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TEA [*CAMELLIA SINENSIS* (L.) KUNTZE] LEAF COMPOST AMELIORATES THE ADVERSE EFFECTS OF SALINITY ON GROWTH OF CLUSTER BEANS (*CYAMOPSIS TETRAGONOLOBA* L.)

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Abstract

The pot experiment was carried out to evaluate the effect of tea compost on plant growth under salinity. Plants were grown in clay pots filled with sandy loam soil and irrigated by saline water (0, 50 and 100mM NaCl) with and without tea compost amendments. Soil evapotranspiration (ET), vegetative and reproductive growth and biochemical parameters were studied in this experiment. ET rate was increased with increasing salinity, whereas, it decreased with application of tea compost under all salinity. Vegetative (shoot height, number of leaves, fresh and dry biomass) and reproductive (number of seeds per plant) growth significantly decline under increasing salinity levels. Tea compost treatment helped in improving all these parameters. Total photosynthetic pigments (chlorophyll a, b, carotenoids and total chlorophyll content) showed reduction under raising salinity levels, while betterment was recorded with application of tea compost. Organic solutes (soluble sugars, proteins, free amino acids and phenolic content) increased with increasing salinity (50-100mM NaCl). Increased soluble sugars were found with tea compost treatment under non-saline control and decreased in salinity. Soluble proteins, amino acids and phenolic content increased with application of tea compost under both control and salinity. It is concluded that tea compost treatment is found to cope with salinity stress and improve plant growth and biochemical parameters by diluting the hazardous effects of salinity.

Key words: Salinity, Tea leaves compost, Growth, Guar, Evapotranspiration, Photosynthetic pigments.

Introduction

Excessive salts in soil leads to increased salinity in agricultural lands. Salinity is a global problem which results reduction in agricultural crops productivity. Plant growth inhibition varies due to nature and properties of salts in each climatic zone, but severity is confined in arid and semi-arid zones (Rengasamy, 2006). In Pakistan, 16.2 Mha land is irrigated, from which more than 6.7 Mha are affected by salts with different levels of salinity. Increased salts in rhizosphere may inhibit the growth of plant either through osmotic inhibition of water by roots or specific in effects due to which toxicity occur (Saqib, 2012). Decrease in rate of photosynthesis, reduction in cell expansion and leaf area under salinity is well studied (Ashraf *et al*., 2012) also decreased supply of enzymes (like photosynthase) and hormones (Apel & Hirt, 2004).

Among the various salinity management practices, soil amendments, composting and mulching are well known methods to raising fields. Partially decomposed organic mulch improves plant growth under salinity (Saeed & Ahmad, 2009; 2013). Compost is an organic material which decomposes and improves physicochemical properties of soil. Municipal solid waste (MSW) is the common organic compost help to ameliorate the effects of salinization (Lakhdar *et al*., 2009). There are several composts available for bedding of the seasonal plants. Application of vermicompost tea improved biological properties of a peat-perlite media to grow plants in greenhouse and field conditions (Pant *et al*., 2011). Compost tea is a liquid extract of compost which adds valuable soil amendments and affects the plant growth (Gharib *et al*., 2008). Compost tea can be applied to the soil or directly to the plant as a foliar spray, that provide nutrients for foliar or soil application.

Coffee (*Coffea arabica* L.) and tea (*Camellia sinensis* (L.) Kuntze) are familiar beverages and considered as a medicine since the ancient times to polyphenolic contents and having high amount of metal chelating substances that may remain in the wastes when extracted with hot water (Morikawa & Saigusa, 2008). In some studies reported that tea has been associated in reducing risk of heart disease and cancers, anti-allergic action and antimicrobial properties (Paola *et al*., 2005). Tea has the complex chemical composition, contain polyphenols, alkaloids (caffeine, theophyline and theobromine), amino acids, carbohydrates, proteins, chlorophylls, volatile compounds, minerals, trace elements and other unknown compounds, from all of these compounds, polyphenols are essential bioactive molecules of the tea (Cabrera *et al*., 2003). When, tea mixed with the soil, it improves the nutrient retention of the soil which can stimulate the plant growth. The increasing amount of the nutrients when available to the root system leads to a stronger and healthier plants. Azza *et al*. (2010) reported that due to presence of soluble nutrients in compost tea and their extracts plant growth was enhanced.

Clusture beans (*Cyamopsis tetragonoloba* (L.) Taub) is commonly known as guar belongs to the family Fabaceae. Due to the benefits of the guar, it is cultivated as an important leguminous crop in Pakistan. An area under cultivation of Guar is about 242.6×103 hectares, and seed production is about 220.7×103 tons/year with an average yield of 909.7 kg/ha (Anon., 1993). It is used for human consumptions as vegetable, forage for cattle and it is also well known as green manure crop. The guar plants have nitrogen fixing bacteria in their root nodules which enhance the soil fertility by adding nitrogen in the soil. Their seeds are the great source of galactomannan gum, which is used for paper manufacturing, food processing, textile printing and in pharmaceutical industries. This guar gum is also used in food as thickeners and in pharmaceutical (Raymond *et al*., 2009). The present study is designed to determine the effect of tea leaves compost on growth and biochemical content of cluster beans (guar) plants.

Materials and Methods

Present study was conducted in completely blocked designed in Department of Botany at Federal Urdu University of Arts, Science and Technology, Karachi. Used tea of the single brand was collected from the offices and homes for composting. This tea was kept wet for decomposition about 3 months after then used in experiment (100 g in 1 Kg soil). Surface sterilized seeds of cluster beans were sown in clay pots having diameter 16cm and depth 14cm with basal hole for leaching. Pots were filled by one Kg sandy loam soil and cow dung manure (1:9). Initially plants were irrigated with tap water (non-saline control), later salinity treatments were started by acclimatizing through 10 mM NaCl to the desired salinity (50 mM and 100 mM NaCl solutions).

Amount of irrigation water and Evapotranspiration (ET) was calculated as Ünlükara *et al*. (2008).

$$I = \{(W_{bi} - W_{fc})/ \rho w\}/1\text{-}LF$$

where, I is amount of irrigation water applied (litre), LF is the leaching fraction {30% (at which $EC_w = EC_e$) as proposed by Ayers & Westcot (1989)}, W_{fc} is the pot weight at field capacity (kg), W_b is the pot weight before irrigation, and ρ_w is density for water (1.0 kg/litre).

Weight of each pot (containing growing plants) of different saline conditions and treatment were recorded before and after irrigation. Use the following formula was used to calculate ET.

$$E\,T = (I - D) + (W_b\text{-}W_e) / \rho_w$$

where, I is amount of irrigation water applied, D is amount of drained water, W_b and W_e are the pot weights (g) at the beginning and at the end of the experiment, respectively, ρ_w is water density (1.0 kg/litre),

Electrical conductivity of saturated soil extracts was recorded by EC meter (Adwa, AD 32/31, UK).

Plant material: Plants were harvested after 45 days of the treatments and growth parameters viz., shoot height (cm), fresh and dry biomass (g) was recorded. Leaf samples were collected from each plant and biochemical contents were determined as,

Photosynthetic pigments (chlorophyll contents by Arnon, 1949; and carotenoids by Duxbury & Yentsch, 1956) were determined in 0.1g leaf tissue extracted in 80% acetone, absorbance was recorded by spectrophotometer (JENWAY 6305) and values were calculated by following formulae (mg g^{-1}F.wt).

Chla = $12.7\ A_{663} - 2.69\ A_{645} / W * V$
Chlb = $2.9\ A_{663} - 4.68\ A_{645} / W * V$
Total Chl = $20.2\ A_{663} + 8.02\ A_{645} / W * V$
Carotenoid$_{x+c}$ = $7.6\ A_{480} - 2.63\ A_{510} / W * V$

The soluble sugars in alcoholic extracts were estimated by the method of Yemm & Willis (1954). 0.1g leaf tissues were macerated in 5ml ethanol. It was boiled in water bath for 10-15min and allowed to cool at room temperature. One ml of extract was added in 5ml of freshly prepared Anthrone reagent and boiled for 30 minutes. Cool at room temperature and recorded the absorbance at 620 nm against reagent blank by spectrophotometer (JENWAY 6305). Amount of soluble sugars was calculated from standard curve in mg glucose g^{-1} fresh weight.

Total phenolic content was estimated according to Iqbal *et al*. (2005). One ml ethanolic extract of leaves was added in 5ml of Folin reagent (1:9 in distilled water). Mixed well and added 4ml of 7.5% Na_2CO_3. The samples were incubated at room temperature for 30 minutes. The absorbance was measured at 765nm against reagent blank by spectrophotometer. The amount of phenols expressed in mg of Galic acid g^{-1} F.wt.

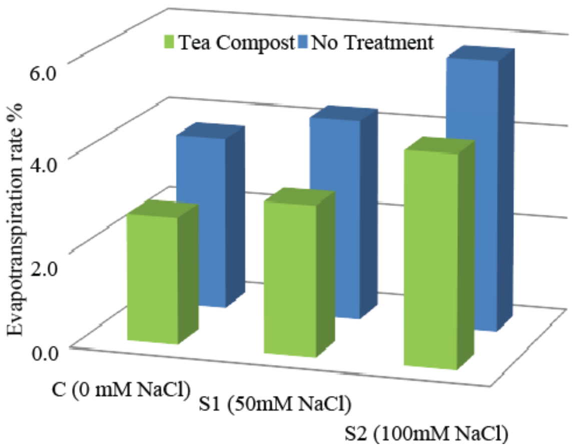
Total soluble proteins were estimated according to the method of Bradford (1976). 0.1g of leaf tissues were macerated in four ml of chilled sodium phosphate buffer (pH 7.0), centrifuged and adjust final volume as 10ml. 05ml of diluted Bradford Reagent (1:5) was added in 0.1 ml of extract. Absorbance was recorded at 595 nm after 30 minutes incubation at room temperature by spectrophotometer. The protein content in the tissue was determined by using standard curve of BSA (Bovin Serum Albumin) and expressed in mg g^{-1} F.wt.

Total free amino acids were determined by the ninhydrin method of Hamilton & Vanslyke (1943). One ml of sample extract (prepared as proteins) was mixed with one ml of 10% pyridine and one ml of 2% ninhydrin solution. The samples were boiled for 30 minutes and cooled at room temperature. The absorbance was measured at 570 nm and free amino acids were calculated from standard curve of leucine in mg g^{-1}F.wt.

Analysis of variance and Duncan's multiple range tests (Duncan, 1955) for the means at $LSD_{0.05}$ were performed by SPSS ver. 20 (Anon., 2011).

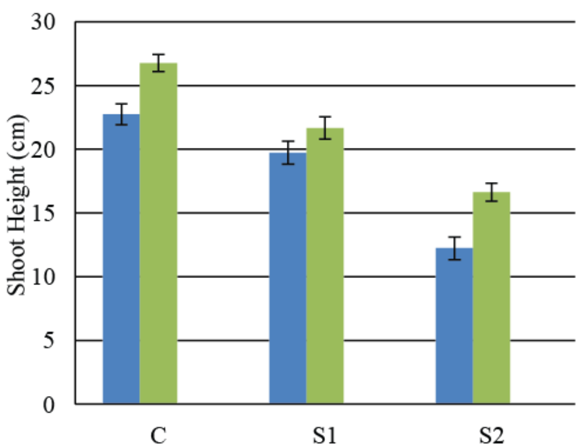
Results and Discussion

The effects of saline water irrigation and tea compost treatment on the evapotranspiration rate is given in Fig. 1. The present study showed significant increase in evapotranspiration (ET) rate at higher salinities ($F = 39.194$, $p<0.0001$) without tea compost treatment. Whereas, decrease was recorded due to tea compost treatment ($F = 19.135$, $p<0.0001$) under both non-saline control and salinity. Interaction of salinities and tea compost treatment was found significant ($F = 3.305$, $p<0.05$). The decrease in ET resulted increase in growth and yield of the plant and conserved moisture in plants as compared to control. Increased water consumption by vegetable plants and decreased water use efficiency was reported by increasing salinity level in irrigation water (Ünlükara, 2008 and 2010).

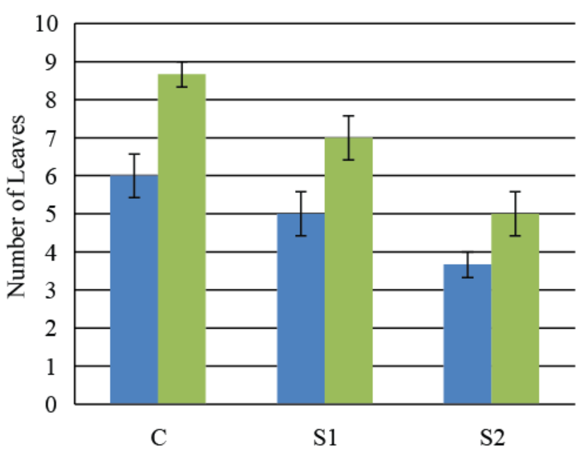


F-values at $LSD_{0.05}$, Salinity = 39.194***, Treatment = 19.135***, Salinity x Treatment = 3.305*

Fig. 1. Effect of salinity and tea compost treatment on the rate of evapotranspiration.



F-values at $LSD_{0.05}$, Salinity = 111.777***, Treatment = 11.371**, Salinity x Treatment = 0.852^{ns},



F-values at $LSD_{0.05}$, Salinity = 34.824***, Treatment = 14.529***, Salinity x Treatment = 0.588^{ns}

Fig. 2. Effect of salinity and tea compost treatment on the shoot height and number of leaves of *Cyamopsis tetragonoloba* (L.) Taub.

Effects of salinity and tea compost treatment on growth of *Cyamopsis tetragonoloba* (L.) Taub presented in Fig. 2. It showed significant decrease in shoot height and number of leaves with increased salinity levels. However, tea compost treatment resulted improved plant growth by increasing shoot height (F= 11.371, p<0.001) and number of leaves (F = 14.529, p<0.0001) under all salinity levels (0, 50 and 100 mM NaCl) as shown in Fig. 5. Kanwal *et al*. (2013) observed significant reduction in the growth and germination of mung beans at 50 mM of NaCl. Saeed & Ahmad (2013) found increased okra yield with organic mulch under non-saline as well as saline conditions. Ali, *et al*. (2014) and Pant *et al*. (2011) reported increase in number of leaves and shoot height of red beans with application of organic compost amendments.

Fresh and dry biomass of *Cyamopsis tetragonoloba* (L.) Taub. grown under salinity and treatment are presented in Fig. 3. The data showed significant decrease in fresh and dry biomass with increasing salinities (0, 50 and 100mM NaCl). Application of tea compost showed significant increase in both parameters in all irrigation regimes. The interaction of the salinity and tea compost treatment was also found significant for fresh and dry biomass (F = 2.423, p<0.01; F= 2.347, p<0.01 respectively). The results of the present study are in agreement with the findings of other workers. Haleem & Mohammed (2007) observed the reduction in fresh and dry biomass of mung bean plants under salinity. Kanwal *et al.* (2013) found decrease in biomass of mung bean plant by applying the 50mM NaCl through root medium.

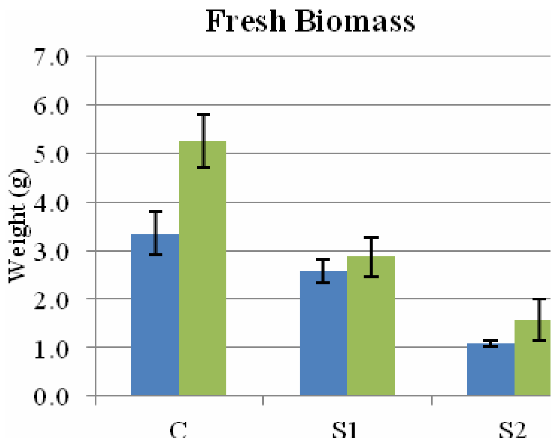
Table 1 showed significant decline in number of seeds per plant under salinity. An increase in seeds number (F = 26.737, p<0.0001) was found in plants treated with tea compost under non-saline as well as saline water irrigation. Tea compost treatment was significant for seeds production (F = 6.833, p<0.001). Several studies showed that reproductive growth or fruit yield is dependent on vegetative growth of the plants. Generally plants yield decreased with increasing salinity of the rooting medium (Saeed & Ahmad, 2013; Saeed & Ahmad, 2009; Läuchli & Grattan, 2007). Organic material incorporation in soil leads to improve soil fertility and availability of the minerals to the plants. According to Hirich *et al*. (2014) and Pant *et al*. (2011), organic amendments showed increased crop yield and productivity even under saline soil.

Total photosynthetic pigments (F = 915.192, p<0.0001), Chl a (F = 764.852, p<0.0001), Chl b (F = 378.144, p<0.0001) and carotenoids (F = 113.654, p<0.0001) decreased significantly whereas, tea compost treatment showed significant increase in all photosynthetic pigments (Fig. 3) under non-saline as well as saline water treatments. Reduction in photosynthetic pigments under salinity which ultimately affects the reduction in biomass, crop growth and yield is reported by Siddiqi *et al*. (2009). Present study showed reduction in chlorophyll content and carotenoids under lower salinity level (50mM) with the application of tea compost. Increased chlorophyll content with application of organic amendment under salinity was also reported in *Hordeum maritimum* by Lakhdar *et al*. (2008) and in *Solanum esculentum* by Saeed & Ahmad (2009). Fayed (2010) reported significant increased leaf pigment contents by foliar application of the compost tea which is decreased by its application in soil.

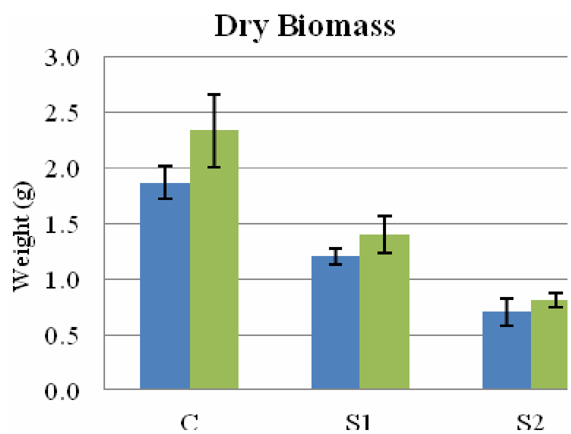
Table 1. Salinity profile of rooting medium and number of seeds produced per *Cyamopsis tetragonoloba* (L.) Taub. under tea treatment.

Salinity	Treatment	ECe Soil dSm^{-1}	Number of seeds produced per plant (mean)
Control (0mM NaCl)	Without treatment	1.6 ± 0.088	54.333 ± 4.485
ECiw = 0.5 dSm^{-1}	Tea compost	1.8 ± 0.058	93.667 ± 4.978
S1 (50mM NaCl)	Without treatment	5.2 ± 0.153	33.000 ± 3.215
ECiw = 3.32 dSm^{-1}	Tea compost	5.4 ± 0.176	45.667 ± 1.764
S2 (100mM NaCl)	Without treatment	7.9 ± 0.173	17.000 ± 3.786
ECiw = 7.43 dSm^{-1}	Tea compost	7.5 ± 0.115	26.333 ± 2.404

F-Values for Number of Seeds/ plants: (S = 207.729***, T=26.737***, SxT = 6.833**)



F-values at $LSD_{0.05}$, Salinity = 54.908***, Treatment = 4.480*, Salinity x Treatment = 2.423*,



F-values at $LSD_{0.05}$, Salinity = 75.683***, Treatment = 7.971**, Salinity x Treatment = 2.347*

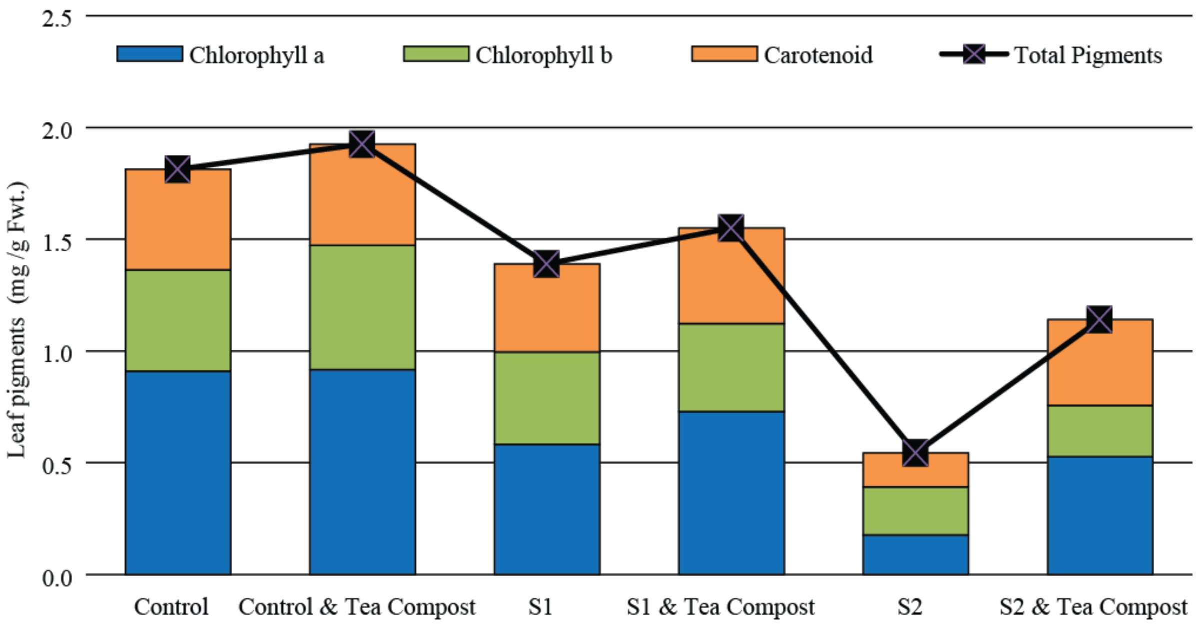
Fig. 3. Effect of salinity and tea compost treatment on fresh and dry biomass of *Cyamopsis tetragonoloba* (L.) Taub.

Plants adopt several mechanisms to enhance their tolerance and accumulate several compatible osmolytes like, sugars, amino acids, proteins, some antioxidants like carotenoid, anthocyanins and phenolics etc. The present study showed significant changes in leaf biochemical (soluble sugars, proteins, free amino acids and phenolic content) under salinity and tea compost treatment (Table 2 and Fig. 4). Total soluble sugars were significantly decreased under salinities (F = 34.509, p<0.0001). Lesser reduction in sugars was found at 100mM NaCl than at 50mM NaCl. Little increase with application of tea compost was found in non-saline control, whereas, decrease was recorded under both salinity levels (F = 0.798, P = ns). Similar results were also observed in leaves of okra (*Abelmoschus esculentum* (L.) Moench) grown under salinity and organic mulch treatment (Saeed & Ahmad, 2012). Generally soluble sugars in leaves were found to be higher under salinity and worked as osmoticum. Certain plants showed compartmentation or partitioning of these sugars to increase their tolerance (Pattanagul & Thitisaksakul, 2008). Our results are in agreement with others (Saeed & Ahmad, 2012 and El-Quesni *et al*., 2010). Organic amendments improve metabolic activities to improve crop yield. Tea compost treatment reduced the total soluble carbohydrates in the plants under higher salinity level as compared to the untreated plants (Saeed and Ahmed, 2009; Elham, 2006).

Antioxidants are the important class of the chemical compounds to provide defense against stress included anthocyanin, polyphenols, vitamin C, flavonoids, phenolics etc. (Longo & Vasapolo, 2006). The present study showed increased phenolic content (F = 18.149, p<0.0001) in the leaves of *Cyamopsis tetragonoloba* (L.) Taub. Tea compost treatment also showed an increase in phenolic contents (F = 0.626, P = ns) under non-saline as well as all salinity levels (Table 2). Klados & Tzortzakis (2014) found increased total phenols in *Cichorium spinosum* under increasing salinity. Increased phenols and polyphenols under salinity to protect against oxidative stress by salinity reported by other scientists (Klados & Tzortzakis, 2014 and Abdelhamid *et al*., 2010. Increased synthesis of phenolic compounds like, flavonoids and anthocyanins improve fruit quality with application of organic compost (Theunissen *et al*., 2010 and Zhao *et al*., 2009).

Proteins are the building blocks and their synthesis is directly affected by salinity. Significant reduction found in soluble proteins under increasing salinities (Table 2). Effect of tea leaves compost showed increase leaf proteins under both non-saline control and salinities (F=12.045, p<0.0001). Whereas, non-significant (F= 0.363, P = 0.832) interaction of salinity and tea leaves compost treatments was observed. Our study is in agreement with Kong-Ngern *et al*. (2005). Klados & Tzortzakis (2014) found decrease protein in perlite treatment with salinity.

Significant increase in free amino acids was found in leaves under increasing salinity levels of irrigation water. Application of tea compost revealed significant increase in control and lower salinity (50mM NaCl), whereas, reduction was noted at higher salinity (100mM NaCl). Interaction of both salinity and tea compost treatment was found significant ($F = 3.319$, $p<0.01$) on free amino acids of *Cyamopsis tetragonoloba* (L.) Taub. According to Hussain *et al*. (2007) and Silva *et al*. (2008) increasing amino acids under saline conditions were due to the reduction in protein synthesis and further depletion of protein. Saeed & Ahmad (2012) have found increase in amino acids under increased salinity and little reduction was found in leaves of okra grown with organic mulch under saline water irrigation.



F-values at $LSD_{0.05\%}$

Source	Chl-a	Chl-b	Carotenoid	Total Chl	Totalpigment
Salinity	764.852***	378.144***	113.654***	1505.401***	915.192***
Treatment	202.923***	14.478**	75.427***	250.553***	214.982***
Salinity x Treatment	72.18***	17.527***	46.965***	43.41***	60.855***

Fig. 4. Effect of salinity and tea compost treatment on photosynthetic pigments (Chlorophyll a, b, carotenoids mg $g.Fwt^{-1}$ and total pigments) in leaves of *Cyamopsis tetragonoloba* (L.) Taub.

Table 2. Effect of salinity and tea compost treatment on the biochemical parameters (Total soluble carbohydrates, total phenolic contents, proteins and amino acids) in *Cyamopsis tetragonoloba* (L.) Taub.

Salinity	Treatments	Soluble sugars (mg/g Fwt.)	Phenolic content (mg/g Fwt.)	Free aminoacids (mg/g Fwt.)	Soluble proteins (mg/g Fwt.)
Control (0mM NaCl)	Without treatment	8.400 ± 0.088	1.000 ± 0.289	3.500 ± 0.289	0.367 ± 0.033
	Tea compost	8.700 ± 0.529	1.053 ± 0.147	4.833 ± 0.145	0.473 ± 0.29
50mM NaCl	Without treatment	7.800 ± 0.163	1.233 ± 0.049	5.833 ± 0.667	0.268 ± 0.021
	Tea compost	7.120 ± 0.150	1.900 ± 0.145	6.233 ±.606	0.343 ± 0.015
100mM NaCl	Without treatment	8.200 ± 0.809	1.377 ± 0.232	6.567 ± 0.371	0.230 ± 0.012
	Tea compost	7.343 ± 0.345	1.500 ± 0.235	5.90 ± 0.208	0.307 ± 0.009
$LSD_{0.05}$		F-values			
Salinity		34.509***	18.149***	8.925**	35.636***
Treatment		0.798^{ns}	0.626^{ns}	3.739*	12.045***
Salinity x treatment		0.678^{ns}	0.249^{ns}	3.319*	0.363^{ns}

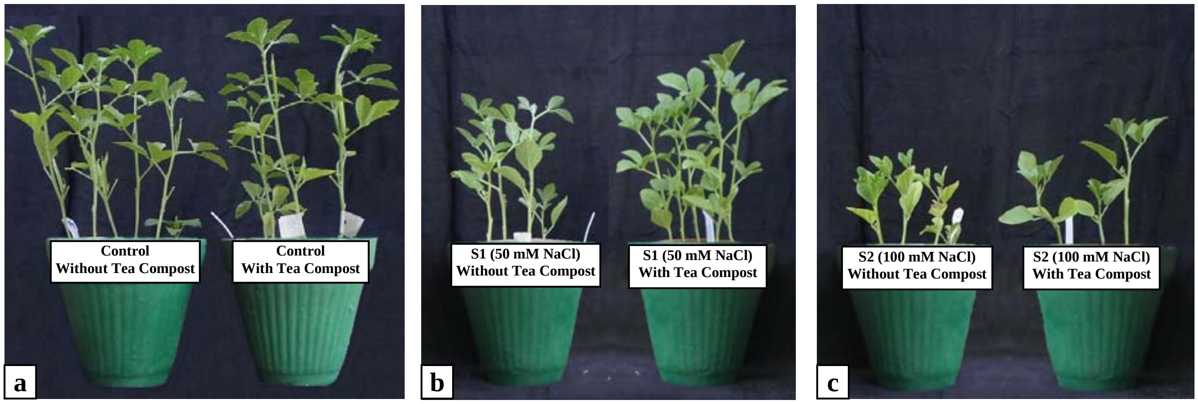


Fig. 5 (a, b & c). Effect of tea compost on the growth of *Cyamopsis tetragonoloba* (L.) Taub. under control (c), 50mM (S1) and 100mM (S2) NaCl salinity respectively.

Conclusion

It was concluded from the present study that recycling of organic waste materials like tea from the industries, offices and homes composted and used for crop cultivation. This research could be helpful in improving salinity tolerance or avoidance mechanisms in plants under salinity.

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