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ORIGINAL RESEARCH

The effect of vermicompost leachate on morphological, physiological and biochemical indices of *Stevia rebaudiana* Bertoni in a soilless culture system

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Abstract
Purpose A comparative study was carried out to assess the impact of vermicompost leachate (VCL) and inorganic fertilizer either alone or in combination on growth and biochemical parameters in *Stevia rebaudiana* (Bertoni) through a soilless culture system.
Methods The cattle manure-based vermicompost was processed by earthworms and the resulting leachate was brewed for 48 h and then collected in the system reservoir for use in soilless culture. An inorganic fertilizer was also prepared by dissolving a water soluble fertilizer in distilled water.
Results Most of the parameters measured were influenced by fertilizer type and growth media. The results indicated that VCL could increase some growth attributes like biomass production, plant height and leaf area, but not as much as what observed in the combined treatment of inorganic fertilizer and VCL. Whereas the carbohydrate content of the leaves did not significantly change in all fertilizer type treated plants, the photosynthetic efficiency and the activities of superoxide dismutase (SOD), (proxidase) POX and (catalase) CAT markedly increased in VCL treated plants.
Conclusion Due to the equivalent effects of both VCL and inorganic fertilizer in the current investigation, it could be suggested that replacing inorganic fertilizer with VCL or developing an equilibrated fertilization strategy that combines the proper ratios of inorganic fertilizer and VCL could be justified for sustainable *Stevia* cultivation.

Keywords Antioxidant activity · Fertilizer · Photosynthetic efficiency · *Stevia rebaudiana* · Sustainable agriculture · Vermicompost leachate

Abbreviations

SOD	Superoxide dismutase
VCL	Vermicompost leachate
POX	Proxidase
CAT	Catalase

Introduction

The global demand for high potency sweeteners is expected to increase. *Stevia rebaudiana* (Bertoni) is a small shrubby perennial herb which belongs to the family of Asteraceae. It grows in sandy soils, up to 65 cm in height and has 2–3 cm long leaves. The leaf extract of Stevia as a natural sweetener with zero calories and proven non-toxic effects on human health, has been used traditionally in many remedial applications (Brandle et al. 1998; Megeji et al. 2005; Andolfi et al. 2006; Earanna 2007; Sic Zlabur et al. 2013; Dushyant et al. 2014). Some sweeteners such as stevioside and rebaudioside are present in concentrations around 3–10 and 1–3 % of the leaf dry matter, respectively. They can be used as a sweetening agent in all foods because they are highly stable at high temperatures and in acid environments. Beside, stevioside has characteristics

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similar to saccharose, but has no calories and no effect on blood sugar, so it is of large industrial and therapeutic value and helpful for diabetes (Farooqi and Sreeramu 2001; Megeji et al. 2005; Andolfi et al. 2006; Earanna 2007; Dushyant et al. 2014; Ramya et al. 2014). The sweetener isolated from the leaves of *Stevia* is up to 300 times sweeter than sucrose (Megeji et al. 2005; Liu et al. 2011; Ramya et al. 2014). Furthermore, the leaf extract of *Stevia* has been reported to contain high levels of antioxidant and antimicrobial activity (Singh et al. 2012; Taleie et al. 2012; Ramya et al. 2014).

Different organic and inorganic cultivation techniques have been used to increase plant yield and quality in *Stevia*. The results showed that organic cultivation improved the root activity and enhanced rate of photosynthesis in the plants (Liu et al. 2011; Ma and Shi 2011; Yang et al. 2013; Dushyant et al. 2014). Application of agro-chemicals since the green revolution of the 1960s enhanced food productivity with the cost of environment. Besides, chemically grown crops have in some instances, caused health problems among consumers. Therefore, the scientific communities are looking for alternatives to replace agro-chemicals (Savci 2012; Rekha et al. 2013). Vermicompost contains the most nutrients in plant—available forms such as nitrate, exchangeable phosphorus, potassium, calcium and magnesium. It is the result of the conversion of organic waste into bio-fertilizers through the action of earthworm. During the process of composting with earthworms, the important plant nutrients, such as nitrogen, potassium, phosphorous and calcium present in field material are transformed into forms which are much more soluble and available to plants than those in the parent compounds (Edwards and Burrows 1988; Reinikainen 1993; Domynguez et al. 1997; Manyuchi et al. 2013; Rekha et al. 2013). Vermiculture is a promising approach to increase soil productivity. Earthworms ameliorate soil fertility and raise crop productivity by excreting beneficial soil microbes and secreting polysaccharides, proteins and other nitrogenous compound into the soil (Ansari 2008; Hatti et al. 2010; Lazcano and Dominguez 2011; Rekha et al. 2013). Vermicompost also contains some plant growth hormones and humic acids which improve the growth and yield of plant crops (Atiyeh et al. 2002). Dushyant et al. (2014) reported that a combined application of vermicompost and bone meal showed the maximum height of plant, the highest number of leaves as well as maximum fresh and dry weight of leaves in *Stevia* among the all treatments. Organic fertilizers positively affect soil structure and improve nutrient availability, resulting in enhanced yield and quality of crops and are also less costly than synthetic fertilizers (Thy and Buntha 2005; Rekha et al. 2013). The final product of vermicomposting has high electrical conductivity (EC), which results in enhanced soil salinity with sequential utilization. To reduce EC, VCL and vermivash have been developed (Gutierrez-Miceli et al. 2007; Lazcano and Dominguez 2011; Ayyobi et al. 2013, 2014). VCL as a bio-fertilizer is a liquid nutrient collected after transmission of water through a mass of vermicompost which is a collection of excretory products of earthworms that are very useful for plant growth (Hatti et al. 2010; Nath and Singh 2012; Quaik et al. 2012; Samadhiya et al. 2013; Rekha et al. 2013; Ayyobi et al. 2014). Several epithets such as vermiwash, vermicomposting leachate, vermi-leachate, worm bed leachate and worm tea have been used to describe the liquid derived from the vermicomposting process (Quaik and Ibrahim 2013). Use of VCL as a liquid fertilizer provides the advantage of homogeneity when applied to growth media as compared to application of solid fertilizer (Quaik et al. 2012). A significant increase in the growth of vermiwash treated plants could be due to the proper ratio of macro and micronutrients available in the vermiwash (Hatti et al. 2010). Arthur et al. (2012) reported that VCL could serve as a potential substitute for P and K deficiency. Govindarajan and Prabakaran (2012) have also reported the potent antimicrobial activity of vermiwash. Greenhouse and field investigations have been achieved in terms of the effects of VCL on French dwarf bean (Ayyobi et al. 2014), peppermint (Ayyobi et al. 2013), maize (Carlos et al. 2008; More et al. 2013), kharif crops (Nath and Singh 2012), tomato (Arthur et al. 2012; Allahyari et al. 2014; Marquez-Quiroz et al. 2014), pak choi (*Brassica rapa* Cv Bonsai, Chinensis group) (Pant et al. 2011), lemongrass (Leon-Anzueto et al. 2011), black gram (Rekha et al. 2013) spinach, onion, potato (Ansari 2008), certain summer vegetable crops (Nath and Singh 2009), mango (Sathe and Patil 2014), cow pea and rice (Rajan and Murugesan 2012). Most of these studies confirmed that VCL positively influenced plant yield and quality, resulting in enhanced crop production. The growth promoting effects of vermiwash on the germination of some vegetable crops have also been evaluated (Fathima and Sekar 2014).

The VCL is increasingly being famous among farmers as it is efficient in raising the productivity of crops for both hydroponic systems and foliar sprays. Therefore, its efficiency in improving *Stevia* production needs to be evaluated. Considering the above facts and limited number of studies on VCL as a liquid fertilizer and its potential in hydroponic culture systems, this investigation was undertaken to assess the efficiency of VCL as a nutrient supplement in *Stevia* plants. The objective of this experiment was to increase *Stevia* productivity using of an organic nutrient source in a suitable soilless culture.

Materials and methods

Plant preparation and treatment procedure

The investigation was conducted from 14 May 2014 to 16 August 2014 at the experimental greenhouse and tissue culture laboratory of the agricultural college, Isfahan University of Technology, Isfahan, Iran. Plantlets of *S. rebaudiana* (Bertoni) obtained from in vitro micro-propagated and grown cultures were used as the source of plant materials. The experiment comprised of 9 treatments with the application of VCL and inorganic fertilizer (water soluble fertilizer, a product of melspring Holland—www.melspring.com) (Table 1) either alone or in combination, used on different soilless culture materials namely perlite, coco peat and perlite + coco peat (1:1). The treatments consisted of 9 fertilizer type: F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate.

An open aggregate hydroponic system, namely container culture was established using PVC pipes 9 cm in diameter containing growing beds which were stranded in lines on the greenhouse floor horizontally and they were sealed at both ends after being filled with the above mentioned growth media. Holes were made in the upper surface of each pipe for the introduction of transplants of *Stevia* and two small slits were made on each side for drainage or leaching. Some moisture was also introduced into each pipe before planting. Drip irrigation of the nutrient mixes with a capillary tube leading from the main supply line to each plant was adopted. This open aggregate hydroponic system permitted accurate and uniform delivery of nutrient solutions.

The experiment consisted of a completely randomized design with nine treatments in which the sampling was done after a 15 day interval. Drainage from the beds was tested frequently during the experiment. Each PVC pipe holding 6 plants served as one treatment in which each treatment was replicated three times; therefore, the hydroponic system being used to conduct the experiment consisted of 27 PVC pipes in total.

Table 1 Characteristic of vermicompost leachate obtained from cattle manure, compared with inorganic fertilizer

Characteristic	Vermicompost leachate	Inorganic fertilizer (www.melspring.com)
pH	7.56	7.5
Electrical conductivity (ds m^{-1})	5.42	–
N (%)	1.3	20
P_2O_5 (%)	0.7	20
K_2O (%)	0.9	20
Cu (%)	0.05	0.01
Zn (%)	0.09	0.01
Mn (%)	0.18	0.1

These values are subjected to variations depending on the cattle feeding

Vermicompost leachate preparation and characterization

Vermicompost (VC) and vermicompost leachate (VCL) were prepared according to the method recommended by Ayyobi et al. (2013). The cattle manure-based vermicompost was processed by Earthworms (*Eisenia fetida*) at a rate of 25 g earthworms per 1 kg of cattle manure and vermicomposted for 2 months. The vermicompost (100 kg) was flushed with 50 l of water and leachate (vermiwash) brewed for 48 h and then collected in the system reservoir. An inorganic fertilizer was prepared by mixing 2 g of water soluble fertilizer a product of melspring Holland (www.melspring.com) to each 1 l of distilled water. Table 1 shows the characteristics of vermicompost leachate derived from cattle manure and inorganic fertilizer used in the experiment.

Analysis of growth parameters

The plants from each treatment were uprooted carefully and then shoot height was measured in cm, leaf area was measured in cm^2. Plants were also washed with tap water to remove adhering foreign particles and fresh mass of shoots and roots were recorded after surface drying the shoots and roots. The shoots and the roots were dried in an oven at 80 °C for 48 h till constant dry weight was achieved. The sampling was done after 15 day intervals followed by fertilizer treatments except the shoot and root fresh and dry weights which were measured just 60 days after treatment. Some growth and biochemical parameters such as chlorophyll, protein, carbohydrate and antioxidant enzymes were determined in third and fourth leaf from the top according to Patil (2010). The leaves were harvested and frozen in liquid N_2 and kept at −80 °C until being used for biochemical and physiological measurements.

Carbohydrate content assay

The amount of carbohydrate present in the samples was estimated using the anthrone method following the procedure used by Nazarli and Faraji (2011). Therefore, to measure the content of carbohydrate, 0.5 g of dry leaves was ground in a mortar and pestle with 5 ml of 95 % ethanol, the solution was filtered by Whatman filter paper. The residues in the filter paper were washed with 95 % ethanol and ground again. After filtering, all filtrates were mixed and residues were discarded. Centrifugation was achieved at 3000 RPM for 15 min to obtain a clear supernatant. Then 1 ml of alcoholic extract preserved in the refrigerator was mixed with 3 ml anthrone reagent (150 mg anthrone, 100 ml of 72 % sulfuric acid, W/W). The samples were placed in boiling water bath for 10 min and the optical density was taken at 625 NM against the reagent blank. Content of soluble carbohydrate was determined using glucose standard and expressed as mg g^{-1} DW of leaves.

Total soluble protein content assay

The content of soluble protein was assayed from the leaf sample based on the color change (red to blue) of the dye coomassie Brilliant Blue G250 ($C_{47}H_{48}N_3NaO_7S_2$) upon binding to added protein following the method of Bradford (1976) and expressed as mg g^{-1} fresh weight. The leaf sample of 0.5 g was macerated with 10 ml of phosphate buffer (0.1 M, pH 7.0). The extract was centrifuged at 10,000 RPM at 4 °C for 20 min. 0.1 ml of supernatant was taken and 5 ml of dye mixture was added and used for sample analysis. The solution was mixed well and kept aside for 15 min. The color intensity was recorded at 595 nm optical density. For dye mixture preparation, 100 mg of coomassie brilliant blue (G 250) was dissolved in 50 ml of 95 % ethanol and 100 ml of ortho phosphoric acid was added and made up to 200 ml using distilled water. 1 ml of dye solution was taken and 4 ml of distilled water was added and this was used for sample analysis.

Chlorophyll fluorescence and chlorophyll measurements

Chlorophyll fluorescence was measured using a fluorometer (Walz, Effeltrich, Germany). The photochemical efficiency of PSII was calculated as the ratio Fv/Fm for each segment. The relative chlorophyll (Chl) content was measured with a portable leaf chlorophyll meter (SPAD 502, Minolta *Co*., Osaka, Japan).

Antioxidant enzymes assay

Frozen-leaf samples (0.2 g) were ground in liquid nitrogen and stored at −80 °C until assay. The enzyme extract for superoxide dismutase (SOD), proxidase (POX) and catalase (CAT) was prepared by mixing frozen samples with 2 ml extraction buffer containing 0.1 M potassium phosphate buffer, pH 7.5 and 0.5 mM ethylenediamintetra acetic acid (EDTA). The extract was centrifuged for 20 min at 12,000×*g* and 4 °C. Then the supernatant was used for enzymatic assay. Assay of SOD activity (expressed as unit per milligram of protein) was based on reduction of nitroblue tetrazolium (NBT) according to the method used by Padmaja et al. (2011). A complete reaction mixture contained 1 ml of the 125 mM sodium carbonate, 0.4 ml of 25 μM NBT and 0.2 ml of 0.1 mM EDTA added to 0.5 ml of plant extract. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride and the absorbance was read at 560 nm using a spectrophotometer at 5 min intervals. Units of SOD were expressed as the amount of enzyme required for inhibiting the reduction of NBT by 50 %. Catalase activity was measured by the titrimetric method applied by Padmaja et al. (2011). The reaction mixture comprised of 5 ml of 300 μM phosphate buffer (pH 6.8) containing 100 μM hydrogen peroxide (H_2O_2) and 1 ml of plant extract was prepared and left at 25 °C for 1 min. The reaction was stopped by adding 10 ml of 2 % sulfuric acid and residual H_2O_2 as titrated with potassium permanganate (0.01 N) till pink color was obtained. Enzyme activity was measured by calculating the decomposition of μM H_2O_2 per min per mg protein. Assay of peroxidase was also achieved according to the method used by Padmaja et al. (2011). 3.5 ml of phosphate buffer (pH 6.5) was taken into a clean, dry cuvette, 0.2 ml of plant extract and 0.1 ml of freshly prepared O-dianisidine solution were added to it at 28–30 °C and absorbance was recorded at 430 nm. Then 0.2 ml of 0.2 mM H_2O_2 was added and mixed and then the absorbance was read at every 30 s intervals up to 3 min. A graph was plotted with increase in absorbance against time. The enzyme activity was expressed per unit time per mg of protein.

Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses were carried out with SAS and MSTAT-C computer programs. The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by LSD test. Differences at $p \leq 0.05$ were considered as significant.

Results and discussion

Although several researchers have studied the effect of VCL on some crops (Ansari 2008; Carlos et al. 2008; Nath and Singh 2009; Singh et al. 2010; Gutiérrez-Miceli et al. 2011; Leon-Anzueto et al. 2011; Pant et al. 2011; Arthur et al. 2012; Nath and Singh 2012; Rajan and Murugesan 2012; Abduli et al. 2013; Ayyobi et al. 2013; More et al. 2013; Rekha et al. 2013; Allahyari et al. 2014; Ayyobi et al. 2014; Marquez-Quiroz et al. 2014; Avila-Juarez et al. 2015), our study is the first to evaluate VCL as a nutrient source for *Stevia*. Overall, the morphological attributes of *Stevia* such as plant height, leaf area, shoot and root fresh and dry weight were higher after 30, 45 and 60 days, in the combined application of VCL and inorganic fertilizer containing F_7, F_8 and F_9 treatments compared with inorganic fertilizer or VCL, separately (Figs. 1, 2, 3, 4, 5, 6). Among the treatments, the application of sole VCL (F_4, F_5 and F_6 treatments) showed the minimum plant height (Fig. 1). The combination of inorganic fertilizer and VCL further enhanced plant height relative to their application, separately. Our results agree with those obtained by Parthasarathi et al. (2008) who found that supplementation of N, P and K with vermicompost enhanced the growth and yield in black gram (*Vigna mungo*). Under the organic cultivation system the yield is 10–30 % lower than the yield achieved by conventional farming (Stanhill 1990), but the lower productivity can be offset by the higher price. However, there was a significant interaction effect of fertilizer type and growth media in which higher plant height was obtained with the mixture of perlite and coco peat relative to their application alone (Fig. 1). Pant et al. (2011) also found that there was a significant interaction between vermicompost tea and growth media on *Brassica rapa* cv. Bonsai. The effect of fertilizer treatments in terms of leaf area followed a trend similar to plant height across the growth media (Fig. 2). The higher leaf area was recorded with the combined treatment of VCL and inorganic fertilizer in the mixture of perlite and coco peat (Fig. 2). These results agree with those reported by Patil (2010). Singh et al. (2010) reported that some morphological attributes such as plant height and leaf area was improved by application of vermicompost leachate in strawberry. However, Ayyobi et al. (2013) reported that differences between peppermint plants treated with organic fertilizer was not significant for leaf area index and shoot dry weight. In the current investigation, the leaf area of VCL treated plants grown in the mixture of perlite and coco peat (F_6 treatment) after 60 days showed the highest record (12.87 cm^2) among those fertilized with inorganic fertilizer and VCL, separately. However, there was no significant difference between that of F_6 treatment and the results obtained from the combination of VCL and inorganic fertilizer treatments across the all growth media (Fig. 2). The effect of fertilizer types, growth media and their interaction on shoot and root fresh and dry weight showed a similar tendency to the results obtained in terms of plant height and leaf area. The biomass (shoot and root fresh and dry weights) was significantly higher with the plants treated with the combined application of inorganic fertilizer and VCL across the all growth media (Figs. 3, 4). Patil (2010) also found similar trends as total fresh biomass production was highest with combined application of biofertilizer when compared to the sole application. The highest increases in shoot dry weight (52.11 g) due to VCL and the inorganic fertilizer mixture were observed with the combined perlite and coco peat (F9 treatment), while VCL and inorganic fertilizer exhibited no significant difference

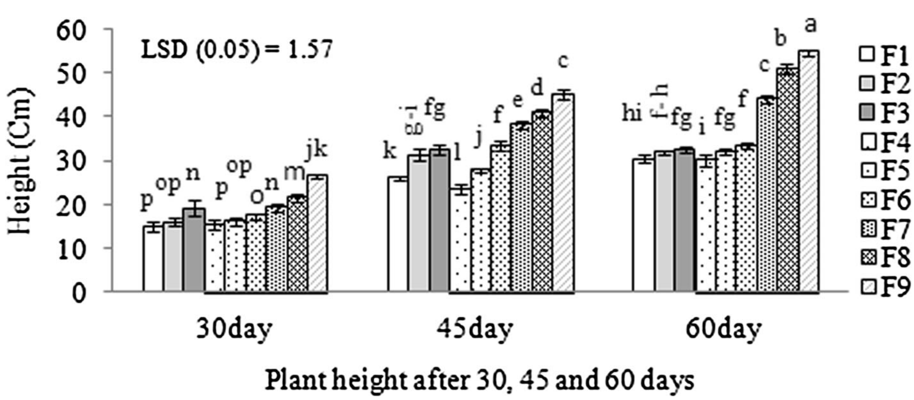

Fig. 1 Effect of fertilizes on plant height of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

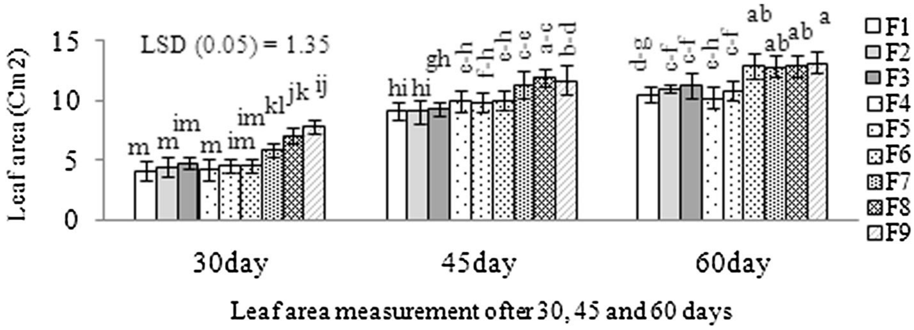

Fig. 2 Effect of fertilizes on leaf area of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

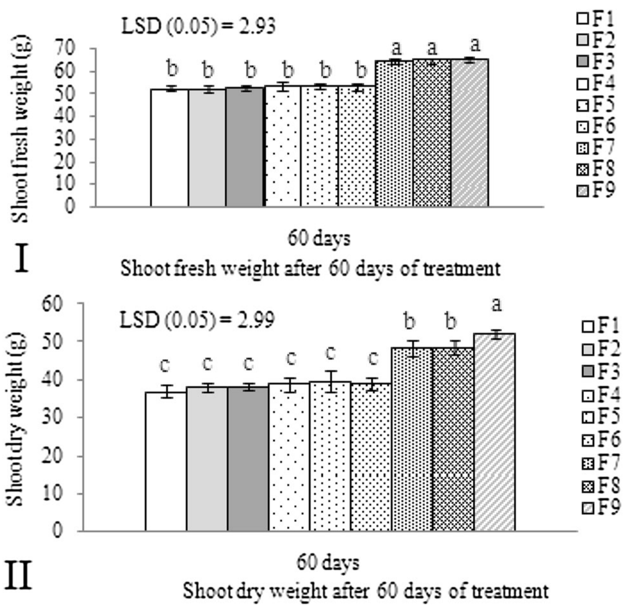

Fig. 3 Effect of fertilizes on shoot fresh (I) and dry weight (II) of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

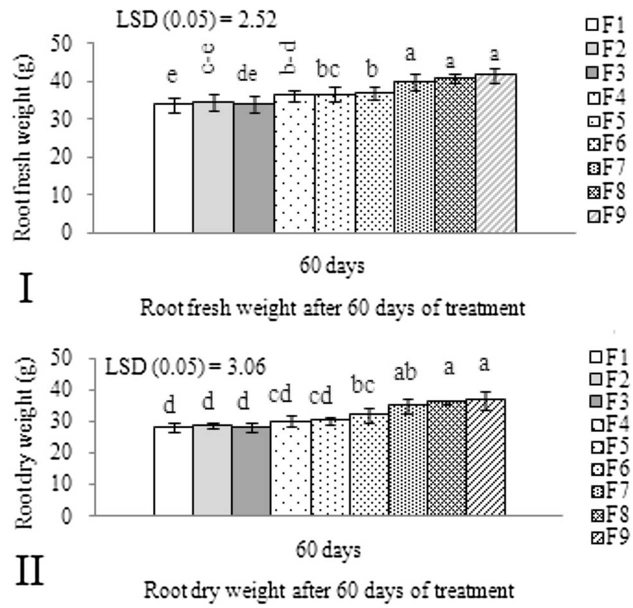

Fig. 4 Effect of fertilizes on root fresh (I) and dry weight (II) of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

among treatments across the media separately (Figs. 3, 4). This result was also in line with results of Liu et al. (2011) who used organic manure and inorganic fertilizer on *S. rebaudiana.* VCL could improve the plant biomass by supplementing plant nutrients and producing growth hormones (Liu et al. 2011). Pant et al. (2011) asserted that all vermicompost extracts, regardless of extraction methods, provided similar effect on plant growth and nutrient concentration in the peat–perlite medium.

In this investigation, application of VCL resulted in an almost equal carbohydrate content as that of inorganic fertilization, as the addition of VCL to the growth media of *Stevia* produced no significant difference in carbohydrate content of the leaves at all intervals compared to inorganic

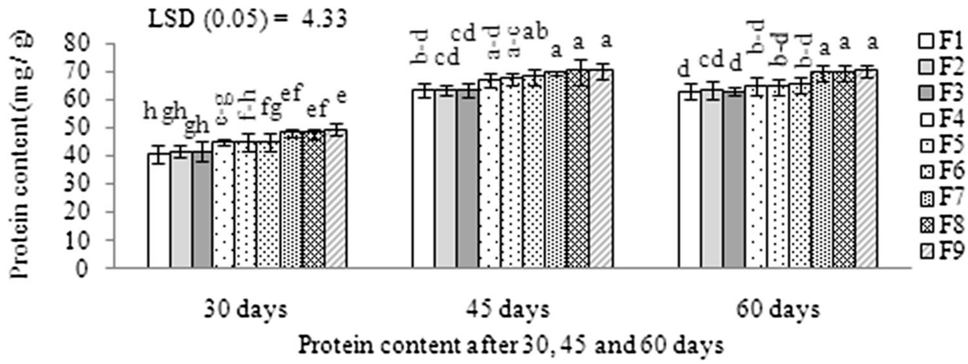

Fig. 5 Effect of fertilizes on protein content (mg/g) in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

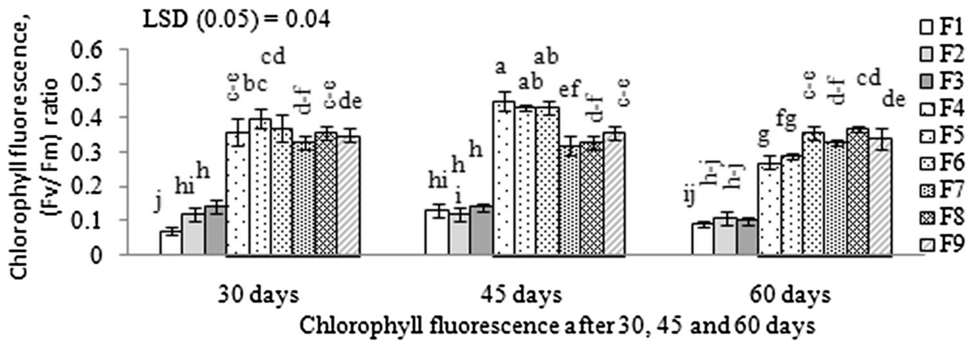

Fig. 6 Effect of fertilizes on photosynthetic efficiency in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

fertilizer (data not shown). Patil (2010) obtained the highest content of carbohydrate of *Stevia* plants with the combination of NPK (inorganic fertilizer) and vermicompost. Table 2 indicates the positive correlation between carbohydrate content and biomass production of *Stevia* (shoots and root fresh and dry weight). For all growth media, addition of VCL enhanced the content of protein more than inorganic fertilizer, although an insignificant decrease was observed after 60 days of the experiment (Fig. 5). This was similar to findings by Patil (2010) in which the protein content of *Stevia* in the middle phase of life cycle showed the highest amount. A close link between carbon metabolism and protein synthesis is necessary to prevent acute carbon starvation in growing tissues where most of protein synthesis contributes to building the new biomass (Smith and Stitt 2007; Piques et al. 2009). VCL may be effective in strengthening this link as the positive correlation (0.75**) between carbohydrate and protein content in the current investigation in Stevia plants (Table 2) proved this hypothesis. The use of VCL in growth media also enhanced significantly the photosynthetic efficiency of leaves compared to inorganic fertilizer and the highest ratios (0.45, 0.43 and 0.43) after 45 days were recorded, respectively, with F_4, F_5 and F_6 treatments (Fig. 6). This result was also corroborated with works of Liu et al. (2011) who reported that organic manure enhanced the rate of photosynthesis in terms of *Stevia* plants. Addition of combined treatments of inorganic fertilizer and VCL produced a significant increase in chlorophyll content after 45 and 60 days of the experiment as the highest increases in chlorophyll content was recorded with F_8 and F_9 treatments at these intervals (Fig. 7). Our results agree with those obtained by Patil (2010) who obtained the highest protein content of *Stevia* plants in the combined treatment of NPK (inorganic fertilizer) and vermicompost.

Table 2 Correlation of studied parameters in *S. rebaudiana* (Bertoni) grown on a soilless culture system using VCL and inorganic fertilizer

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Height	1	0.88**	0.83**	0.82^{ns}	0.82**	0.81**	0.76**	0.82**	0.18^{ns}	0.55**	0.81**	0.81**	0.73**
2. Leaf area		1	0.86**	0.88**	0.84**	0.84**	0.84**	0.91**	0.16^{ns}	0.57**	0.83**	0.88**	0.81**
3. Shoot fresh weight			1	0.98**	0.99**	0.97**	0.96**	0.74**	-0.02^{ns}	0.22*	0.72**	0.91**	0.89**
4. Root fresh weight				1	0.97**	0.98**	0.96**	0.77**	0.03^{ns}	0.25*	0.74**	0.93**	0.91**
5. Shoot dry weight					1	0.98**	0.95**	0.71**	-0.003^{ns}	0.17^{ns}	0.70**	0.91**	0.89**
6. Root dry weight						1	0.96**	0.71^{ns}	0.06^{ns}	0.17^{ns}	0.69**	0.92**	0.90**
7. Carbohydrate content							1	0.75**	0.02^{ns}	0.19*	0.73**	0.93**	0.91**
8. Protein content								1	0.21^{ns}	0.75**	0.89**	0.81**	0.73^{ns}
9. Chlorophyll fluorescence									1	0.2^{ns}	0.32**	0.30**	0.34**
10. Chlorophyll content										1	0.63**	0.33**	0.20*
11. Catalase activity											1	0.81**	0.75**
12. Peroxidas activity												1	0.96**
13. Superoxide dismutase activity													1

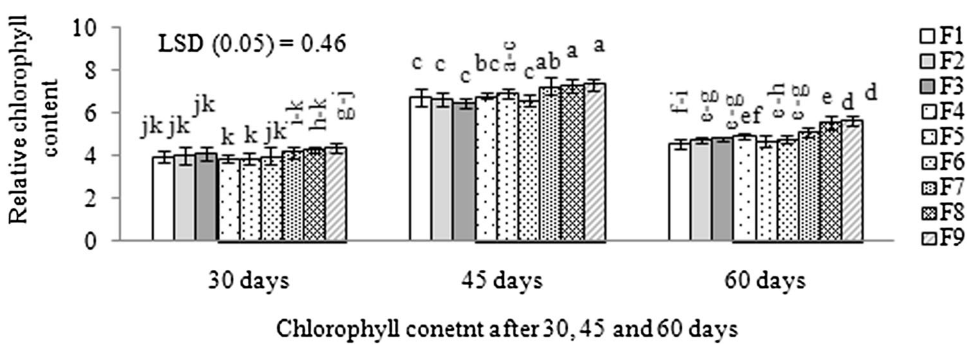

Fig. 7 Effect of fertilizes on chlorophyll content in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

Generally, combined treatments of VCL and inorganic fertilizer at 3:1 ratio had beneficial effects on plant growth and improved quality of *Stevia*. These results indicate that better plant growth with the lower doses of inorganic fertilizer (25 %) and the higher amount of VCL (75 %) can be obtained compared to each fertilizer type separately. Contrary to this, More et al. (2013) suggested that 75 % N through inorganic fertilizer +25 % N through bio-compost could be adopted to minimize the cost of production along with sustained corn yield.

In this study, the activity levels of several antioxidant enzymes were also assessed during the experiment. The results of the assays showed that fertilizing with VCL led to significant changes in the antioxidant status of *Stevia*. The response of *Stevia* with VCL showed better results than with inorganic fertilizer in terms of CAT activity (Fig. 8). The effect of sole VCL, as well as combination treatments significantly improved POX activity of *Stevia* at all intervals (Fig. 9). Among all the treatments, the best results were shown after 60 days by the combinations of VCL and inorganic fertilizer across all the media (Fig. 9). At this interval (60 days of treatments), the plants under treatment F_6 (VCL across the mixture of perlit and coco peat) had no significant difference compared to the combined treatments of VCL and inorganic fertilizer (F7, F8 and F9 treatments) (Fig. 9). Ma and Shi (2011) asserted that potassium is an element essential to the proficiency of multifold plant enzyme functions. In the current study, the catalyst effect of VCL on enzyme activities of *Stevia* might be due to the high potassium content of this bio-fertilizer.

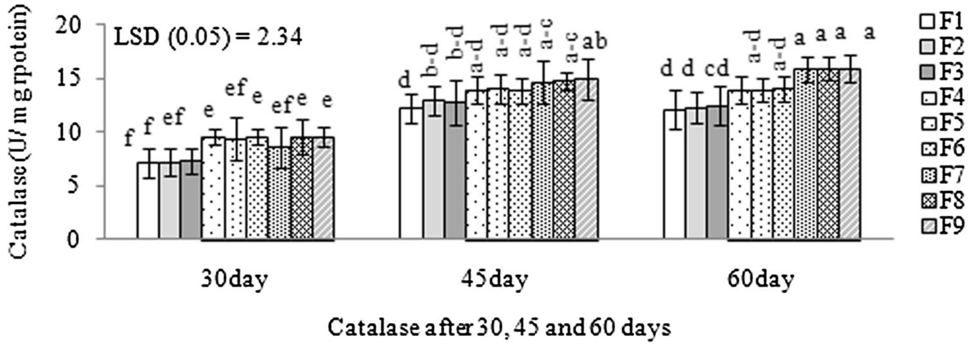

Fig. 8 Effect of fertilizes on catalase activity in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

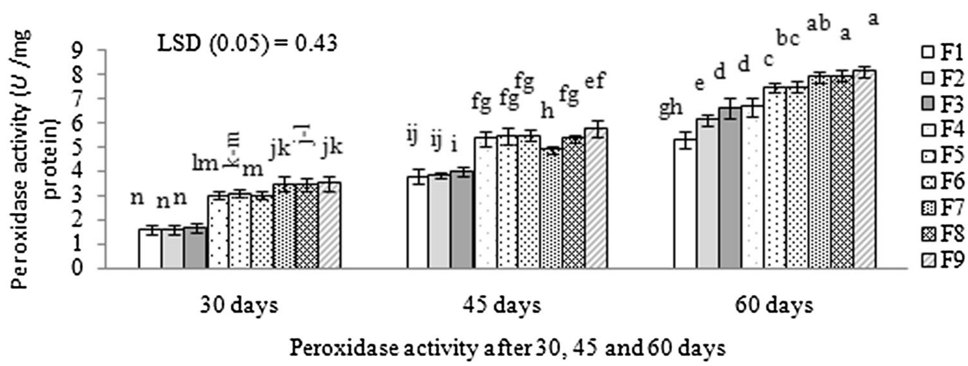

Fig. 9 Effect of fertilizes on peroxidase activity in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

The plants treated with F4, F5, F6, F7, F8 and F9 showed significantly higher SOD activity compared with other treatments at all intervals, although no significant difference was observed among these treatments (Fig. 10). The plants treated with sole inorganic fertilizer (F1, F2 and F3 treatments) had lower SOD activity than the sole VCL and the combined VCL and inorganic fertilizer (Fig. 10). As shown in Table 2, there was a significant positive correlation between enzyme activities and chlorophyll fluorescence, which could be concluded that VCL enhanced antioxidant activities in *Stevia* plants. In this investigation, organic source of fertilizer in the form of VCL was concluded to be effective and comparable with the chemical source of fertilizers in improving the growth of *Stevia*. Using organic manure in growing *Stevia* plants, Liu et al. (2011) asserted that lower growth rate of the organic manure cultivation than the inorganic fertilizer cultivation in the early growth stage was due to nutrient deficiency caused by the organic manure in which it has not been fully decomposed. The current results also showed that the problem of initial nutrient deficiency has been solved using vermicompost leachate.

From this investigation, it could be concluded that the use of VCL and inorganic fertilizer at 3:1 ratio was proved to be the best for improving some growth attributes like plant height, leaf area, and biomass production (shoot and rood fresh and dry weight), but in terms of carbohydrate, protein and chlorophyll contents, photosynthetic efficiency and antioxidant activity, the results indicated no significant differences between the sole VCL and the combination treatments of VCL and inorganic fertilizer. Avila-Juarez et al. (2015) similarly asserted that VCL had no effect on the physiological parameters of tomato. In some cases like photosynthetic efficiency and POX activity the plants treated with VCL showed a significant improvement compared to sole inorganic fertilizer.

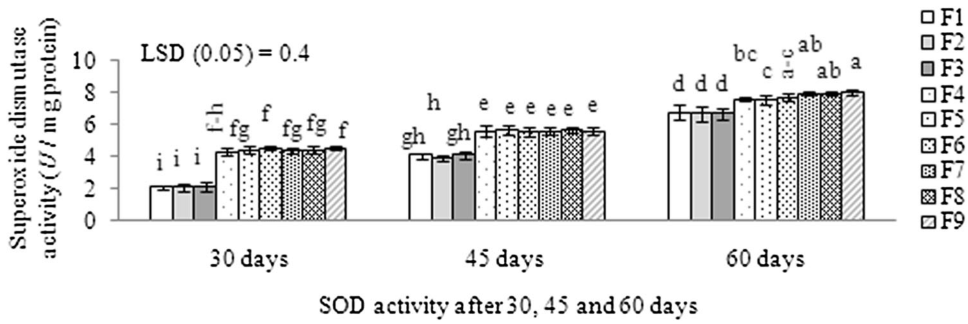

Fig. 10 Effect of fertilizes on superoxide dismutase activity in leaves of *S. rebaudiana*. *Bars* showing the same letter are not significantly different at $p \leq 0.05$ as determined by LSD test. F_1 = perlite + inorganic fertilizer; F_2 = coco peat + inorganic fertilizer; F_3 = a 1:1 mixture of perlite: coco peat + inorganic fertilizer; F_4 = perlite + vermicompost leachate; F5 = coco peat + vermicompost leachate; F_6 = a 1:1 mixture of perlite: coco peat + vermicompost leschate; F_7 = perlite + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_8 = coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate; F_9 = a 1:1 mixture of perlite: coco peat + a 1:3 mixture of inorganic fertilizer: vermicompost leachate

Conclusion

Replacement of the inorganic fertilizer with VCL in the growing beds of *Stevia* by adding appropriate quantities produced a significant improvement in plant growth and development. Due to the similar effects of both VCL and inorganic fertilizer in this investigation, it could be suggested that replacing inorganic fertilizer with VCL or developing an equilibrated fertilization strategy that combines the proper ratios of VCL and inorganic fertilizer (3:1) could be justified in *Stevia* cultivation systems.

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