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EVALUATION OF WASTE STABILIZATION PONDS EFFLUENT EFFICIENCY ON THE GROWTH AND NUTRITIVE CHARACTERISTICS OF CLUSTER BEANS (*CYAMOPSIS TETRAGONOLOBA* L.) TAUB.

MOAZZAM ALI KHAN*, S. SHAHID SHAUKAT, AAMIR ALAMGIR AND SYED ADNAN HASAN

Institute of Environmental Studies, University of Karachi, Karachi-75270, Pakistan
Corresponding author e-mail: sherwanis@hotmail.com

Abstract

South Asian countries including Pakistan are facing chronic shortage of water supply which is anticipated to be aggravated in future. These countries are agribased where the continued water supply is crucial for sustainable economy. One of the possible alternatives to overcome the problems of water scarcity is the used of treated wastewater which is gaining much importance even in the western world. The treated wastewater can be used as a liquid fertilizer which could provide dual benefits both in terms of saving of fresh water as well as inorganic fertilizers. The potential of treated effluent from waste stabilization ponds (WSP) and equivalent basal fertilizer on growth and nutritive quality of cluster beans (*Cyamopsis tetragonoloba* L.) Taub. was investigated under field conditions. Treated effluent significantly increased fresh weight of leaves and stems. Dry weight of stem was also significantly higher with the treatment of WSP effluent as compared to the use of basal fertilizer and fresh water. Fresh and dry fruit weights, number of seeds per fruit and fruit length were also significantly increased in WSP effluent treatment as compared to other two treatments. Treatment with WSP effluent also improved the nutritive characteristics such as crude proteins and total carbohydrates. However, total fat and ash content percentage of *Cyamopsis tetragonoloba* remained unaltered. The application of WSP effluent also increased NPK and organic matter content of the soil after harvesting the crop which would be helpful for succeeding crop. The study demonstrated that treated effluent can be successfully used for unrestricted irrigation in the water deficient areas of Pakistan thereby saving huge quantities of fresh water.

Introduction

Fresh water scarcity is a common problem in countries like Pakistan which are situated in arid and semi arid regions of the world. Water scarcity problem in Pakistan is quite alarming in the face of agri-based economy of the country. One alternative to overcome the acute shortage of water supply is the use of treated wastewater for unrestricted irrigation (Aghtape *et al.,* 2011; Oron *et al.,* 2007; Mohammad & Mazahreh, 2003; Al-Rashed & Sherif, 2000; Vazquez-Montiel *et al.,* 1996; Al-Jaloud *et al.,* 1995; Feigin *et al.,* 1991; Bouwer & Idelovitch, 1987). The major limitation pertaining to the use of treated wastewater is its effective management and treatment to protect public health at an affordable cost (Sipala *et al.,* 2003; Anderson *et al.,* 2001; Agunwamba, 2001; Asano & Levine, 1996; Marcos do Monte *et al.,* 1996; Asano *et al.,* 1992).

The developing countries which cannot afford sophisticated biomechanical systems of wastewater treatment are exploiting waste stabilization ponds (WSP) technology for both restricted and unrestricted irrigation (Khan *et al.,* 2010, Khan *et al.,* 2009, Khan *et al.,* 2008; Khan & Khan, 2007; Alcalde *et al.,* 2003, Mara & Pearson, 1998). The technology provides low operation and maintenance cost with effective removal of pathogens which has made WSP technology as a treatment of choice in many parts of the world specially in tropical and subtropical regions (Alcalde *et al.,* 2003; Mara & Pearson 1998; Khan & Ahmed, 1992; Mara, 1987).

In Pakistan scientific work on WSP technology is very scanty, and the commercial exploitation has not been initiated so far through which the treated wastewater can be converted into economic asset rather than an economic burden (Khan *et al.,* 2010; Khan *et al.,* 2009; Khan *et al.,* 2008; Khan & Khan, 2007).

Cluster bean (*Cyamopsis tetragonoloba* L.), locally known as Guar, is a drought resistant annual crop grown in semiarid region. In Pakistan it is usually cultivated in May-June and harvested in August- September. It is used as a common vegetable in Pakistan where it is considered as summer delicacy. The crop is also used for the production of guar gum for which Pakistan is also one of the major world suppliers (Shaukat *et al.,* 2010) through which the country earns huge foreign exchange (Vahidy & Yousuf Zai 1991). *Cyamopsis tetragonoloba* is also used as green manure owing to its high C/N ratio (Hussain *et al.,* 1995). It increases the amount of nitrogen in soil (Gurusaravanan *et al.,* 2012) besides altering a number of soil characteristics. It is well known that microbial biomass is a sensitive index of tillage and crop-induced changes in the biological characteristics of soils (Mele & Carter, 1995). Ashraf *et al.,* (2002) reported that it can also be cultivated in hyper saline soil because of its inherent genetic potential.

The present study was designed to explore the potential of treated effluent from Waste Stabilization Ponds (WSP) for the growth of *Cyamopsis tetragonoloba*. The study demonstrated that the effluent can be efficiently used for the commercial production of *Cyamopsis tetragonoloba* even in the areas where droughts cause frequent failure of many other crops. The use of treated effluent not only improves the characteristics of soil but also enhances the microbial biomass.

Materials and Methods

Technical details of ponds: The two waste stabilization ponds used in the present study are available at Karachi University Campus that were constructed in consultation with the Institute of Environmental Studies, University of Karachi. The technical details are given in Table 1.

Table 1. Technical details of waste stabilization pond system at Karachi University Campus.

S. No.	Parameters	Characteristics
1.	Pond area (bottom)	$53 m^2$
2.	Pond area (WSP)	$69 m^2$
3.	Pond area (average)	$61 m^2$
4.	Outlet for effluent	1.8m
5.	Influent sump capacity	$203 m^3$
6.	Effluent sump capacity	$67 m^3$
7.	Pond volume at 1.8 m depth	$127 m^3$
8.	Average total retention time	2 days
9.	Total average hydraulic load	$650 m^3$ /day (approx.)
10.	Total average BOD_5 load	250mg/l

Based on average BOD_5 load of 250 mg/l; Khan & Ahmed 1992; Khan & Khan, 2007

The ponds are trapezoidal in shape of equal dimensions and are lined with concrete at the bottom as well as the sides. The ponds are connected in series and the outlet is available at a depth of 1.8 m. The primary ponds are connected with the influent sump which receives the raw domestic wastewater through underground sewerage line of Karachi Water and Sewerage Board. The secondary pond is likewise connected with the effluent sump.

Collection and analysis of effluent samples: The samples of the effluent were collected periodically twice a month and analysed for total Kjeldahl nitrogen (TKN), Phosphate-Phosphorus, potassium and organic matter content in accordance with APHA (Anan., 2005).

Field experiment

Selection of soil: The soil selected for cultivation of *Cyamopsis tetragonoloba* was a sandy loam with pH 7.9 and maximum water holding capacity of 33%. The site is located at the Institute of Environmental Studies, University of Karachi.

Development of experimental plots: In all nine plots were developed each measuring 9.0 m^2. Various treatments were applied including fresh water (A) which represents the control, fresh water with basal fertilizer (0.010 gm/l K_2SO_4; 0.08 gm/l $CaHPO_4$ H_2O; 0.010gm/l g Urea) (B) and WSP effluent (C) . The three treatments were randomized within each of the three blocks in a randomized complete block design.

Sowing of *Cyamopsis tetragonoloba*: Seeds of *Cyamopsis tetragonoloba* (L.) Taub. (BR-99; obtained from Pakistan Agriculture Research Council, Karachi) were sown in 10 rows with a distance of 20.0 cm between rows while distance between the seeds was 30 cm. The seeds were sown on 15th May and the crop was harvested on 15th July (60 days). The thinning was done manually after 15 days of germination to maintain the desired plant spacing and to avoid competition between plants.

Irrigation of plots: The plots were irrigated only twice with 30 gallons of water per plot. This amounts to 0.0027 (approx.) acre inch. Plots of treatment A were irrigated with fresh water, those of treatment B with fresh water containing basal fertilizer while plots of treatment C were irrigated with WSP effluent. This would mean that the levels of NPK applied through basal fertilizer were K= 1.4 , P= 2.5 and N= 2.8 kg/ha. respectively.

Analysis of soils: The soil samples were analysed before sowing and at post harvest. The soil parameters analysed were pH, total Nitrogen, Phosphorus, K, organic matter and microbial biomass-N. Soil samples were collected from each plot from a depth of 0-20 cm using a soil auger from 3 random points within a plot. Soil texture was determined by the method described in USDA (Anon., 1951). Maximum water holding capacity was determined in accordance with the method of Keen (1931). Soil pH was determined by direct pH meter (Model WTW 82362, Weliheim, Germany) after preparing a suspension in the ratio of 1:5 w/v (soil; distilled water). Total nitrogen was determined by Kjeldahal method (Anon., 2005) whereas inorganic phosphate was determined in accordance with Fogg & Wilkinson (1958). Organic matter content was estimated by the loss on ignition method (Dean, 1974). K was determined using EIL flame photometer.

To determine the microbial biomass-N the collected soil samples were pre-conditioned for seven days at 28^{o}C in laboratory and provided with nearly 100% humidity in a container and alkali (KOH) was kept in a small glass tube for the removal of CO_2. The container was opened daily for 5 minutes for the purpose of aeration. Microbial biomass-N was determined by the chloroform fumigation extraction technique developed by Vance *et al.,* (1987), using pure $CHCl_3$ treatment followed by extraction with 0.5M potassium sulphate of both fumigated and non-fumigated soils. Subsequently microbial biomass- N was determined in accordance with Singh *et al.,* (2007).

Growth studies: The growth characteristics recorded were fresh and dry weights of leaves, stem and roots. Similarly, reproductive characteristics including fruit (pod) weight, number of seeds per fruit and fruit length were also determined.

Nutritive characteristics of seeds: Additionally, nutrient quality of crop (dry matter) was determined by using the following parameters. Seed protein was determined by AOAC crude protein (Anon., 2000a), fat content (Anon., 2000b), total carbohydrate (Clegg, 1958) and ash content (Anon., 2000c).

Statistical analysis: Data of individual variables were subjected to two-way analysis of variance ANOVA (Zar, 2008). As a follow up of ANOVA, Duncan's multiple range test was performed.

Results and Discussion

Analysis of WSP effluent: The results of WSP analysis are shown in Table 2. In all 13 samples were collected between 12:00-13:00 hours when the flow discharge was maximum.

Table 2. Descriptive statistics of WSP effluent quality used for irrigation of *Cyamopsis tetragonoloba* crop.

Variables mg/l	Mean	Median	Min.	Max.	Lower quartile	Upper quartile	Std. Dev.	SE
Total Kjeldahl Nitrogen	17.21	17.21	12.62	21.64	14.75	19.87	3.07	± 0.85
Phosphate-Phosphorus	3.92	3.92	2.22	5.40	3.28	4.63	0.99	± 0.27
Potassium	14.13	7.13	5.62	7.87	6.75	7.54	25.80	± 7.15
Organic matter	590	587	534	673	561	610	43.90	± 12.17

Table 2 shows that WSP effluent has sufficient amounts of N, P, K and organic matter. The treated effluent can be successfully used as a liquid fertilizer which can be helpful in reducing the cost incurred on the application of inorganic fertilizer. Additionally, the equivalent quantity of fresh water can be saved by the application of the treated effluent. In the present study, WSP effluent was tested prior to the irrigation of plots for the organisms of public health importance (Total coliforms and Total faecal coliforms) and heavy metals. The coliforms were found to be well within the guidelines of WHO (Blumenthal *et al.,* 2000) and as such no advanced wastewater treatment was used. Most heavy metals including As, Co, Cr, Cd, Hg, Pb, and Ni were absent while concentrations of Cu, Mn and Zn were less than 0.05 mg/l that are within the permissible limits of health and safety standards (personnel observations, data not presented).

The average concentrations of total nitrogen, phosphate phosphorus, potassium and organic matter in the effluent were 17.21, 3.92, 6.87, 590.88 mg/l respectively. Based on these concentrations of nutrients and the total amount of effluent used for irrigation of *Cyamopsis tetragonoloba* crop, the total quantities of N, P and K turned out to be 16.36, 2.90 and 11.39 kg/ha respectively. The economic potential of the treated WSP effluent (Liquid fertilizer) in Pakistan climatological conditions have already been established and considered better than inorganic fertilizer (Urea nitrogen, Potassium and Phosphorus; Khan *et al.,* 2012; Khan *et al.,* 2010; Khan *et al.*, 2009; Khan *et al.,* 2008; Khan & Khan 2007).

Analysis of soil: Triplicate samples were collected from each plot and the samples were pooled together to obtain one composite sample from each plot. Soils were loamy sands that on an average comprised of 78% sand, 13% silt and 9% clay by weight. The soils were mostly alkaline with ample amounts of $CaCO_3$ (10-14%) The results of soil analysis are shown in Figs. 1-4. Soil nitrogen increased significantly ($p<0.05$) at post harvest compared to pre-sowing in basal fertilizer and WSP effluent treatments. Phosphorus content of soil was found to be significantly elevated at post-harvest over that at pre-sowing. On the other hand, the level of soil potassium was found significantly ($p<0.05$) higher at post-harvest compared to pre-sowing in basal fertilizer as well as WSP effluent treatments. Organic matter content of soil increased significantly at harvest ($p< 0.001$) only in WSP treatment. Agunwamba (2001) also reported elevated mineral content of soils irrigated with wastewater.

Cyamopsis tetragonoloba grows best in light sandy loam soil with the pH ranging between 7.5-8 and at temperature of 21-30^{o}C at planting. (Tyagi, 1982; Ibrahim *et al.,* 2011). Mekki *et al.,* (2006) reported that the use of treated wastewater tends to increase the density of soil microorganisms including bacteria, fungi and actinomycetes that helps in nutrient availability of plants. The microbial biomass –N was significantly (p at the most 0.05) elevated at post- harvest time over that of pre-sowing (Fig. 5). The size and activity of the microbial biomass is a crucial factor regulating the rate of N-mineralization (Azmal *et al*., 1996; Singh *et al.*, 2007). Besides N- mineralization, degradation of herbicides can also be caused by the microbes (Sorenson *et al.,* 2003; Holtze *et al*., 2007).

Vegetative characteristics of *Cyamopsis tetragonoloba* crop: It can be seen from Fig. 5 that irrigation of *Cyamopsis tetragonoloba* crop with the WSP effluent significantly (p at the most 0.05) increased fresh weight of leaves and stem while fresh weight of roots remained unchanged. Similarly, dry weight of stem was significantly higher ($p<0.05$) with the treatment of WSP effluent. However, dry weights of leaves and roots only showed slight but no significant increase (Fig. 6). No significant difference between control (treatment A) and basal fertilizer (Treatment B) were found with regard to fresh and dry weights of leaves, stem and roots. Fresh and dry fruit weights were also significantly higher by WSP effluent than those of treatments A and B. However, treatment B and C increased fresh weight of fruit compared to control (Fig. 7). Number of seeds per fruit and fruit length were also significantly higher in treatment with WSP effluent than those of Treatments A (fresh water) and B (Basal fertilizer) (Figs. 8-9). These vegetative characters are mainly influenced by NPK and organic matter which was adequately available in treated effluent. Thus it is demonstrated that the treated effluent improved the plant growth. Crouch *et al.,* (1993) suggested that the liquid fertilizer obtained from seaweed can also be used as liquid fertilizer as it contains high concentration of organic matter and micro and macro elements. Thirumaran, *et al.,* (2009) also reported that seaweed liquid fertilizer applied on *Cyamopsis tetragonoloba* crop increases the growth, yield and soil nutrients. This could be mainly due to the greater nutrient availability of plants through the improvement of soil microorganism populations in the rhizosphere (Mekki *et al.,* 2006). The use of WSP effluent also increased the mineral contents of soil (Agunwamba, 2001). Moreover, in the experimental soil phosphorus concentration was low which was markedly enhanced after the irrigation with treated effluent. It can be argued that dual benefits of using treated effluent were achieved as its application significantly enhanced plant vegetative characteristics and the available phosphorus in the soil would further increase the yield of succeeding crop.

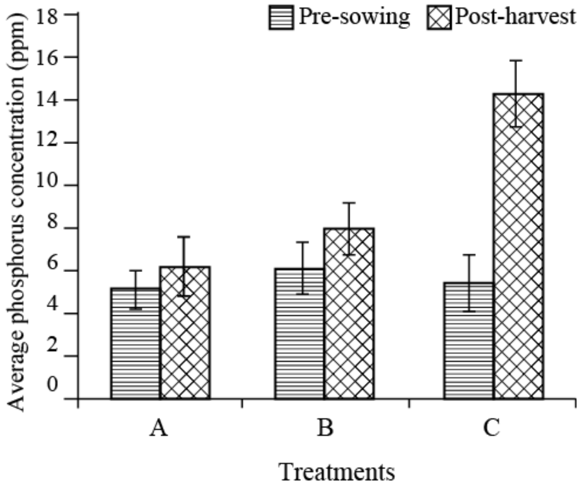

Fig. 1. Average nitrogen concentration in soil samples at pre-sowing and post-harvest.

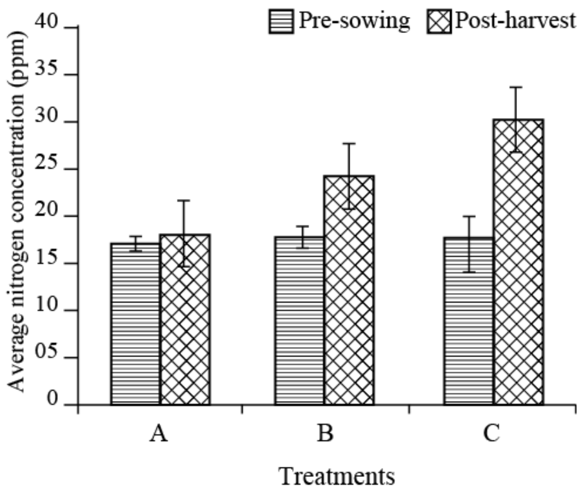

Fig. 2. Average phosphorus concentration in soil samples at pre-sowing and post-harvest.

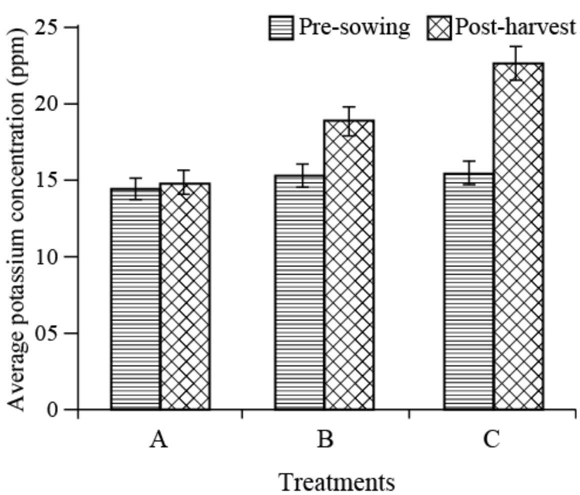

Fig. 3. Average potassium concentration in soil samples at pre-sowing and post-harvest.

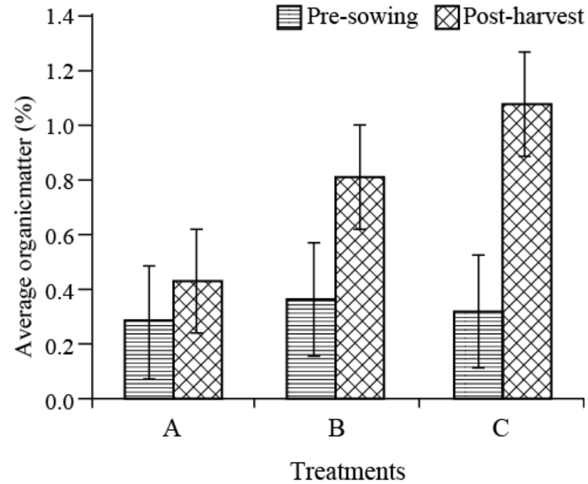

Fig 4. Average organic matter concentration in soil samples at pre-sowing and post-harvest.

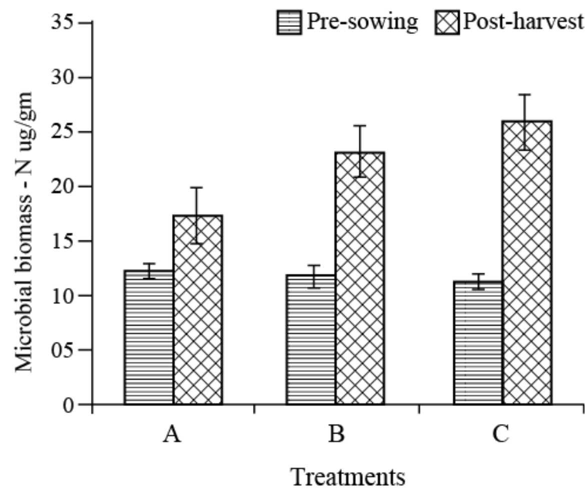

Fig. 5. Average microbial biomass-N in soil samples.

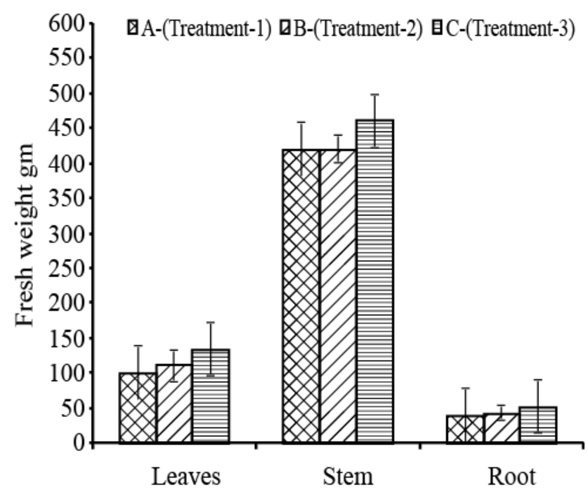

Fig. 6. Fresh weights of leaves, stems and roots of *Cyamopsis tetragonoloba*.

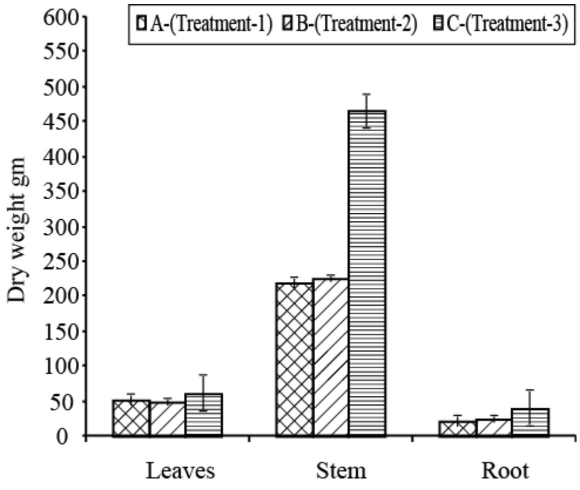

Fig. 7. Dry weights of leaves, stems and roots of *Cyamopsis tetragonoloba*.

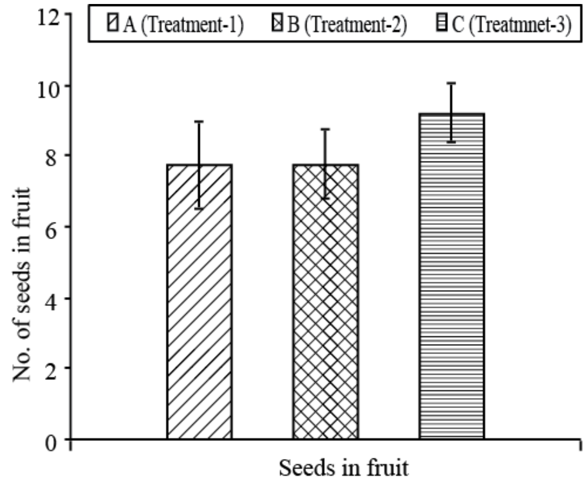

Fig. 9. Number of seeds per fruit of *Cyamopsis tetragonoloba* after 3 treatments.

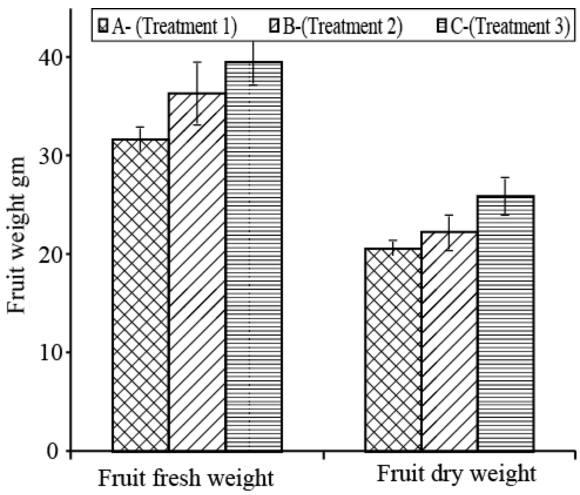

Fig. 8. Fruit weights of *Cyamopsis tetragonoloba* after 3 treatments.

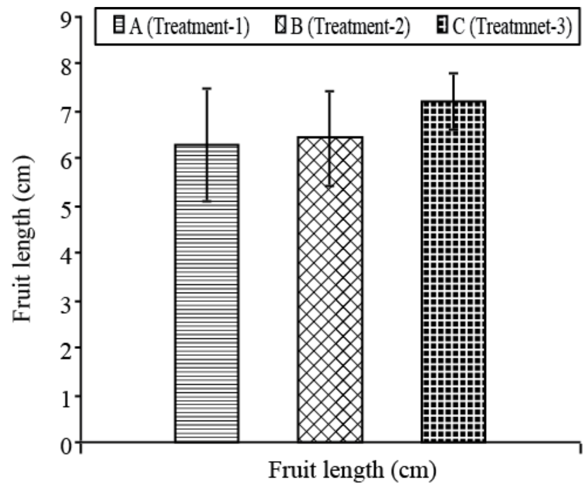

Fig. 10. Fruit length of *Cyamopsis tetragonoloba* after 3 treatments.

Ibrahim *et al.*, (2011) reported increased phosphorus content of the seed after inoculation of *Bradyrhizobium* strain in guar cultivars. Ibrahim *et al*., (2011) also demonstrated that inoculation of *Bradyrhizobium* in guar cultivars significantly increased plant height, fruiting branches/plant, seed weight and oil contents in seeds. However, protein and ash content remained unaffected.

Nutritive characteristics of *Cyamopsis tetragonoloba* crop: Nutritive characteristics of seeds using three treatments are presented in Fig. 10. Among the nutritive characteristics crude proteins and total carbohydrates contents with basal fertilizer (B) and WSP effluent were significantly enhanced ($p<0.05$) compared to controls. Total fat and ash content percentage remained unaltered by the treatments (i.e. Basal fertilizer and WSP effluent). The availability of crude protein in plant is dependant on harvesting. Mahala *et al*., (2012) reported highest concentration of crude protein at 60 days harvesting. However, acid detergent fibre decreased at 45 and 60 days. High content of crude protein is likely to be increased due to the treatment with WSP effluent and the days of harvesting. Shubhara *et al.*, (2004) claimed that phosphorus application increased chlorophyll and sugar contents of *Cyamopsis tetragonoloba*. Shubhara *et al.*, (2004) further reported that gum content is increased after phosphorus application. This may be due to the fact that phosphorus enhances the polysaccharide synthesis in seeds. The WSP effluent contains adequate concentration of phosphorus which could help in improving seed nutritive characteristics (Fig. 11). The present study also revealed increased number of seeds per fruit and fruit length following application of WSP effluent in which appreciable quantity of phosphorus is present. One factor which increases guar seed yield was the row spacing. In the present study 30 cm of row spacing was maintained as recommended by Akhtar (2012) and Sharma *et al.*, (1984). The grain yield of *Cyamopsis tetragonoloba* in arid soil is, however, low because of low availability of nutrients particularly phosphorous. This can be increased by enhancing phosphorus availability in the soil by the application of phosphate fertilizer (Trafdar *et al.*, 1995; Aggarwal & Venkates warlu, 1989). Increased seed yield could be due to the fact that plant used organic phosphorus sources (Trafdar & Classen, 1988; Sharpley, 1985) by the release of phosphatase enzyme produced by the plant roots and available microorganisms in the rhizosphere.

Kathju *et al.*, (1987), reported that *Cyamopsis tetragonoloba* did not respond to phosphate application. They suggested that uniform distribution of precipitation had more favourable influence on plants. However, succeeding crop of *Cyamopsis tetragonoloba* increased its yield.

Available carbohydrates are mainly controlled by genetic and environmental factors (Murwan *et al.,* 2012). Murwan *et al.,* (2012) reported 83.3-87.5% carbohydrate in guar. Selvaraj *et al.*, (2013) proved that total soluble sugar and protein content were increased in *Cyamopsis tetragonolaba* after the application of seaweed powder(*Gracillaria corticata*). The WSP effluent is rich in green algae which not only increase the organic matter of the soil but also helps in reducing toxic contaminants present in domestic effluent if any. The removal of toxicants from the effluent is mainly attributed to bioadsorbant ability of green algae.

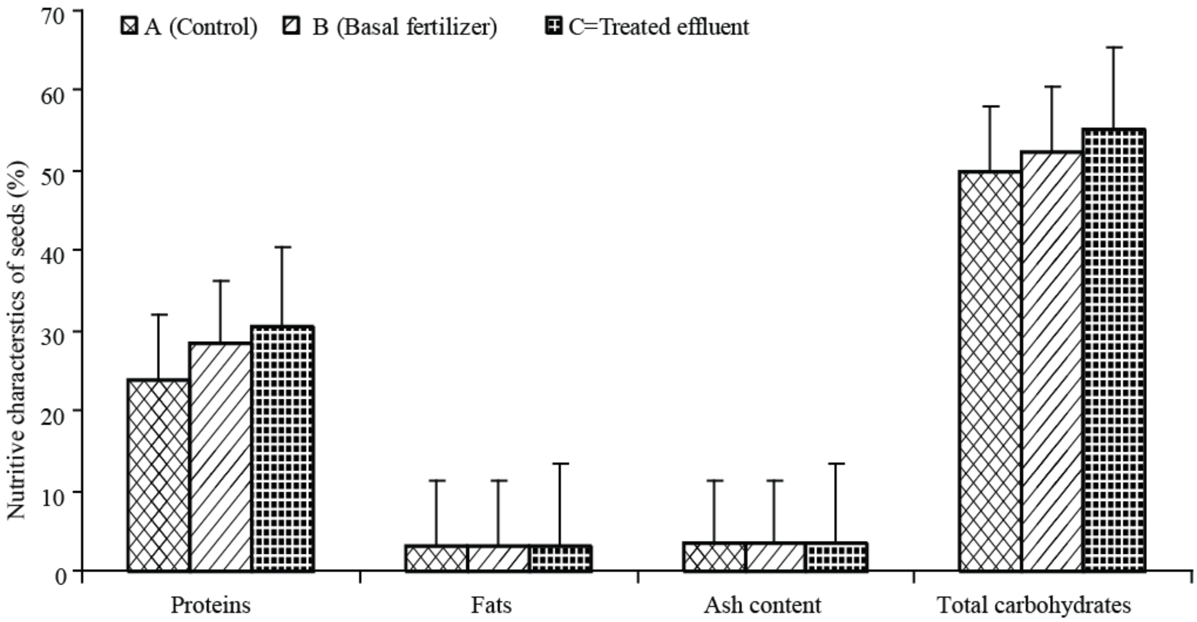

Fig. 11. Nutritive characteristics of seeds of *Cyamopsis tetragonoloba* after 3 treatments.

Conclusions

The study demonstrates that the vegetative and nutritive characteristics of *Cyamopsis tetragonoloba* can be enhanced by the application of treated effluent from WSP as compared to either freshwater or freshwater containing basal fertilizer.The treated wastewater generated through the WSP was shown to be as effective as the inorganic fertilizer and provides good yield of *Cyamopsis tetragonoloba* crop. Since the treated wastewater is sufficiently rich in NPK nutrients, the cost of inorganic fertilizer can be saved. The most important advantage in the use of treated wastewater is that it can avoid environmental problems of discarding it into adjacent water bodies. Thus considerable quantities of fresh water can be saved for human consumption. Besides, the WSP technology can be used for sustainable production of *Cyamopsis tetragonoloba* and other crops.

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