



Biochemical, physiological, and yield responses of lady's finger (*Abelmoschus esculentus* L.) grown on varying ratios of municipal solid waste vermicompost

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Abstract

Purpose In the present study, effect of earthworm-processed MSW was seen on biochemical, physiological, and yield responses of *Abelmoschus esculentus* L.

Methods Plants were grown on different amendment ratios of municipal solid waste vermicompost (MSWVC). Pot experiments were conducted by mixing MSWVC at 0, 20, 40, 60, 80, and 100% ratios to the agricultural soil.

Results An increase in photosynthetic rate and stomatal conductance of plants grown at 20 and 40% MSWVC amendment ratios was observed. Total chlorophyll, carotenoid, and protein contents also increased significantly in 20, 40, and 60% amendment ratios at 65 days after germination (DAG). Likewise, proline, peroxidase, and lipid peroxidation increased with increasing levels of MSWVC at both 45 and 65 DAG.

Conclusion The study suggests that MSWVC could be used as organic amendment in soil depicted by good yield and anti-oxidative response of lady's finger (*A. esculentus*) at different amendments of MSWVC (up to 60% w/w ratios). Furthermore, agricultural utilization of MSWVC will help in managing dreadful effects of the burgeoning amount of organic solid waste.

Keywords Municipal solid waste · Vermicompost · *Abelmoschus esculentus* L. · Heavy metals · Physiology

Introduction

Management of the burgeoning amount of organic solid waste is a challenging problem in the contemporary scenario around the globe. The improper handling and unscientific disposal of organic waste has led to countless problems posing a threat to the ecosystems (soil, air, and water) and environmental sustainability (Vergara and Tchobanoglous 2012, Srivastava et al. 2015, 2016). Therefore, it is imperative to find a solution to this problem that would not only focus on managing its quantity, but will also help in sustaining

the environment with social acceptability. Organic wastes constitute major fraction of municipal solid waste (MSW) in most of the developing countries. Thus, land application of organic fraction of MSW offers a good option for waste management and its recycling. For example, land application of organic waste such as sewage sludge is a common practice nowadays (Singh and Agrawal 2008; Lee et al. 2018; Sharma et al. 2018). However, there is always a potential threat as it may contain various toxic heavy metals, organic compounds such as antibiotics, pharmaceuticals and personal care products (PPCPs), and pathogens in traces (Bibby and Peccia 2013, Kang et al. 2013, Prosser and Sibley 2015). The prolonged use of sewage sludge or organic wastes in agricultural field may result in heavy metal accumulation in soil that can be taken up by the crops which may cause many health issues when transferred to higher trophic levels (Srivastava et al. 2017). Therefore, composting of organic wastes/biosolids is a more interesting option for recycling of wastes. Nowadays, composting/vermicomposting of organic fraction of municipal solid waste is gaining attention among workers as it decreases the stabilization time of organic wastes (Fernández et al. 2014, Weber et al. 2014). The quality of the

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compost largely depends on factors such as feedstock source, presence of toxic contaminants/heavy metals, stabilization time, and composting design (Hargreaves et al. 2008, Srivastava et al. 2016).

Cherif et al. (2009) examined the effect of municipal solid waste compost, MSWC (40 and 80 Mg ha⁻¹), on wheat growth and noticed a significant increase of grain yield in both the amendments (58.96 and 60.21 Mg ha⁻¹, respectively) compared to the control (17.65 Mg ha⁻¹). Based on the treatment effectiveness index, 40 Mg ha⁻¹ of MSWC was recommended for agricultural practices. Similarly, agricultural application of MSWC and its effects on the yield of various crops has been reported in various studies such as winter squash (Warman et al. 2009), lettuce (Fagnano et al. 2011), wheat (Lakhdar et al. 2011), and spring triticale (Weber et al. 2014). Though the agricultural application of MSWC has immense potential for recycling of such type of organic solid waste; however, the presence of heavy metals and other pollutants in MSWC has always been a matter of concern (Singh and Kalamdhad 2013, Alvarenga et al. 2015).

Hence, there is a growing interest in vermicomposting technology which has emerged as a new biotechnological tool for recycling of different kind of organic wastes through the action of earthworms (Wu et al. 2014, Lim et al. 2016). Both MSW compost (MSWC) and vermicompost have many advantages when compared to inorganic fertilizers which badly affect the soil's physico-chemical and microbial properties (Srivastava et al. 2016). Agricultural utilization of MSW compost/vermicompost (VC) ameliorates the soil's nutrient profile, texture, water holding capacity, buffering capacity, soil microbial response, etc. (Weber et al. 2014, Bouzaiane et al. 2014). MSWC/VC has good organic matter content, nitrogen, phosphorus, and humic substances which are important for maintaining the soil quality (Hargreaves et al. 2008). Moreover, it improves the activity of different soil enzymes such as dehydrogenase, urease, phosphatase, protease, phosphodiesterase, arylsulphatase, etc. (Perucci 1990, Bhattacharyya et al. 2003). According to Sim and Wu (2010), it has been demonstrated that through vermicomposting, MSW could be sustainably transformed into an organic fertilizer known as vermicompost that provides great benefits to the agricultural soil and plants. Atiyeh et al. (2001) studied the effect of earthworm-processed pig manure on germination, growth, and yield response of *Lycopersicon esculentum* under greenhouse environment. Plants were grown in the standard potting medium amended with various ratios (0–100%) of pig manure vermicompost (VC). Germination rates were increased significantly by 20–40% in the VC amended soil than the control. Similarly, tomato seedlings grown in 50% VC had greater number of leaves and biomass in comparison to control. However, a sharp decline in growth, number of leaves, and biomass was

noticed in 100% vermicompost and the largest marketable yield was noticed in 20% pig manure vermicompost.

Plenty of literature are available on the effect of organic waste vermicompost on growth and yield of different plants (Atiyeh et al. 2001, Arancon et al. 2004, Lim et al. 2015, Sangwan et al. 2010). However, studies on antioxidative response of plants grown on different organic waste vermicompost are still in its infancy. Organic waste vermicompost may contain significant amount of trace metals which could pose phytotoxicity (Gupta and Garg 2008, Roodbergen et al. 2008, Mohee and Soobhany 2014, Atiyeh et al. 2000). Therefore, the present work was aimed to assess the biochemical, physiological, and yield responses of lady's finger (*Abelmoschus esculentus* L.) grown on soil amendment with different ratios of MSW vermicompost. Also, the potential of MSWVC application in agricultural practices was comprehensively evaluated.

Materials and methods

Study area

The experiments were performed at the experimental field of Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, situated in the eastern Gangetic plain of the Indian subcontinent at 25°14'N latitude, 82°3'E longitude, and 76.19 m above the sea level. The experiments were performed during August–October 2016. This period of the year is characterized by mean monthly maximum temperatures between 29.0 and 36 °C and mean monthly minimum temperatures between 18.0 and 27.0 °C. The total rainfall was 216.90 mm. Maximum relative humidity showed variation from 80.0 to 100% and minimum from 27 to 93%.

Experimental design and raising of plants

All the experiments were carried out in earthen pots of 30 cm diameter and 30 cm depth. The MSWVC used in the present study was prepared by mixing organic waste and cow dung (1:1 ratio). MSW was collected from secondary waste collection points of municipal corporation, Varanasi district. The vermicomposting of organic fraction of MSW (including vegetable wastes, fruit wastes, flower wastes, paper wastes, and leaf litter) was performed using *Eisenia fetida* (an earthworm spp.). Three replicates of six different amendments i.e., control (unamended soil), 20% MSWVC (w/w), 40% MSWVC (w/w), 60% MSWVC (w/w), 80% MSWVC (w/w), and 100% MSWVC (w/w) designated as S, A, B, C, D, and E respectively were used. The MSWVC was mixed uniformly with soil and left for 10 days in the field before filling the pots. Lady's finger (*Abelmoschus esculentus* L.

var. F1 GS-126, Virgo), a common vegetable consumed in central India was used as a test plant in the present study. Required moisture level (25%) was maintained prior to the sowing of lady's finger seeds and thereafter six seeds were sown at equal distances in each pot. After germination, thinning was performed to maintain three plants in each pot. The pots were placed in open field condition to provide identical light and temperature to all the amendments and were irrigated with equal amount of water to maintain identical water regime throughout the growth period of plants.

Soil sampling and analysis

Triplicate soil samples collected from each amendment were air dried, grounded, and sieved through 2-mm mesh size and physico-chemical properties were analyzed. The pH of the soil at different amendments was measured in the suspension of 1:5 (w/v) with the help of pH meter (Model 802, Systronics, India) standardized with pH 4, 7, and 9.2 reference buffers. Electrical conductivity was measured by electrical conductivity meter (Model 303, Systronics, India). Soil organic carbon was determined by Walkley and Black's rapid titration method (Allison 1973) and total nitrogen content of samples was measured by Pelican automatic nitrogen analyzer (Model KEL PLUS India). Total phosphorous was estimated by method described by Allens (1974). For heavy metal analysis, 1 gm of soil and VC samples were acid digested with 20 ml of triacid mixture ($\text{HNO}_3:\text{H}_2\text{SO}_4:\text{HClO}_4::5:1:1$) following Allen et al. (1986). The filtered acid digests were analyzed by atomic absorption spectrophotometer (AA 240 FS, Varian).

Estimation of biochemical and physiological parameters

For biochemical analysis, fresh leaves were plugged manually at 45 and 65 days after germination (DAG) and stored in deep-freezer till further analyses. Chlorophyll and carotenoid contents were estimated by the method of Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively, and expressed as mg g^{-1} dry leaf. Protein content in the fresh leaves was analyzed following Lowry et al. (1951). Foliar ascorbic acid and proline contents were measured by the method of Keller and Schwager (1977) and Bates et al. (1973). Peroxidase activity was measured using the method of Britton and Mehley (1955). Total phenol and thiol contents were measured following Fahey et al. (1978) and Bray and Thorpe (1954), respectively. The MDA (malondialdehyde) level representing the index of lipid peroxidation was measured by the method proposed by Heath and Packer (1968). Physiological parameters of lady's finger plants such as the rate of photosynthesis, stomatal conductance, and transpiration rate were measured using portable

photosynthetic system (LI-6200, LI-COR, Inc. Lincoln, NE, USA) directly on intact plants in the pot at ambient climatic conditions. Randomly selected three plants were sampled from the each experimental setup for estimation of morphological parameters at 45 DAG. Morphological parameters such as root and shoot length, number of leaves, and leaf area of each plant were estimated. Measurement of leaf area was performed using leaf area meter (Systronics 211, India). Dry weights of root, shoot, and leaves were also estimated after drying the samples at 80 °C till constant weight was achieved. The biomass yield was calculated as fresh weight at the time of harvest at 65 DAG.

Chemicals and quality control

All the chemicals used in this study were of analytical grades and used without further purification. The analytical quality control was guaranteed through the use of laboratory quality assurance and quality control protocols and standards. Standard operating procedures were followed for the calibration with standards, analysis of reagent blanks, and recovery of known spiked samples. The reagent blanks and known standards were used throughout the metal analysis and used to correct the analytical results. Precision and accuracy of heavy metal analysis were assessed through repeated analysis of the samples against National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the heavy metals. The results were found to be within $\pm 2\%$ of the certified value. All the experiments were carried out in triplicate.

Data analysis

Statistical analysis was performed using the SPSS version 16 (Illinois, USA) software for windows program. Treatments were compared using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was performed to test the significance of difference between the treatments. The graphs were drawn using Sigma Plot version 10 software.

Results and discussion

The MSWVC used in the study had almost neutral pH (7.12) and high electrical conductivity (2.24 mS cm^{-1}), total organic carbon (31.46%), total N (1.4%), total P (15.81 g kg^{-1}), total K (15.36 g kg^{-1}), and total Fe ($984.13 \text{ mg kg}^{-1}$) contents (i.e., 100% MSWVC) (Table 1). The concentrations of heavy metals of Cr, Pb, Ni, Cu, Cd, and Zn were 28.68, 2.40, 10.07, 37.7, 1.07, and 294.6 mg kg^{-1} , respectively, in ready MSWVC. The physico-chemical properties and nutrient profile of ready vermicompost largely depend on the source of substrates/



Table 1 Selected physico-chemical properties of unamended and municipal solid waste vermicompost amended soil (values \pm SE)

Parameters	Soil	A (20%VC)	B (40%VC)	C (60%VC)	D (80%VC)	E (100%VC)
pH	8.05 \pm 0.04 ^a	7.94 \pm 0.03 ^b	7.85 \pm 0.01 ^c	7.67 \pm 0.008 ^d	7.50 \pm 0.01 ^e	7.12 \pm 0.01 ^f
EC (mS cm ⁻¹)	0.26 \pm 0.01 ^f	0.45 \pm 0.01 ^e	0.56 \pm 0.008 ^d	0.87 \pm 0.01 ^c	1.09 \pm 0.01 ^b	2.24 \pm 0.008 ^a
TOC (%)	0.63 \pm 0.01 ^e	6.33 \pm 0.36 ^d	7.83 \pm 0.32 ^d	14.68 \pm 0.43 ^c	22.29 \pm 1.27 ^b	31.46 \pm 0.63 ^a
TKN (%)	0.24 \pm 0.01 ^e	0.26 \pm 0.00 ^{de}	0.28 \pm 0.01 ^d	0.36 \pm 0.01 ^c	0.41 \pm 0.01 ^b	1.40 \pm 0.01 ^a
TP (g kg ⁻¹)	4.14 \pm 0.11 ^e	4.82 \pm 0.07 ^c	6.88 \pm 0.26 ^d	9.37 \pm 0.15 ^c	11.09 \pm 0.22 ^b	15.81 \pm 0.50 ^a
TK (g kg ⁻¹)	5.85 \pm 0.25 ^e	6.54 \pm 0.21 ^{de}	6.84 \pm 0.85 ^d	7.99 \pm 0.12 ^c	9.60 \pm 0.44 ^b	15.36 \pm 0.30 ^a
Cr (mg kg ⁻¹)	6.45 \pm 1.51 ^d	7.62 \pm 0.29 ^d	8.4 \pm 1.00 ^{cd}	13.50 \pm 0.40 ^{bc}	15.17 \pm 1.43 ^b	28.68 \pm 3.43 ^a
Pb (mg kg ⁻¹)	0.98 \pm 0.33 ^b	1.00 \pm 0.18 ^b	1.88 \pm 0.16 ^a	2.13 \pm 0.16 ^a	2.05 \pm 0.25 ^a	2.40 \pm 0.12 ^a
Ni (mg kg ⁻¹)	5.20 \pm 0.51 ^b	5.30 \pm 0.43 ^b	5.88 \pm 0.26 ^b	6.38 \pm 0.42 ^b	7.27 \pm 0.32 ^b	10.07 \pm 1.50 ^a
Cu (mg kg ⁻¹)	13.97 \pm 0.68 ^d	14.43 \pm 0.23 ^d	15.82 \pm 0.41 ^d	18.98 \pm 0.68 ^c	21.28 \pm 0.29 ^b	37.70 \pm 1.72 ^a
Fe (mg kg ⁻¹)	432.35 \pm 21.66 ^d	425.28 \pm 4.5 ^d	468.17 \pm 12.38 ^{cd}	493.77 \pm 5.51 ^c	552.63 \pm 7.33 ^b	984.13 \pm 33.02 ^a
Cd (mg kg ⁻¹)	0.66 \pm 0.13 ^a	0.67 \pm 0.04 ^a	0.70 \pm 0.19 ^a	0.72 \pm 0.07 ^a	0.90 \pm 0.09 ^a	1.07 \pm 0.15 ^a
Zn (mg kg ⁻¹)	52.45 \pm 0.33 ^d	51.63 \pm 1.03 ^d	60.17 \pm 2.96 ^d	91.42 \pm 3.43 ^c	114.25 \pm 7.60 ^b	294.6 \pm 8.07 ^a

Different letters in each group show significant difference at $p < 0.05$

waste used in the vermicomposting process (Srivastava et al. 2015, 2016). For example, Garg et al. (2006) showed variations in pH of different organic wastes vermicompost ranged between 7.7 (sludge) and 8.3 (kitchen waste). Similarly, EC was ranged between 0.7 mS cm⁻¹ (institutional waste) and 2.3 mS cm⁻¹ (kitchen waste) depending on the nature of waste.

In the present study, physico-chemical analysis of MSWVC amended soil showed a significant decrease in pH and increase in EC with increasing levels of VC as compared to unamended soil (Table 1). This trend may be attributed to lower pH and higher EC of MSWVC used in the study. The release of humic substances might be the reason for such trends (Suthar et al. 2015). Atiyeh et al. (2001) observed similar results in application of pig manure vermicompost for horticultural uses. Soil organic carbon, total nitrogen, total phosphorus, and total potassium contents were increased in VC amended soil (Table 1). Improvement in the nutrient profile of soil due to amendment of organic waste compost/vermicompost has been reported previously (Sangwan et al. 2010, Avramidou et al. 2013). However, higher doses of MSWVC treatments led to increased concentration of heavy metals in soil (Table 1). The concentrations of heavy metals in the MSWVC amended soils were found in the order of Fe > Zn > Cu > Cr > Ni > Pb > Cd. Zn is an important micronutrient for different metabolic functions in plants; however, its toxic levels in soil lead to reduced plant growth, leaf chlorosis, photosynthesis impairment, and reactive oxygen species (ROS) production that poses harm to membrane integrity and permeability (Cambrollé et al. 2012, Subba et al. 2014, Srivastava et al. 2017). Moreover, high concentrations of Zn adversely affect dehydrogenase, β -D-glucosidase, urease, catalase, and invertase activities in soil (Kunito et al. 2001, Yang et al. 2006, Ciarkowska

et al. 2014). Previous studies have shown positive correlation between plant heavy metal uptake and total heavy metal content in the soil (Singh and Agrawal 2007, 2009, 2010a, b). Therefore, MSWVC amendments may pose risk to soil and plant health at higher doses.

Compared to the control, the total chlorophyll content of plants at 45 and 65 DAG was increased in MSWVC amended soil (Fig. 1). Total chlorophyll content in *A. esculentus* was 1.84, 1.97, 2.51, 2.28, 1.71, and 0.99 mg g⁻¹ dry weight in S, A, B, C, D, and E, respectively, at 65 DAG. Maximum increase of 61.25 and 36.41% was observed in total chlorophyll content of plants grown in 40% MSWVC (B) at 45 and 65 DAG. However, significant decrease in total chlorophyll was noticed in 100% MSWVC (E) treatment and a decrease of 40.96 and 46.20% was recorded at 45 and 65 DAG, respectively. Lakhdar et al. (2012) studied the effect of MSW compost on photosynthetic performance of *Triticum durum*, following application up to 300 t ha⁻¹. An increase of 14 and 15% was recorded at 40 and 100 t ha⁻¹ application rate, followed by a progressive decline in total chlorophyll content at higher doses (200 and 300 t ha⁻¹). Similarly, Sangwan et al. (2010) demonstrated increased level of total chlorophyll content in marigold at different application rates of cow dung and filter cake (sludge from sugar mill waste water treatment) + horse dung vermicomposts. Decrease in photosynthetic rate may be attributed to elevated levels of heavy metal accumulation that can adversely affect electron transport as well as photosynthetic metabolism (Burzyhski and Zurek 2007).

Carotenoids are non-enzymatic antioxidants, which protect the photosynthetic pigment against oxidative stress posed by heavy metal-induced reactive oxygen species (ROS) (Halliwell 1987). Carotenoid content increased significantly at both the ages and maximum increase of

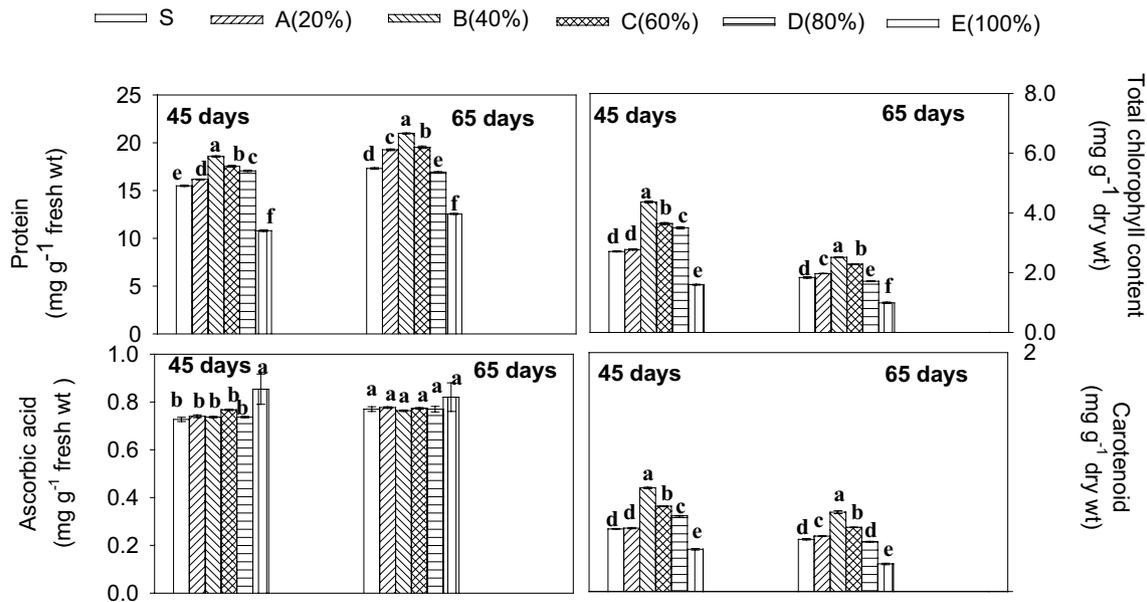


Fig. 1 Protein, ascorbic acid, total chlorophyll, and carotenoid content in lady’s finger plants grown at different MSWVC amendments at two different ages (Mean ± 1 SE). Bars with different letters in each group show significant difference at $p < 0.05$

65.38 and 50.80% was found in 40% MSWVC (B) at 45 and 65 days, respectively, as compared to control (Fig. 1). Increase in carotenoid content depicted that the plant defense system is actively responding to oxidative stress; however, a decrease of 32.69% in 100% MSWVC (E) at 45 days showed that the plant defense mechanism collapsed and failed to defend the plant against stress.

Photosynthetic (Ps) and transpiration rate increased significantly in 40% MSWVC (B), whereas stomatal conductance increased significantly in 40 and 60% MSWVC (B and C) compared to control (Table 2). An increase of 71.60% in photosynthetic rate of plants grown in 40% MSWVC (B) was observed, whereas it declined to 31.49% in 100% MSWVC. Lakhdar et al. (2012) reported increased CO₂ net assimilation in *Triticum durum* plants grown at 40 and 100 t ha⁻¹ MSW compost, whereas a significant reduction was seen at 300 t ha⁻¹. Increased photosynthetic rate in plants is ascribed to nutrient rich profile of MSWVC (Chen et al. 2005); however, bioavailability of heavy metals to the plants and

salinity due to higher dose of MSWVC might have resulted in reduced photosynthetic rate (Myśliwa-Kurdziel et al. 2002). Similarly, the transpiration rate also increases due to adequate nutrient supply (Adamtey et al. 2011). Ascorbic acid is a powerful antioxidant, which functions as a redox buffer and prevents plants from free oxygen radicals. It also works as a cofactor for enzymes participating in photosynthesis (Smirnoff and Wheeler 2000). In this study, no significant changes were noticed in ascorbic acid content of *A. esculentus* grown in different MSWVC amendments. Singh and Agrawal (2010c) found similar kind of trends for ascorbic acid content while studying the response of *Oryza sativa* L. grown at different sewage sludge application rates (Fig. 1). It is presumed that reactive oxygen free radicals might have oxidized ascorbic acid to dehydroascorbic acid (DHA) resulting in reduction and a non-significant response of ascorbic acid content of plants. Similarly, Rao and Sresty (2000), during their study on seedlings of *Cajanus cajan* (L.) Millspaugh under Zn and Ni stresses found negative

Table 2 Variation in selected physiological characteristics of *A. esculentus* plants grown in different amendment rates of MSW vermicompost (values ± SE)

Amendments	Photosynthetic rate (μmol m ⁻² s ⁻¹)	Transpiration rate (mmol m ⁻² s ⁻¹)	Stomatal conductance (cm s ⁻¹)	Ci/Ca
Soil	7.43 ± 0.22 ^b	3.55 ± 0.12 ^b	0.12 ± 0.00 ^{cd}	0.72 ± 0.03 ^a
A (20%VC)	7.78 ± 0.11 ^b	3.62 ± 0.04 ^b	0.13 ± 0.01 ^c	0.71 ± 0.01 ^{ab}
B (40%VC)	12.75 ± 0.28 ^a	4.78 ± 0.14 ^a	0.25 ± 0.01 ^a	0.72 ± 0.02 ^a
C (60%VC)	6.49 ± 0.35 ^c	3.85 ± 0.11 ^b	0.16 ± 0.02 ^b	0.64 ± 0.03 ^b
D (80%VC)	5.24 ± 0.20 ^d	3.41 ± 0.23 ^b	0.10 ± 0.01 ^{cd}	0.75 ± 0.04 ^a
E (100%VC)	5.09 ± 0.37 ^d	3.63 ± 0.35 ^b	0.09 ± 0.01 ^d	0.64 ± 0.02 ^b

correlation between ascorbic acid content and increasing levels of heavy metals.

Protein content increased significantly in plants with increasing rate of MSWVC (A, B, C, and D) compared to control; however, a significant decrease was noticed in 100% MSWVC (E) amendment at both 45 and 65 DAG. The total protein content in *A. esculentus* plants was 17.31, 19.26, 20.97, 19.52, 16.92, and 12.55 mg g⁻¹ fresh weight in S, A, B, C, D, and E, respectively, at 65 DAG. Maximum increments of 19.96 and 21.14% were observed in 40% MSWVC (B) at 45 and 65 DAG, respectively. Whereas in 100% MSWVC (E), a sharp decline of 30.30, and 27.50% in total protein content was noticed at 45 and 65 DAG. High organic matter content in MSWVC might be the main reason for increased level of protein content as it slows down the release of N in VC amended soil (Singh and Agrawal 2010c). Also, heavy metal toxicity may induce heat shock proteins (Kim et al. 2014). The elevated level of heavy metals at higher doses of MSWVC might be the reason for decreased protein content.

Increased level of malondialdehyde (MDA) acts as marker of lipid peroxidation. MDA content was increased significantly with increasing level of MSWVC when compared with the control plants at both the ages (Fig. 2). The maximum increase of 125% was noticed in MDA content of plants grown in 100% MSWVC at 65 DAG. Increased bio-availability of heavy metals to the plants at higher doses of MSWVC led to ROS production that could have damaged the plasma membrane (Cuypers et al. 2011). Singh and Agrawal (2007) reported increased level of lipid peroxidation in *Beta vulgaris* grown at 20 and 40% sewage sludge amendments. It was observed that peroxidase content increased significantly in plants with increasing doses of MSWVC; the increments were more prominent in 60, 80, and 100% MSWVC in 45 and 65 DAG (Fig. 2). Similar findings have been reported earlier in *Beta vulgaris* (Singh and Agrawal 2007) and in *Oryza sativa* (Singh and Agrawal, 2010c) grown in different sewage sludge amendments. Phenols, a secondary metabolite and antioxidant, play a crucial role in defense mechanism of plants against oxidative and abiotic stresses.

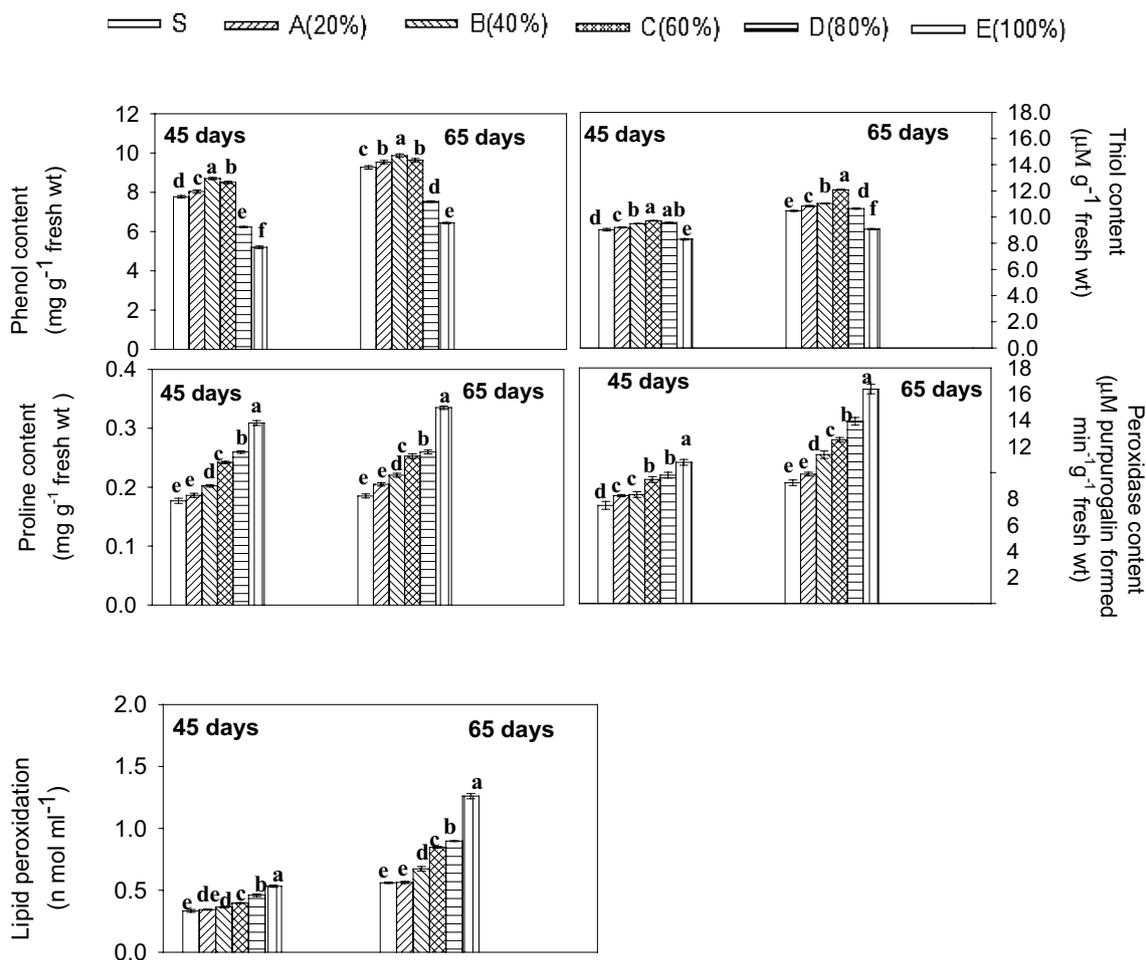


Fig. 2 Phenol, proline, thioli, peroxidase, and lipid peroxidase content in lady's finger plants grown at different MSWVC amendments at two different ages (Mean \pm 1 SE). Bars with different letters in each group show significant difference at $p < 0.05$



Table 3 Morphological characteristics (at 45 DAG) and yield* response (at 65 DAG) of lady's finger (values \pm SE)

Amendment	Root length (cm)	Shoot length (cm)	Leaf area (cm ²)	No. of leaves (plant ⁻¹)	Root biomass (g plant ⁻¹)	Shoot biomass (g plant ⁻¹)	Leaf biomass (g plant ⁻¹)	Total biomass (g plant ⁻¹)	Yield* (g plant ⁻¹)
S	6.20 \pm 0.62 ^{bc}	28.03 \pm 1.21 ^b	136.17 \pm 4.15 ^e	5.00 \pm 0.00 ^{bc}	0.05 \pm 0.01 ^{cd}	0.32 \pm 0.04 ^b	0.30 \pm 0.02 ^{cd}	0.67 \pm 0.07 ^{bc}	121.33 \pm 2.09 ^{cd}
A	7.27 \pm 0.41 ^b	34.87 \pm 1.52 ^{ab}	206.07 \pm 7.39 ^c	6.00 \pm 0.58 ^b	0.10 \pm 0.00 ^b	0.63 \pm 0.07 ^b	0.37 \pm 0.03 ^{bc}	1.05 \pm 0.08 ^b	138.33 \pm 8.44 ^{bc}
B	9.77 \pm 0.07 ^a	43.83 \pm 2.33 ^a	432.0 \pm 7.42 ^a	8.0 \pm 0.58 ^a	0.19 \pm 0.03 ^a	1.72 \pm 0.40 ^a	1.08 \pm 0.13 ^a	2.99 \pm 0.55 ^a	196.64 \pm 7.23 ^a
C	8.8 \pm 0.50 ^a	30.53 \pm 6.08 ^b	280.83 \pm 5.20 ^b	5.67 \pm 0.67 ^b	0.05 \pm 0.01 ^{cd}	0.54 \pm 0.07 ^b	0.58 \pm 0.06 ^b	1.17 \pm 0.11 ^b	147.70 \pm 4.09 ^b
D	7.13 \pm 0.15 ^b	28.73 \pm 3.42 ^b	182.97 \pm 4.03 ^d	5.33 \pm 0.33 ^b	0.06 \pm 0.01 ^{bc}	0.31 \pm 0.03 ^b	0.46 \pm 0.06 ^{bc}	0.84 \pm 0.10 ^{bc}	115.46 \pm 6.76 ^d
E	5.13 \pm 0.20 ^c	12.73 \pm 0.94 ^c	41.03 \pm 6.89 ^f	3.67 \pm 0.33 ^c	0.01 \pm 0.00 ^d	0.11 \pm 0.03 ^b	0.09 \pm 0.01 ^d	0.23 \pm 0.04 ^c	72.01 \pm 6.85 ^e

Different letters in each group show significant difference at $p < 0.05$

Phenol content increased significantly in plants at different amendment rates (20, 40, and 60% MSWVC); however, a decrease was noticed in 80 and 100% MSWVC indicating failure of plant defense system at higher doses at 45 DAG (Fig. 2). Similarly, increased level of polyphenol content in *Mesembryanthemum edule* grown at 40 t ha⁻¹ of MSW compost was reported by Lakhdar et al. (2011).

Proline is an osmolyte which plays a pivotal role during abiotic and oxidative stress in plants and acts as metal chelator, antioxidant, and a signaling molecule (Hayat et al. 2012). Proline content was increased significantly in plants with increasing levels of MSWVC at both the ages with maximum increase in 100% MSWVC compared to control. This could be due to either high salt concentration, plant phytotoxicity in higher dose of VC, and/or heavy metal accumulation in amended soil (Arancon et al. 2004, Sangwan et al. 2010, Srivastava et al. 2016). Heavy metals in plants may affect the permeability of membranes leading to water stress environment causing proline accumulation (Singh and Agrawal 2007, 2009). Similarly, thiol content increased at different amendment ratios of MSWVC (A, B, C, and D) and was declined at higher doses (E) at both 45 DAG and 65 DAG (Fig. 2).

Root length and shoot length increased significantly in 40% MSWVC whereas it declined in 100% MSWVC at 45 DAG (Table 3). Reduction in root and shoot length may be ascribed to increased levels of heavy metals in 100% MSWVC amendments. Increased levels of heavy metals might have decreased the mitotic activity within the root cells leading to suppressed root growth in 100% MSWVC (Thounaojam et al. 2012). Furthermore, high concentrations of heavy metals caused more ROS production that posed negative effects on plant physiology resulted in reduced shoot growth (Srivastava et al. 2017). Similarly, Singh and Agrawal (2007) reported significant decrease in root lengths of *Beta vulgaris* plants at 20 and 40% (w/w) sewage sludge amendments (SSA), whereas shoot lengths were decreased significantly at 40% SSA due to heavy metal stress. Leaf areas were increased significantly with increasing MSWVC (A, B, C, and D), while decrease in leaf area was noticed in E amendment at 45 DAG. An increment of 217.25% was

seen in leaf area of B, but it was declined by 69.87% in E amendment compared to control. Likewise, total biomass was increased significantly in B, but reduced significantly in plants grown at E amendment. Yield response recorded at 65 DAG showed significant increase at 40 and 60% MSWVC soil amendments; however, it was decreased in 80 and 100% MSWVC amendments.

Conclusion

The present study clearly suggests that MSWVC showed a positive effect on soil nutrient profile represented by increased organic carbon and NPK. Higher doses of MSWVC led to either salinity stress, plant phytotoxicity or bioavailable heavy metal accumulation depicted by increased level of antioxidative plant response, lipid peroxidation, and peroxidase activity. However, MSWVC showed positive effect on biochemical, physiological, and yield responses of *A. esculentus* (Lady's finger) up to 60% MSWVC amended soils represented by increased rate of photosynthesis, stomatal conductance, and improved antioxidative response. In addition, the leaf area, total biomass, and yield responses increased significantly with respect to control. The present study concludes that up to 60% amendments of MSWVC can be used as manure for improving soil fertility in agricultural applications. This could help in the sustainable management of the burgeoning amount of organic waste.

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