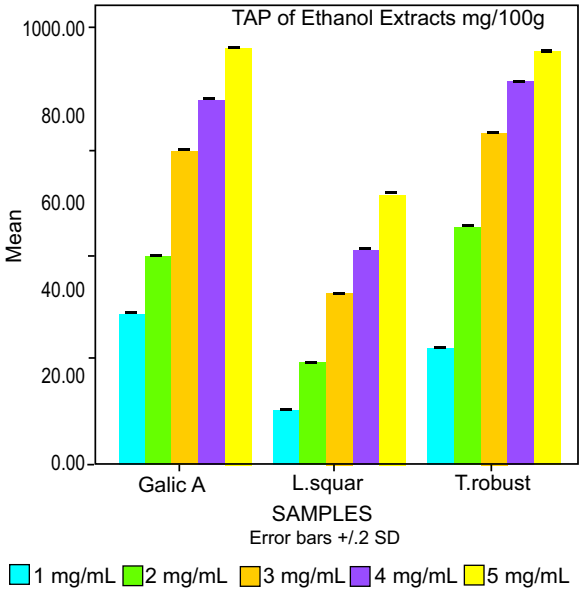

Fig. 9c. Total antioxidant property in (ETOH).

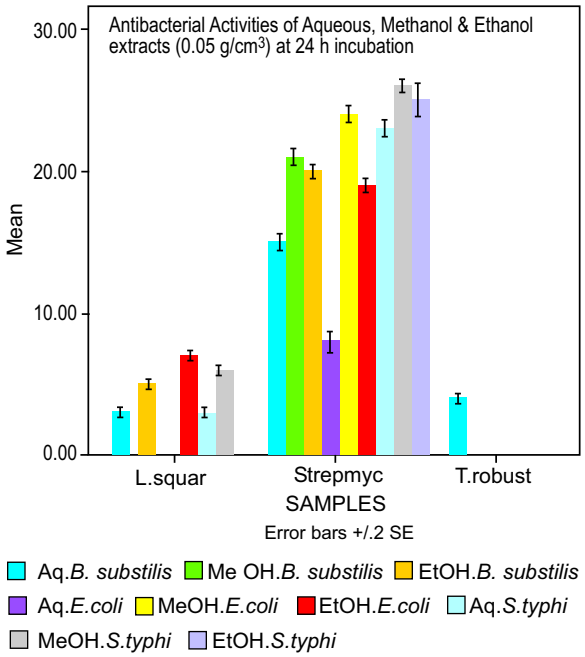

Fig. 10a. Total antioxidant property in (ETOH).

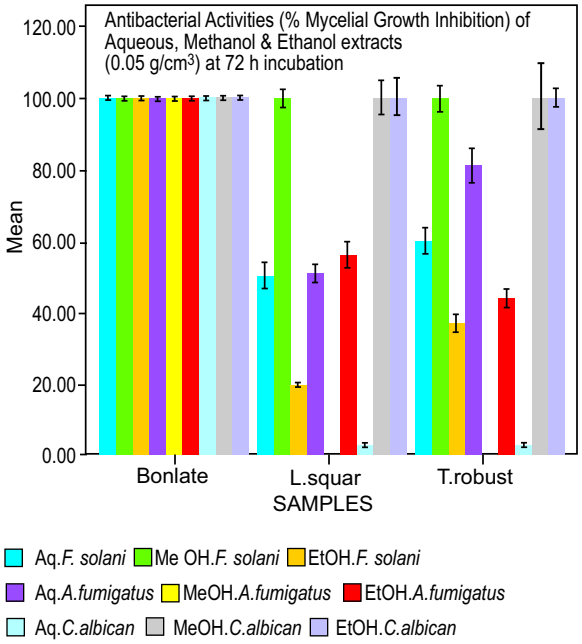

Fig. 10b. Antioxidant property of Mycelial growth.

Conclussion

T. robustus and *L. squarrosulus* possessed good antioxidant and antimicrobial properties in this study and a general strong and positive association was observed

between antioxidant activities and total phenolic content as well as total flavonoids content. The inhibitory actions of extracts could be attributed to the presence of phytochemicals and phenolic compounds. Higher efficiency in recovery of polyphenols using suitable solvents will enhance the inhibitory actions. Thus the mushrooms can be potential natural sources of antioxidant and antibiotic agents. The results were positive at the laboratory level and further work can be carried out to find out the antioxidant strength of various phytochemicals using modern analytical methods and more antioxidant models and their effects in biological systems could be achieved.

References

Abdullah, N., Lau, C.C., Ismail, S.M. 2015. Potential use of *Lentinus squarrosulus* mushroom as fermenting agent and source of natural antioxidant additive in livestock feed. *Journal of the Science of Food and Agriculture*, **96:** 1459-1466.

Alvarez-Parrilla, E., de-laRosa, L.A., Martínez, N.R., González, A.G.A. 2007. Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico. *Ciencia Y. Tecnología Alimentaria*, **5:** 329-334.

Ames, B.M., Shigenaga, M.K., Hagen, T.M. 1993. Oxidants, antioxidants and the degenerative diseases of aging. In: *Proceedings of the National Academy of Sciences*, pp. 7915-7922.

Arbaayah, H.H., Umi, K.Y. 2013. Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere*, **4:** 661-673.

Aremu, M.O., Basu, S.K., Gyar, S.D., Goyal, A., Bhowmik, P.K., Datta, B. 2009. Proximate composition and functional properties of mushroom flours from *Ganoderma* spp., *Omphalotus olearius* (DC.) Sing. and *Hebeloma mesophaeum* (Pers.) Quél. used in Nasarawa State, Nigeria. *Malaysian Journal of Nutrition*, **15:** 233-241.

Barros, L., Falcão, S., Baptista, P., Freire, C.,Vilas-Boas, M., Ferreira, I.C.F.R. 2008. Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. *Food Chemistry*, **111:** 61-66.

Barros, L., Ferreira, M.J., Queirõs, B., Ferreira, I.C.F.R., Baptista, P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemisty*, **103:** 413-419.

Colombo, M.L., Bosisio, E. 1996. Pharmacological activities of *Chelidonium majus* (Papaveraceae). *Pharmacological Research*, **33:** 127-134.

Cook, N.C., Samman, S. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutritional Biochemistry*, **7:** 66-76.

Dharmendra, S., Manish, M., Monika, G., Poonam, S., Abhishek, G., Rajeev, N. 2012. Nitric oxide radical scavenging assay of bioactive compounds present in methanol extract of *Centella asiatica*. *International Journal of Pharmacy and Pharmaceutical Science Research*, **2:** 42-44.

Duh, P.D., Tu, Y.Y., Yen, G.C. 1999. Antioxidant activity of aqueous extract of Hamjyur (*Chrysanthemum morifolium* Ramat). *Lebensmittel-Wissenchaft & Technologie*, **32:** 269-277.

Faweya, E.B., Ayeni, M.J., Kayode, J. 2015. Accumulation of natural radionuclides by some edible wild mushrooms in Ekiti State, Southwestern, Nigeria. *World Journal of Nuclear Science and Technology*, **5:** 107-110.

Gulati, K., Ray, A., Masood, A., Vijayan, V.K. 2006. Involvement of nitric oxide (NO) in the regulation of stress susceptibility and adaptation in rats. *Indian Journal of Experimental Biology*, **44:** 809-815.

Guno, S.C. 2009. Free radical scavenging activity of *Aesculus indica* leaves. *International Journal of PharmTech Research*, **1:** 524-526.

Halliwell, B. 1996. Antioxidants in human health and disease. *Annual Review of Nnutrition*, **16:** 33-50.

Hamowia, A.M., Salfat, A.M. 1994. Pharmacological studies on *Vernonia amygdalina* (Del) and *Tithonia diversifolia* (Gray). *Journal of Veterinary Medicine*, **42:** 91-97.

Hearst, M., David, N., Graham, M., Linda, M., Ballard, B., Cherie, M., Sara, M., Stephen, M., John, E.M., Juluri, R.R. 2010. Antimicrobial properties of protein extracts from wild mushroom fungi and native plant species against hospital pathogens. *Journal of Pharmacognosy and Phytotherapy*, **2:** 103-107.

Heo, K.S., Lim, K.T. 2004. Antioxidative effects of glycoprotein isolated from *Solanum nigrum* L. *Journal of Medicinal Food*, **7:** 349-357.

IAEA, 1994. Intervention criteria in a nuclear or radiation emergency. International Atomic Energy Agency,

Vienna, *Safety Series* No. 109.

Iwalokun, B.A., Usen, U.A., Otunba, A.A., Olukoya, D.K. 2007. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology*, **6:** 1732-1739.

Iweala, E.J., Okeke, C.U. 2005. Comparative study of the hypoglycemic and biochemical effects of *Catharanthus roseus* (Linn) *G. apocynaceae* (*Madagascar periwinkle*) and chlorpropamide (Diabenese) on alloxan-induced diabetic rats. *Biokemistri*, **17:** 149-156.

Jagetia, G.C., Rao, S.K., Baliga, M.S., Babu, K.S. 2004. The evaluation of nitric oxide scavenging activity of certain herbal formulations in vitro: a preliminary study. *Phytotherapy Research*, **18:** 561-565.

Jain, P.K., Himanshu, J. 2012. Coumarin: chemical and pharmacological profile. *Journal of Applied Pharmaceutical Science*, **2:** 236-240.

Jayaprakasha, G.K., Mandadi, K.K., Shibu, M., Poulose, Y., Jadegoud, G.A., Nagana, G., Bhimanagouda, S. 2007. Inhibition of colon cancer cell growth and antioxidant activity of bioactive compounds from *Poncirus trifoliata* (L.) Raf. *Bioorganic and Medicinal Chemistry*, **15:** 4923-4932.

Jonathan, S.G., Amos-Tautua B.M.W., Olawuyi O.J. 2011. Food values, heavy metal accumulation, aflatoxin contamination and detection of exo-polysaccharrides in *Lentinus Squarrosulus* Berk, a Nigerian mushroom. *African Journal of Agricultural Research*, **6:** 3007-3012.

Keleş, A., Koca, I., Gençcelep, H. 2011. Antioxidant properties of wild edible mushrooms. *Journal of Food Process Technology*, **2:** 130-136.

Koffi, E., Sea, T., Dodehe, Y., Soro, S. 2010. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *Journal of Animal and Plant Sciences*, **5:** 550-558.

Kosani, M.C, Rankovi, C.B., DaSi, C.M. 2013. Antioxidant and antimicrobial properties of mushrooms. *Bulgarian Journal of Agricultural Science*, **19:** 1040-1046.

McCutcheon, A.R., Ellis, S.M., Hancock, R.E.W., Tower, G.H.N. 1994. Antifungal screening of medicinal plants of British Columbian native people. *Journal of Ethnopharmacology*, **44:** 157-169.

Nedelkoska, D.N., Nataija, A.P., Haris, A., Dafina, V., Emiija, I., Mitko, K., DzokoKungulovsk, I. 2013. Screening of antibacterial and antifungal activities of selected macedonian wild mushrooms. *Journal of Natural Science*, **124:** 333-340.

Obodai, M., Ferreira, I.C.F.R., Fernandes, Â., Barros, L., Mensah, D.L.N., Dzomeku, M., Urben, A.F., Prempeh, J., Takli, R.K. 2014. Evaluation of the chemical and antioxidant properties of wild and cultivated mushrooms of Ghana. *Molecules*, **19:** 19532-19548.

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: pecific application to the determination of vitamin E. *Analytical Biochemistry*, **269:** 337-341.

Prior, R.L., Cao, G. 1999. Variability in dietary antioxidant related natural product supplements: the need for methods of standardization. *Journal of American Nutraceutical Association*, **2:** 46-56.

Priya, L.J., Srinivasan, V. 2013. Studies on the antibacterial activities of mushroom. *International Journal of Current Microbiology and Applied Sciences*, **2:** 184-189.

Pulido, R., Bravo, L., Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, **48:** 3396-3402.

Rajan, S., Mahalakshmi, S., Deepa, V.M., Sathya, K., Shajitha, S., Thirunalasundari, T. 2011. Antioxidant potentials of *Punica granatum* fruit rind extracts. *International Journal of Pharmacy and Pharmaceutical Science*, **3:** 82-88.

Rao, J.R., Smyth, T.J., Millar, B.C., Moore, J.E. 2009. Antimicrobial properties of shiitake mushrooms (*Lentinula edodes*). *International Journal of Antimicrobial Agents*, 3**3:** 591-592.

Rice-Evans, C.A., Miller, N.J., Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science*, **2:** 152-159.

Silva, A.R.H., Moreira, L.R., Brum, E.S., Freitas, M.L., Boligon, A.A., Margareth, L.A., Roman, S.S., Mazzanti, C.M., Brandão, R. 2014. Biochemical and hematological effects of acute and sub-acute administration to ethyl acetate fraction from the stem bark *Scutia buxifolia* Reissek in mice. *Journal of Ethnopharmacology*, **153:** 908-916.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation

substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **299:** 152-178.

Srinivasan, D., Nathan, S., Suresh, T., Perumalsamy, P.L. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology*, **74:** 217-220.

Umamaheswari, M., Chatterjee, T. K. 2000. In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicine*, **5:** 61-73.

Ursini, F., Maiorino, M., Morazzoni, P., Roveri, A., Pifferi, G. 1994. A novel antioxidant flavonoid (idb 1031) affecting molecular mechanisms of cellular activation. *Free Radical Biology and Medicine*, **16:** 547-553.

Yamac, M., Bilgili, F. 2006. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. *Pharmaceutical Biology*, **44:** 660-667.

Yun-Zhong, F., Sheng, Y., Guoyao, W. 2002. Free radicals, antioxidants, and nutrition. *Nutrition*, **18:** 872-879.

Zhishen, J., Mengcheng, T., Wu, J. 1999. The Determination of flavonoid contents in mulberry and their scanvenging effects on superoxide radicals. *Food Chemistry*, **64:** 555-559.

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Comparative Study of *Tribolium castaneum* H. (Coleoptera: Tenebrionidae) Occurred on Different Wheat Varieties in Pakistan

Muhammad Waqar Hassan*, Javaid Iqbal and Moazzam Jamil
University College of Agriculture and Environmental Sciences,
The Islamia University of Bahawalpur, Pakistan

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Abstract: Five wheat varieties *i.e.,* AASS, CHAKWAL, FARID, MIRAJ and GALAXY in Pakistan were campared to survey the red flour beetle population occurrence during one month period. Only sound grains were taken as medium of population growth for the red flour beetles. Study parameters included number of grains per unit weight, beetles population growth/survival percentage in relation to different wheat varieties and percent weight loss, if any in grains of these wheat varieties due to beetles feeding. Wheat varieties had significantly different number of grains per unit weight. Red flour beetles' population growth was checked in a 30 day time period. Beetles could not grow within the food medium of sound wheat grains of these varieties in this time span and most of these died. Survival percentage of beetles was measured in relation to different wheat varieties which did not vary significantly for these varieties. Within a given span of time percent weight loss due to beetle feeding in grains was nominal in all varieties and it also did not vary significantly between varieties. From these results it is concluded that red flour beetles being secondary stored grain insect pests were unable to multiply on sound grain wheat varieties and caused minimal weight loss in these varieties.

Keywords: wheat varieties, number of grains, weight loss, storage insects, red flour beetle, population growth

Introduction

Annual post-harvest losses of stored cereals range from 10-20% of the overall production, in which insect damage, microbial deterioration and other factors play the major role (Phillips and Throne, 2010). A primary factor in these losses is due to ravages of stored product insect pests that can reduce the quality and quantity of grains (Weaver and Subramanyam, 2000). Stored product insects have been associated with human activities since the earliest civilizations, and methods for their diagnosis and control have been reported for over a century (Levinson and Levinson, 1985). There are many safe, effective, and relatively simple prevention and control methods available to manage populations of stored-product insect pests without the use of chemical insecticides (Phillips and Throne, 2010). In integrated pest management system, alongside other management practices, host plant resistance against different insect pests is very important part to effectively reduce pest populations without the indiscriminate use of toxic insecticides. It has been reported that varietal resistance is one of the basic components that should be seriously taken into account when a stored-product integrated pest management-based strategy is planned (Throne *et al*., 2000). It has been stated that substrate type, germplasm and cracked kernels in the stored materials should be give due care for the management of serious stored grain insect pests (Athanassiou *et al*., 2010).

Wheat is staple food for the people in many countries across the globe. In Pakistan wheat is harvested in May and is stored for the entire season during which it is likely to be infested by different stored grain insect pests. Presence of broken grains in stored wheat is either an indication of an earlier infestation by primary pests (Arbogast *et al*., 2000) or otherwise it may be due to physical or mechanical injuries met with during harvesting, transport or storage process. As soon as a cereal contains cracked grains it may become vulnerable to attack by secondary stored product pest insects which primarily feed on broken grains.

Red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a cosmopolitan pest of wheat especially wheat by-products. Its presence in wheat flour makes it responsible for change in colour and odour and is also pollute the host with its excreta and cast skins (Bosly and Kawanna, 2014). Consumption of the contaminated edibles can cause serious health issues (Gorham, 1979; 1975). Red flour beetles are primarily secondary stored product pests which can rarely feed or damage sound grains of cereals (Walter, 1990). However other authors indicated that red flour

*Author for correspondence; E-mail: waqar_722@hotmail.com

beetles over a certain period of time caused noticeable damage and weight loss to sound wheat grains in different wheat varieties and its population growth occurred in sound grains of wheat over a given time span (Ali *et al*., 2012; 2009).

This study was therefore started to check red flour beetles population increase or decrease in a 30 day time period and any weight loss in five different wheat varieties namely *i.e.,* AASS, CHAKWAL, FARID, GALAXY and MIRAJ containing sound grains. Results would reveal the ability of red flour beetles to make damage and weight loss to different wheat varieties having sound grains during this one month period keeping in view the sound nature of grains in this study. Unlike some primary storage pest insects whose feeding makes substantial amount of damage in sound grains, the red flour beetle may cause minimal damage during this short period of one month. Therefore, even a small weight loss after one month period may indicate feeding on sound grains and damage to them by red flour beetles. Results would reveal relative feeding and damage as weight loss by red flour beetles in five wheat varieties over one month period. Any significant difference in weight loss among these varieties shall reveal some resistant or susceptible germplasm (s) or varieties relative to each other against this pest that may pinpoint their importance for their inclusion in integrated control programme for this pest in wheat showing resistance at this stage

Materials and Methods

Insect source. Red flour beetles were taken from laboratory culture maintained at 30±2 °C and 65± 5% R.H., in Department of Entomology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Adults of *T. castaneum* were taken and allowed to lay eggs in separate plastic jars containing sterilized whole wheat flour for a period of two weeks. After that adults were sifted out and flour containing eggs of *T. castaneum* was left for egg development inside the laboratory. These jars were kept for about one month for adult emergence. After one month from the date of sifting parent adults, emerging adults were of homogenous age and about one week or so older because according to Rees (2007) red flour beetles take about 25 days from egg to adult at 32.5 °C and > 70 relative humidity.

Experimental-setup: Five wheat varieties namely *i.e.,* AASS, CHAKWAL, FARID, GALAXY and MIRAJ were obtained from Regional Agriculture Research Institute, (RARI) Bahawalpur, Pakistan. Experiments were performed to execute the current research aim. In first experiment 5 g weight measured with sensitive electrical weighing balance was taken for all varieties. Number of grains per 5 g weight was counted for every variety. This process was replicated four times to get mean values. In second experiment 10 adult red flour beetles (We could use 10 same age adult beetles sample size due to scarcity of culture) in the same day-age were released into 5 g weight of each variety (only sound grains per 5 g were used in experiments). This experimental setup (10 beetles released within wheat grains) were then poured in to a 400 mL plastic jar for a period of 30 days to see any weight loss in grains and the increases or decrease in the final population of these adults during this time. There were also four replications for this experiment to get mean values. Finally weight of wheat varieties was measured with sensitive electrical weighing balance to see any change in final weight due to insect feeding that was calculated in the form of percent weight loss.

Data recording: After a period of one month jars were examined to count the number of beetles in each replication by visual counting and separation of adults from wheat grains was done in glass petri dishes by using camel hair brush. Percent survival was calculated by using formula:

$$\text{Survival (\%)} = \frac{\text{Number of live beetls}}{\text{Number of beetles released}} \times 100$$

After separation of beetles, percent weight loss in final weight of grains was calculated by using formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Data from number of grains, survival percentage and percent weight loss subjected to analysis of variance using Statistix software (version 8.1.). Means were compared by least significance difference test at P =0.05.

Results and Discussion

Results showed that mean number of grains per 5 g weight was significantly different (Table 1; $P < 0.05$) in five wheat varieties. Maximum mean number of

grains was found in FARID (197.25 ± 4.57) followed by CHAKWAL (179.00 ± 4.23) being statistically similar. Minimum grains were in GALAXY (140.75 ± 9.30) followed by MIRAJ (162.50 ± 1.18) and AASS (166.00 ± 10.00) in ascending order. Variety GALAXY had significantly less number of grains than that in FARID, CHAKWAL and AASS (Table 2, $P < 0.05$; Fig. 1). Red flour beetles were unable to grow on whole wheat grains in any of five wheat varieties. Even their population was decreased from 10 adults released initially for 30 day period. Therefore, percent survival of beetles was calculated from 10 adults used for the experiment. Results showed that beetles' survival was non-significant in five varieties (Table 1; $P > 0.05$). Maximum survival was in CHAKWAL (32.50 ± 9.45%) followed by GALAXY (15.00 ± 4.08%), AASS (7.50 ± 1.58%), FARID (7.50 ± 3.02%) and MIRAJ (0.00 ± 0.00%) in decreasing order (Table 2, Fig. 1). Finally we tested percent weight loss in grains of five varieties which showed that percent loss in grain weight was nominal and non-significant among varieties (Table 1; $P > 0.05$). Maximum weight loss occurred in AASS (2.42 ± 1.92%) followed by GALAXY (2.42 ± 0.31%), FARID (2.17 ±0.77%), CHAKWAL (0.92 ± 0.53%) and MIRAJ (0.33 ± 0.21%) in decreasing order (Table 2, Fig. 1).

Significant difference in number of grains per unit weight (5 g in this study) can be due to difference in size of grains in different wheat varieties. It can be common that different wheat varieties have different size of grains. Thus a significant difference in number of grains per 5 g weight of five wheat varieties was obtained. These results are similar to other studies in which they found number of grains per unit weight varied significantly and a clear proportion of damaged and healthy grains per unit weight for different wheat varieties was established (Ali *et al.,* 2009).

It is important to note that when the beetles, only sound grains were used as test medium and any damaged or broken grains were removed to check beetle's survival and weight loss in healthy grains of wheat varieties. In a 30 day period, beetles were unable to grow and reproduce on any of the five varieties of wheat. Results showed mortality of insects occurred in all varieties and survival out of initial ten insects in different varieties remained non-significant as counting of survived adults out of initial ten adults showed that their survival was non-significant in different wheat varieties. Maximum percent survival was 32.5% and minimum was 0.00%. Red flour beetles are secondary pests of stored food

Table 1. Analysis of variance for number of grains, survival and weight loss by beetles for different wheat varieties

		Number of grains		Survival (%)		Weight loss (%)	
Source	DF	F	P	F	P	F	P
Variety	4	7.68	0.0014*	2.61	0.0779n.s	0.79	0.5502n.s
Error	15						
Total	19						
Grand mean	CV	169.10	8.69	12.500	122.64	1.6480	131.96

* = significance; n.s = non-significant; DF, F, and P = Survival % of no. of live beetles

Table 2. LSD all-pair wise comparisons tests of number of grains, survival (%) and percent weight loss by red flour beetles in different wheat varieties

Variety	Number of Grains	Survival (%)	Weight loss (%)
FARID	197.25 ± 4.57a	7.50 ± 3.03n.s	2.42 ± 0.32n.s
CHAKWAL	179.00 ± 4.23ab	32.50 ± 9.45	0.92 ± 0.54
AASS	166.00 ± 10.00 b	7.50 ± 1.58	2.42±1.92
MIRAJ	162.50 ± 1.18bc	0.00 ± 0.00	0.33 ± 0.21
GALAXY	140.75 ± 9.30c	15.00 ± 4.08	2.42 ± 0.32

Alpha 0.05; n.s= non-significant; DF, F, and P = Survival % of no. of live beetles

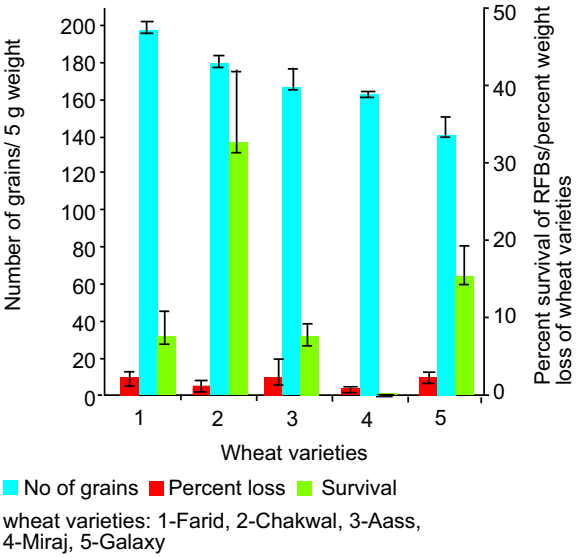

Fig. 1. Number of grains, percent weight loss and percent survival of *T. castaneum* on different wheat varieties

materials and are usually found infesting grain by-products such as wheat flour and other farinaceous compounds. Their minute damage in sound grains may be detected and measured by using X-rays and other sensitive techniques (Karunakaran *et al.* 2004). It is not certain that their feeding on sound grains can leave prominent marks on those grains. Further finding damaged grains due to feeding by red flour beetles and other secondary stored grain insects having such weaker mouth parts may require special techniques as described by Karunakaren *et al*. (2004). Therefore alternatively percent weight loss in unit weight of wheat varieties over a given period of time can be an indication of their attack on sound grains. According to our results percent weight loss remained slight and non-significant on different wheat varieties and it ranged from 0.42 to 0.32 %. These results show that red flour beetles being secondary stored grain insect pests caused minimal or negligible weight loss in sound wheat grains in five wheat varieties in a one month period and their pest status needs further evaluations for sound wheat grains keeping in view one month test period. Our results cannot be compared with Ali *et al*. (2009) firstly because they used different set of varieties and secondly the test period in their study was about five months in contrast with our studies which lasted for one month. A study was conducted by Renteria-Gutierez *et al.* (2000) to test the population growth of the lesser grain borer, *Rhyzopertha dominica* (F.) and the red flour beetle, *T. castaneum* (H.) under laboratory conditions. Both species were reared using whole grain and flour from four wheat varieties and four wheat groups. Although both species were able to complete development on both diet types *e.g.,* whole grains and flour from four wheat varieties but *T. castaneum* adult populations was reduced on whole wheat grains compared with flour from four wheat varieties. Thus these results are in agreement with our current findings to some extent that *T. castaneum* population cannot sustain much on whole grains compared with flour. Further investigations would be beneficial in this research area for this cosmopolitan pest of wheat.

Conclusion

Current results show that red flour beetles could not induce major weight loss in thirty day period in wheat varieties containing little or no broken grains. Research should also be extended about investigation on red flour beetles population growth and damage in wheat containing different degree or percentage of cracked kernels to evaluate the effect of percentage of cracked kernels on damage or weight loss caused by these insects and on the population growth or survival percentage of red flour beetles and other secondary stored grain insect pests. Further it is also desirable to include more varieties under such investigation and testing period may be more then one month to see any weight loss to investigate resistance phenomenon in wheat against red flour beetles. It can help in eco-friendly management of such secondary stored grain insect pests and avoidance of residues due to pesticide usage in these commodities.

References

Ali, A., Sarwar, M., Khanzada, S., Abro, G.H. 2011. Evaluating resistance of wheat germplasms to attack by red flour beetle, *Tribolium castaneum*. H (Coleoptera). *Pakistan Journal of Zoology*, **43:** 793-797.

Ali, A., Sarwar, M., Khanzada, S., Abro, G.H. 2009. Reaction of certain wheat varieties to the action of red flour beetle, *Tribolium castaneum*. H (Coleoptera) under insectary conditions. *Pakistan Journal of Zoology*, **41:** 51-56.

Arbogast, R.T., Kendra, P.E., Mankin, R.W., McGovern, J.E. 2000. Monitoring insect pests in retail stores by trapping and spatial analysis. *Journal of Economic Entomology*, **93:** 1531-1542.

Athanassiou, C. G., Opit, G. P., Throne, J. E. 2010. Influence of commodity type, percentage of cracked kernels, and wheat class on population growth of stored-product psocids (Psocoptera: Liposcelidae). *Journal of Economic Entomology*, **103:** 985-990.

Bosly, H.A.Q., Kawanna, M.A. 2014. Fungi species and red flour beetle in stored wheat flour under Jazan region conditions. *Toxicology and Industrial Health*, **30:** 304-310.

Gorham, J.R. 1979. The significance for human health of insects in foods. *Annual Review of Entomology*, **24:** 209-224.

Gorham, J.R. 1975. Filth in foods: Implications for health. *Journal of Milk and Food Technology*, **38:** 409-418.

Karunakaran, C., Jayas, D. S., White, N.D.G. 2004. Identification of wheat kernels damaged by red flour beetle using X-ray images. *Biosystems Engineering*, **87:** 267-274.

Levinson, H. Z., Levinson, A. R., 1985. Storage and insect species of stored grain and tombs in ancient

Egypt. *Zeitschrift für angewandte Entomologie*, **100:** 321-339.

Phillips, T.W., Throne, J.E. 2010. Biorational approaches to managing stored product insects. *Annual Review of Entomology*, **55:** 375-397.

Rees, D. 2007. *Insects of Stored Grain*. CSIRO Publishing, Canberra, Austration.

Renteria-Gutierez, T. R., Moreno-Martinez, E. J., Barron-Hoyos, M. 2000. Population growth of *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in different wheat varieties and groups commercially produced in Sonora Mexico. *Southwest Entomology*, **25:** 213-220.

Throne, J. E., Baker, J. E. Messina, F. J., Kramer, K. J. Howard, J. A. 2000. Varietal Resistance, In: Alternatives to pesticides in stored-product IPM. Bh. Subramanyam and D. W. Hagstrum (eds.), pp. 166-192, Kluwer Academic Publishers, Boston, MA, USA.

Walter, V.E. 1990. Stored Product Pests. In: *Handbook of Pest Control* (K. and Story Moreland D. (eds.). OH, pp. 526-529. Franzak & Foster Co., Cleveland, USA.

Weaver, D.K., Subramanyam, B. 2000. Botanicals. In: *Alternatives to Pesticides in Stored Product*, IPM, B.H. Subramanyam and D.W. Hagstrum (eds.). Kluwer Academic Publishers, Dordrecht, pp. 303-320.

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Impact of Pesticide Quality Control Programme in Southern Punjab, Pakistan

Muhammad Naeem Akhtar[a], Ashfaq Ahmed Rahi[a], Khuram Mubeen[b]*, Shahid Munir[a], Naeem Sarwar[c], Hafiz Muhammad Rafique[a] and Muhammad Waseem Akhtar[d]

[a]Pesticide Quality Control Laboratory, Multan, Punjab, Pakistan

[b]Department of Agronomy, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan

[c]Department of Agronomy, Faculty of Agriculture and Environmental Sciences, Bahauddin Zakaria University, Multan, Punjab, Pakistan

[d]Department of Seed Technology, University of Agriculture, Faisalabad, Punjab, Pakistan

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Abstract. This study was taken to evaluate pesticide quality and their impact on crop. Total 14336 number of pesticide samples were received for quality evaluation from 2006 to 2012. The data was analysed statistically at 5% level of significance and treatment means were worked out. The results revealed that out of 14336 analysed samples, 13541 (94.5%) were declared fit for crop use whereas the remaining 795 (5.5%) were found to be unfit. The trend in generic and branded unfit samples between the years 2006 to 2012 revealed that the agencies marketing branded pesticides had better quality than generic ones.

Keywords: Pakistan, pesticides, quality control

Introduction

There are serious ecological and environmental problems with over reliance on pesticides. Persistence of pesticides in the food chain (Carson *et al*., 1962) and the development of resistance in pests towards pesticides (Brown 1971) are the two serious problems encountered. Pesticide usage has become obligatory for crop protection. However, pest suppression with synthetic chemicals is the quickest and most effective method of pest management. Control through pesticides takes least time in situations of massive pest outbreak when compared with biological and cultural control practices.

The pesticide usage differs with crops in Pakistan, with reduced use of fungicides and herbicides. Pesticide is mainly used in Pakistan for cotton crops (60%) and 40% for all other crops. This has increased steadily and substantially over the years in Pakistan (Table 1 and Fig. 1).

Plant Protection Directorate and Pesticide Division was set up for sample testing and understanding research on quality control of pesticides during 1970 at Faisalabad, Pakistan and quality control programme of pesticides was initiated in Punjab, Pakistan. The private sector started this industry by promoting the sale of pesticide during 1980's. Hence, to strengthen the quality control of pesticides two more Pesticide Laboratories were established at Kala Shah Kaku and Multan during 1984-85.

World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO, 2005) reported that in developing countries about 30% marketing of pesticides amounting to worth US $ 900 million per year do not meet international quality standards. Pesticides with low quality have also been reported to WHO and FAO by national quality control laboratories of pesticides. Developed countries have also shown to have low quality pesticides magnifying the seriousness of the issue. Pesticides with low quality could be owing to many reasons like low standard production technology and poor production admixing of products and poor pre marketing store conditions. Inadequate implementation of rules by law enforcing authorities may also open windows for such mal-practices.

In developing countries like Pakistan, the authorized inspectors from Directorate of Pest Warning and Quality Control and Agriculture (extension and adaptive research) Department collect pesticides samples from market. Inspectors have the authority from the Government under APO Law, 1971 to collect the sample on intelligence basis or complaint basis by the farmer

*Author for correspondence;
E-mail: khurram.mubeen@mnsuam.edu.pk

(DGA, 1997). The collected samples are then sent to Pesticide Quality Control Laboratories for testing. The monitoring programme keeps on assessing the status of sub-standard pesticide samples issued by Pesticide Quality Control laboratory. Afterwards the culprits are dealt as per the law.

Farmers and law enforcing agencies over the past few years, have given serious consideration to this programme. But such information which may provide the guidelines to the stakeholders and general performance or status of pesticide quality control programme over the years is scarce. To address this need data obtained over years has been analysed, presented and discussed here.

Materials and Methods

To check the quality of pesticides in Multan, Dera Ghazi Khan and Bahawalpur Divisions, the Pesticide Quality Control Laboratory (PQCL) Multan, Pakistan was established in 1985. Authorized inspectors collect pesticide samples with different formulations and send to the laboratory. The samples are stored after marking codes. Spectrophotometry, high performance liquid chromatography (HPLC), gas chromatography (GC) and chemical digestion and titration described by Collaborative International Pesticides Analytical Council (AOAC, 1990; EPA, 1987; Ashworth *et al*., 1970) were used for physical and chemical analysis.

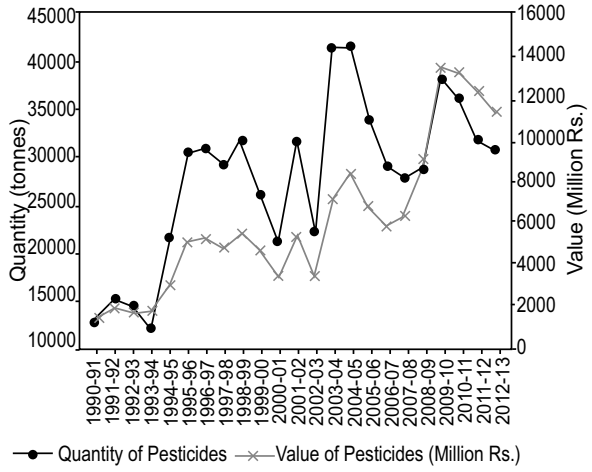

Fig. 1. Import of pesticides (quantity and value) in Pakistan.

Table 1. Crop wise consumption of pesticides over time (million tonnes)

Year	Cotton	Sugarcane	Rice	Maize	Wheat
2006-07	12856	54742	5438	3088	23295
2007-08	11655	63920	5563	3313	21749
2008-09	11819	50045	6952	3593	240333
2009-10	12698	49373	6883	3487	23864
2010-11	11460	55309	4823	3341	24214
2011-12	13595	58038	6160	4271	23517

Active ingredient (a. i.) in the pesticide formulation was calculated as follows:

a. i. (%) = (peak area of sample/peak area of standard) × a.i. content claimed in pesticide formulation

Physical properties like dry sieve test for dustable powders (DP), wet sieve test for wetable powders (WP), granular formulations (GR) and emulsions and oil in water were used for emulsion stability test for emulsifiable concentrates (EC) (Ashworth *et al*., 1970) (Table 2). Emulsion stability test was performed to ensure that a sufficient amount of active ingredients homogeneously dispersed in emulsion to give a satisfactory and effective mixture during spraying.

Pesticide sample fitness on a.i. content was estimated as per tolerance values and appropriate contents as described by FAO (1999). The pesticide inspectors were provided with analytical reports of unfit samples for taking legal action under APO Law, 1971.

The recorded data was analysed statistically by using Fisher's analysis of variance technique and significance of treatment means was compared by least significant difference test at 5% probability level (Steel *et al*., 1997).

Results and Discussion

Standard methods were used to analyse collected pesticide samples by the authorized persons. The samples

Table 2. Emulsion stability limits

Time after dilution	Limits of stability	
0 h (hour)	Initial emulsification	complete
0.5 h	Cream maximum	2%
2.0 h	Cream maximum	4%
	Free oil	nil
24.0 h	Re-emulsification	complete
24.5 h	Cream maximum	nil
Test after 24 h was carried out in case when results at 2 h were in doubt	Free oil	nil

of pesticide received in the PQCL, Multan for analysis improved a lot during 1996-97 to 2004-2005 from 1730 to 3323.

Adoption of integrated pest management (IPM) practices due to Farmer Field Schools reduced number of pesticide samples significantly (Khan *et al*., 2010) and cultivation of *Bacillus thuringiensis* Bt cotton. Furthermore, it resulted in pesticide quality improvement in Dera Ghazi Khan and Multan Division. The number of fit samples on the basis of pesticide analysis has been shown in Fig. 2.

The pesticide import in Pakistan since 1990-1991 to 2012-2013 (Fig. 1) reveals increased import in 2004-2005 by 219% as compared with 1991-92. There is significant reduction in pesticide use afterwards. During each passing year, a reduction in number of unfit samples was recorded i.e. from 247 during 1996-97 to 57 during 2011-12, showing an improvement in quality of pesticide (Fig. 2 and Table 3). However, this improvement is not reflected at farmer level and in the market. A significant and positive change can be achieved by an intelligent market representative sampling by the inspectors through monitoring by Task Force on agriculture and amendment in APO Law, 1971 (DGA, 1997).

Comparison of generic and branded unfit samples during the years 2002-03 to 2012-13 reveals that the number of generic unfit samples was found almost 4 to 5 times more than branded samples. It indicates maintenance of better quality standards of pesticides by the multi-national companies (Fig. 3). Although the pesticide import under generic scheme was with low prices, but at the cost of low quality. Pesticide samples from 21 developing countries were analysed from 1989-1994 where 34% samples were found unfit as per limits of FAO (Kern and Vaagt, 1996).

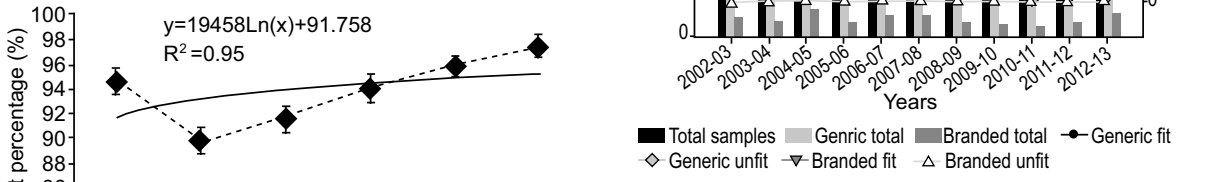

Fig. 2. Quality of pesticide samples analysed in PQCL, Multan, Pakistan.

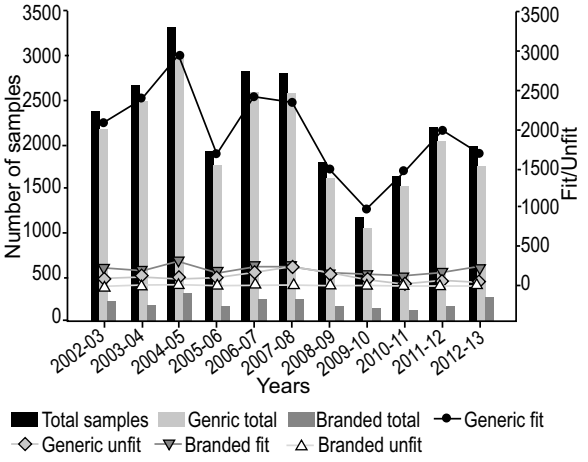

Fig. 3. Trend in generic and branded total, fit and unfit samples (2002-03 to 2012-13).

These results are not in line with those reported by the Pesticide Quality Control Laboratory (PQCL) in Multan (Table 4). It could be owing to biased sampling by the authorized inspectors under the quality control programme.

Table 3. Pesticide samples fitness in southern punjab (2006-07 to 2011-12)

Name of District	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12
Multan	91.04	85.20	88.00	88.85	94.14	94.76
Khanewal	98.29	89.15	87.00	95.40	97.38	97.54
Vehari	89.56	91.71	94.00	93.39	95.28	98.37
Muzzafar Garh	97.08	90.65	95.00	97.96	97.83	97.00
Dera Ghazi Khan	94.00	95.07	94.00	93.41	96.68	98.11
Layyah	97.66	97.94	94.00	100	97.90	99.16
Rajanpur	96.69	93.01	91.00	96.55	95.36	98.20
Total	94.62	89.92	91.55	94.03	95.83	97.38

Table 4. Quality trend (fitness %) of pesticide samples in Southern Punjab over time (2006-07 to 2011-2012)

Year	Total number of samples analysed	Fit samples	Unfit samples	Fit (%)	Unfit (%)
2006-07	1375	1301	74	94.62	5.38
2007-08	1686	1516	170	89.92	10.08
2008-09	1776	1626	150	91.55	8.45
2009-10	1172	1102	70	94.03	5.97
2010-11	1630	1560	68	95.83	4.17
2011-12	2174	2117	57	97.38	2.62

Furthermore, the results of PQCL have been presented at the division level where anti-adulteration campaign is working effectively. The pesticide quality status of the country needs to be accounted to have a clear picture of the pesticide quality control programme at the national level.

Conclusion

Out of sampled pesticides, 94.5% were found fit for use in field crops on arable lands. Whereas the companies marketing branded chemicals assured improved quality of pesticide than generic chemicals. Furthermore, it is suggested that authorized pesticide inspectors should make intelligent pesticide sampling without merely focusing on completing the assigned target so as to avoid the malpractice in pesticide marketing and to further ensure better quality of pesticides being provided to farmers.

References

AOAC, 1990. *Official Methods of Analysis: Agricultural Chemicals. Contaminants, Drugs*, K. Helrich (ed.), pp. 147-231, Association of Official Analytical Chemists, Arlington, Virginia, USA.

Ashworth, R.deB., Henriet, J., Lovett, J.F. 1970. *CIPAC Handbook. Analysis of Technical and Formulated Pesticides*. vol. **1,** pp. 875-893, Collaborative International Pesticides Analytical Council Limited. Plant Pathology Laboratory Hatching Green, Harpenden, Hertfordshire, England.

Brown, A.W.A. 1971. Pest Resistance to Pesticides. In: *Pesticides in the Environment*, R. W. Stevens (ed.), vol. **1,** pp. 457- 552, Dekker, New York, USA.

Carson, R., Darling, L., Darling, L. 1962. *Silent Spring*. Boston: Houghton Mifflin Company, Riverside Press, Boston, USA.

DGA, 1997. *Agricultural Pesticides Ordinance 1971* (amended up to 1997) and Rules. 1973. Directorate General Agriculture (Ext. and AR), Punjab, Lahore, Pakistan.

EPA, 1987. *Manual of Chemical Methods for Pesticides and Devices*. 1st edition, (4th update), Environmental Protection Agency, Publication, Association of Official Analytical Chemists, Arlington, VA, USA.

FAO/WHO, 2005. Amount of poor-quality pesticides sold in developing countries alarmingly high (Press release). Rome, Food and Agriculture Organization of the United Nations, and Geneva, World Health Organization. Retrieved October 25,2016. http://www.fao.org/WAICENT/OIS/PRESS_NE/PRESSENG/2001/pren0105.htm.

FAO, 1999. *Manual on Development and Use of FAO and WHO Specifications for Plant Protection Products*. pp. 17-19, 5th edition, Rome, Italy.

Kern, M., Vaagt, G. 1996. Pesticide quality in developing countries. Pesticide Service Project, Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) GmbH, P.O. Box 5180, 65726 Eschborn , Germany. *Pesticide Outlook Journal*, **7:** 7-10.

Khan, M.J., Zia, M.S., Qasim, M. 2010. Use of pesticides and their role in environmental pollution. *World Academy of Science, Engineering and Technology*. **72:** 122-128.

Steel, R.G.D., Torrie, J.H., Dicky, D.A. 1997. *Principles and Procedures of Statistics. A Biometrical Approach.* pp. 400-428, 3rd edition, Mc Graw Hill International Co., New York, USA.

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Short Communication

Role of Potassium in Reducing Oxidative Damage in Maize under Salt Stress

Muhammad Suhaib[a]*, Asma Mujtaba[b], Muhammad Arshad Ullah[a], Badar-uz-Zaman[a], Imdad Ali Mahmood[a], Hafiz Muhammad Asadullah[c] and Muhammad Jahanzaib[c]

[a]Land Resources Research Institute, NARC, Islamabad, Pakistan

[b]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan

[c]Crop Sciences Institute, NARC, Islamabad, Pakistan

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Abstract. Hydroponic experiment was carried out to assess the effect of K in reducing the oxidative damage in maize (Pioneer-3335) under salt stress. Seedlings were transplanted to three levels of K (3, 6 and 9 mM) with 100 mM NaCl stress. Plant growth parameters, membrane stability index, K^+ / Na^+ ratio and antioxidant enzymes activity were reduced due to salt stress. Addition of K significantly improved the morphological and physiological attributes along with the antioxidant enzymes (SOD, CAT and POD) activity. With increasing K levels an improvement in crop growth was observed but the treatment with 9 mM K was found to give the best results. Maximum shoot and root lengths (61.2 and 30.6 cm) were observed at 9 mM applied K level. The similar trend regarding shoot and root fresh weight was observed for maize genotype. Improved membrane stability was observed at 9 mM K level in (Pioneer-3335) (75.6%) under salt stress. Similarly, improved antioxidant enzymes (SOD, CAT and POD) activity was found in maize plants (35.5, 65.6 and 49.6 unit/g fresh weight) as compared to salt stress at 9 mM K level. The antioxidant enzymes activity was improved with the application of potassium under salt stress which ultimately induced oxidative stress tolerance in maize (Pioneer-3335).

Keywords: maize, salt stress, potassium, oxidative damage, antioxidant enzymes

Saline soil and water are the major constraints in reduced agricultural yield of many crops (Chaum *et al*., 2011; Ashraf, 2009; AzevedoNeto *et al*., 2006; Alam *et al*., 2000). The metabolic activities in most crops ceased due to higher salt concentration in soils that ultimately results in low agricultural productivity (Karsensky and Jonak, 2012; Munns and Tester, 2008; Cramer *et al*., 1996).

Along with osmotic stress, ionic imbalance and specific ion toxicity, the reactive oxygen species (ROS) generation is also coupled with salinity (Ali *et al*., 2011; Nabati *et al*., 2011; Gapinska *et al*., 2008; Mittler, 2002).

Among all other macro nutrients potassium has the significant role in plant survival under salt stress (Mahmood, 2011; Cherel, 2004; Mengel and Kirkby, 2001).

Keeping in mind these factors this study was planned to assess the ameliorative efficiency of potassium under salt mediated oxidative stress.

This study was conducted in the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Three potassium levels (3, 6 and 9 mM) were applied with 100 mM NaCl stress. Maize genotype (Pioneer-3335) was used for this study. Maize seeds were germinated in sand containing trays and were transplanted in thermo pore sheets floating over Hoagland solution containing tubs at two leaf stage (Hoagland and Arnon, 1950). In respective saline treatments salinity was developed after two days of transplantation in 3 increments. 8 h daily aeration was provided through aeration pump and pH was maintained daily from 6-6.5.

After four weeks plants were harvested, recorded the growth attributes and stored in refrigerator for further analysis. The samples were analysed for membrane stability index according to method of Sairam *et al*. (2002). Sodium and potassium ion concentration was analysed using flame photometer.

Antioxidant enzymes activity (SOD, CAT, POD) was recorded using spectrophotometer from enzyme extract (For extracting antioxidant enzymes, 0.5 g fresh leaf samples were ground using a tissue grinder in 5 mL of 50 mM cooled phosphate buffer (pH 7.8) placed in an ice bath. The homogenate is centrifuged at 15000 × g

*Author for correspondence; E-mail: sahirsuhaib@gmail.com

for 20 min at 4 °C. The supernatant is used for determination of antioxidant enzymes) following the method of Giannopolitis and Ries (1977) for SOD by recording the decrease in absorbance of nitro blue tetrazolium 560 nm (1955). Catalase enzyme activity was recorded by calculating the decomposition of H_2O_2 at 240 nm and peroxidase enzyme activity was determined by recording the absorbance at 470 nm after 0 sec for 5 min (Chance and Maehly, 1955). The experiment was laid out using CRD-Factorial arrangement and data was analyzed statistically using statistics 8.1. Plant growth attributes under salt stress have been shown in Table 1.

The data regarding K^+/Na^+ ratio in maize genotype (Pioneer-3335) has been showed in Table 2. These results depicted the fact that saline environment subjects the plants to uptake more Na^+ as compared to K^+ hence decreasing the K^+/Na^+ ratio. This ratio in plants grown in saline soils could be improved by the application of K.

Table 1. Effect of potassium on growth parameters of maize grown under salt stress

Treatments	Pioneer-3335			
	SL (cm)	SFW (g)	RL (cm)	RFW (g)
Control	99.9	45	42.4	6.47
100 mM NaCl	50.1 (50)	21 (47)	22.5 (53)	3.25 (50)
100 mM NaCl +3 mM K	53.3 (53)	23.9 (53)	24.2 (57)	3.67 (57)
100 mM NaCl +6 mM K	58.1 (58)	28 (62)	27 (64)	4.44 (69)
100 mM NaCl +9 mM K	61.2 (61)	31.5 (70)	30.6 (72)	5.12 (79)

Values in () are the percentage of control. (SL, SFW, RL and RFW are the abbreviations of shoot length shoot fresh weight, root length and root fresh weight), respectively.

Table 2. Effect of potassium on K^+/Na^+ ratio and membrane stability index of maize grown under salt stress

Treatments	Pioneer-3335	
	K^+/Na^+ ratio	MSI
Control	2.67	88.6
100 mM NaCl	0.46 (17)	60.2 (68)
100 mM NaCl +3 mM K	0.51 (19)	66.5 (76)
100 mM NaCl +6 mM K	0.56 (21)	71.2 (80)
100 mM NaCl +9 mM K	0.60 (22)	75.6 (85)

Values in () are the percentage of control.

Results regarding antioxidant enzymes (SOD, CAT and POD) activity are presented in Table 3.

Table 3. Effect of potassium on antioxidants enzyme activity (SOD, CAT and POD) of maize grown under salt stress

Treatments	Pioneer-3335		
	SOD	CAT	POD
	(Unit/g FW)		
Control	25.6	64.6	43.8
100 mM NaCl	24.7 (96)	53.4 (82.8)	41.9 (95)
100 mM NaCl +3 mM K	26.9 (105)	58.7 (91)	44 (100)
100 mM NaCl +6 mM K	32.1 (125)	61.9 (95.9)	47 (105)
100 mM NaCl +9 mM K	35.5 (138)	65.6 (101.6)	49.6 (113)

Values in () are the percentage of control.

These results are supported by the facts that under salt stress plant photosynthesis rate, plant growth and biomass production is reduced (Akram *et al*., 2011; Sun *et al*., 2011; Cicek and Cakirlar, 2002). Application of K under saline treatment significantly improved membrane stability and plant growth parameters as supported by the fact that plant growth and yield of the crop is significantly increased by increasing the potassium dose (Fayez and Bazaid, 2013; Kaya *et al*., 2007; Nadia and Bardan, 2006).

From these results it is clear that ROS production is triggered under saline treatment (100 mM NaCl) and suppressing the activity of antioxidant enzymes (Yu and Rengel, 1999; Hernandez *et al*., 1995). Potassium is the major macro-nutrient that helps out the plants to overcome the salt stress conditions and its role in activating the enzymes is clearly depicted in this study that enhanced levels of K were helpful in improving the antioxidant enzymes activity. Such results are also previously revealed by Soleimanzadeh *et al*. (2010); Zheng *et al*. (2008).

References

Akram, M., Afzal, M., Ashraf, M. 2011. Detecting differences in some elite wheat lines for salt tolerance through multi parameters evaluation i.e. morphological and yield parameters. *Pakistan Journal of Botany*, **43:** 435-443.

Alam, S.M., Ansari, A., Khan, M.A. 2000. *Pakistan Salt Effected Soil Survey*. Nuclear Institute of

Agriculture, Tando Jam, Pakistan, pp. 8-21.

Ali, S., Zeng, F., Cai, S., Qiu, B., Zhang, G. 2011. The interaction of salinity and chromium in the influence of barley growth and oxidative stress. *Plant Soil and Environment*, **57:** 153-159.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, **27:** 84-93.

AzevedoNeto, A.D., Prisco, J.T., Eneas-Filho, J., Abreu, C.E.B., Filho, E.G. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, **56:** 87-94.

Chance, B., Maehly, A.C. 1955. Assay of catalase and peroxidase. *Method Enzymology*, **2:** 764-775.

Chaum, S., Pokasombat, Y., Kirdmanee, C. 2011. Remediation of salt-affected soil by gypsum and farmyard manure importance for the production of Jasmine rice. *Australian Journal of Crop Sciences*, **5:** 458-465.

Cherel, L. 2004. Regulation of K^+ channel activities in plants: from physiological to molecular aspects. *Journal of Experimental Botany*, **55:** 337-351.

Cicek, N., Cakirlar, H. 2002. The effect of salinity on some physiological parameters in two maize cultivars. *Bulgarian Journal of Plant Physiology*, **28:** 66-74.

Cramer, G.R., Alberico, G.J., Schmidt, C. 1996. Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Functional Plant Biology*, **21:** 675-692.

Fayez, A.K., Bazaid, S.A. 2013. Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. *Journal of Saudi Society of Agricultural Sciences*, **13:** 45-55.

Gapinska, M., Sklodowska, M., Gabara, B. 2008. Effect of short and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots. *Acta Physiology of Plant*, **30:** 11-18.

Giannoopolitis, C.N., Ries, S.K. 1977. Superoxide dismutase-I. occurrence in higher plants. *Plant Physiology*, **59:** 309-314.

Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F., DelRio, L.A. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Sciences*, **105:** 151-167.

Hoagland, D.R., Arnon, D.I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, **347:** 1-32.

Kaya, C., Tuna, A.L., Ashraf, M., Altunlu, H. 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Environmental and Experimental Botany*, **60:** 397-403.

Krasensky, J., Jonak, C. 2012. Drought, salt and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany*, **63:** 1593-1608.

Mahmood, K. 2011. Salinity tolerance in barley (*Hordeum vulgare* L.) effects of varying NaCl, K^+/Na^+ and $NaHCO_3$ levels on cultivars differing in tolerance. *Pakistan Journal of Botany*, **43:** 1651-1654.

Mengel, K., Kirkby, E.A. 2001. *Principles of Plant Nutrition*, 5th edition, Kluwer academic publisher, Dordrecht, Germany.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sciences*, **7:** 405-410.

Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59:** 651-681.

Nabati, J., Kafi, M., Nezami, A., Moghaddam, P.R., Masoumi1, A., Mehrjerdi, M.Z. 2011. Effect of salinity on biomass production and activities of some key enzymatic antioxidants in kochia (*Kochia scoparia*). *Pakistan Journal of Botany*, **43:** 539-548.

Nadia, M.B. 2006. Effect of potassium rate on barley growth and its mineral content under different salt affected soil conditions. *Research Journal of Agriculture and Biological Sciences*, **2:** 512-519.

Sairam, R.K., Rao, K.V., Srivastava, G.C. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentration. *Plant Sciences*, **163:** 1037-1046.

Soleimanzadeh, H., Habibi, D., Ardakani, M.R., Paknejad, F., Rejali, F. 2010. Effect of potassium levels on antioxidant enzymes and malondialdehyde content under drought stress in sunflower (*Helianthus annuus* L.). *American Journal of Agricultural and Biological Sciences*, **5:** 56-61.

Sun, J.K., Li, T., Xia, J.B., Tian, J.Y., Lu, Z.H., Wang, R.T. 2011. Influence of salt stress on ecophysiological parameters of *Periploca sepium* Bunge. *Plant Soil and Environment*, **57:** 139-144.

Yu, Z., Rengel, Q. 1999. Drought and salinity differentially influence activities of superoxide dismutase in narrow leafed lupines. *Plant Sciences*, **141:** 1-11.

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(Park, 2005; Aksu and Kabasakal, 2004; Evans *et al.,* 2000)

In "List" References :

Evans, W.J., Johnson, M.A., Fujimoto, Cy. H., Greaves, J. 2000. Utility of electrospray mass spectrometry for the characterization of air-sensitive organolan-thanides and related species. *Organometallics,* **19:** 4258-4265.

For Standards:

ASTM, 2007. Standard Test Method for the Determination of Iron Ion Ores and Related Materials (E247-OD) P.O. Box C700, West Conshohocken, USA, *Annual Book of ASTM Standards*, vol, 30.5 pp.163-165.

PCRWR, 2007. *Water Quality Monitoring, Fifth Monitoring Report* 2005-6 . ISBM 978-969-8469-184, Pakistan Council of Research in Water Resources. Pakistan

Books:

Cinar, A., Parulekar, S.J., Undey, C., Birol, G. 2003. *Batch Fermentation: Modeling, Monitoring, and Control,* 250 pp.Marcel Dekker Inc., New York, USA.

Chapters in Edited Books:

Newby, P.J., Johnson, B. 2003. Overview of alternative rapid microbiological techniques. In: *Rapid Microbiological Methods in the Pharmaceutical Industry,* M.C. Easter (ed.), vol. **1**, pp. 41-59, 1st edition, Interpharm / CRC, Boca Raton, Florida, USA.

Papers in Proceedings:

Marceau, J. 2000. Innovation systems in building and construction and the housing industry in Australia. In: *Proceedings of Asia-Pacific Science and Technology Management Seminar on National Innovation Systems,* pp. 129-156,Japan Int. Sci. Technol. Exchange Centre, Saitama, Japan.

Reports:

*SIC, 2016. Annual Report, 2014-15,*Scientific Information Centre, Pakistan Council of Scientific and Industrial Research, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi, Pakistan.

Thesis:

Saeed, A. 2005. Comparative Studies on the Biosorption of Heavy Metals by Immobilized Microalgal Cultures, Suspended Biomass and Agrowastes. *Ph.D. Thesis*, 248 pp., University of the Punjab, Lahore, Pakistan.

Patents:

Young, D.M. 2000. Thermostable Proteolysis Enzymes and Uses Thereof in Peptide and Protein Synthesis, US Patent No. 6,143,517, 7th November, 2000.

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Deming, D; Dynarski, S. 2008. The lengthening of childhood (NBER Paper 14124) Cambridge, MA: National Bureau of Economic Research, Retrieved July 21, 2008 from http://www.nber.org/papers/w14124) DOI No.....

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