# Enhancing soil health and productivity of *Lycopersicon esculentum* Mill. using *Sargassum johnstonii* Setchell & Gardner as a soil conditioner and fertilizer

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Abstract This study was aimed at developing a protocol for improving soil health using Sargassum johnstonii as a conditioner and fertilizer. Tomato (Lycopersicon esculentum) plants were raised on seaweed-amended soil in experimental fields of Department of Botany, University of Delhi, India. Soil was amended with granular (G) and powder (P) seaweed forms in the proportion of 12.5 % ( $G_1$  and  $P_1$ ), 25 % ( $G_2$  and  $P_2$ ), and 37.5 % (G<sub>3</sub> and P<sub>3</sub>) (w/w). To compare the efficacy of seaweed fertilizer with a conventional organic fertilizer, a parallel series (positive control) was run with vermicompost (V) in the above-mentioned proportions. Unamended soil served as control (C). The nutrient status of S. johnstonii and vermicompost was analyzed prior to giving treatments. Physicochemical properties of the amended soils as well as growth, productivity, and biochemical constituents of tomato grown in soil with each treatment were analyzed. Higher concentration of granular form of seaweed  $(G_3)$  in the soil resulted in 144, 268, 122, 138, and 188 % increase in Na, K, Mg, Ca, and Zn, respectively. Seaweed-amended soil had higher porosity and waterholding capacity as compared to C. Tomato plants raised on seaweed (G<sub>3</sub> and P<sub>3</sub>)-amended soil showed an increased overall growth, with earlier flowering and fruiting as compared to control plants. Plants raised on G3-amended soil showed significantly higher levels of proteins (95  $mgg^{-1}$  FW) in leaves, and vitamin C (99.2 mg 100 g<sup>-1</sup>) and lycopene  $(5.78 \text{ mg } 100 \text{ g}^{-1})$  in fruits. The present study showed that S. johnstonii biomass has a high potential to condition and fertilize the soil for improved crop productivity.

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#### Introduction

Conventional farming, which is largely based on chemical fertilizer, pesticide, and herbicide inputs, has played an important role in enhancing food productivity. However, excessive use of the agrochemicals has resulted in soil degradation and decline in yield and quality of crops (Kramer et al. 2006). There is now worldwide interest in alternate ecofriendly practices, known as organic farming, which aim at improving soil health and productivity using organic fertilizers (Lobley et al. 2009). Organic fertilizers improve physicochemical properties of soil such as aeration, moisture, and nutrient retention capacity (Birkhofer et al. 2008) and assist in restoration of natural soil fertility through microbial activity. These organic fertilizers also provide varied macro- as well as micronutrients in soil required for healthy growth and improved crop yield (Rai 2006). Of the whole range of organic fertilizers available in the market, seaweeds are currently being used in various forms, such as spray, granules, powder, drench, and manure (Karthikeyan et al. 2008; Kumari et al. 2011). Seaweeds in the past have been frequently used as solid manure (Chapman and Chapman 1980) but have now gained worldwide popularity as liquid fertilizers (Khan et al. 2009). Recent emphasis on organic farming has led to reinvestigation of the potential of seaweed as a soil conditioner and fertilizer.

Seaweeds contain a diverse range of bioactive substances such as vitamins, minerals, growth regulators, organic compounds, and wetting agents/mucilaginous colloids (e.g., carrageenan, agar, alginic acid, laminarin, and mannitol) that help in retaining moisture and nutrients in the upper layers of soil (Subba Rao et al. 2007). Studies indicate that their application (1) stimulates activity of microorganisms, thereby making soil nutrients readily available to crops; (2) affects soil compaction and aeration (a property of alginic acids); and (3) increases water-holding capacity (due to improved soil texture). Seaweeds also have a positive effect on biological activity (respiration and nitrogen mobilization) of soil (Haslam and Hopkins 1996; Selvaraj et al. 2004) which promotes microbial diversity, thereby creating an environment suitable for root growth (Sarwar et al. 2008). Seaweeds also serve as an external source of macro- and micronutrients that are usually deficient in soil-crop cycle on account of leaching and erosion (Gopinath et al. 2008). Although seaweed fertilizer in liquid form has gained importance in enhancing crop yield (Thirumaran et al. 2009; Kumari et al. 2011), there are few data on soil conditioning and crop productivity through seaweed application in granular/powder form. With global utilization of seaweeds as food, fodder, growth regulators, and biofertilizers on the rise, availability of seaweed biomass in large amount is one of the major constraints. To fulfill the current agricultural demand, "drift" seaweeds can be collected and processed for agro-based industries. The seaweed biomass can also be raised through mass cultivation which plays important role in nutrient-removal system to reduce eutrophication problems (Lüning and Pang 2003). In many countries, including France, Spain, Vietnam, Thailand, Indonesia, Bangladesh, Malaysia, Philippines, Korea, Japan, and China, mass cultivation of seaweed is common (Tseng 1993).

Tomato (Lycopersicon esculentum Mill.) is a fruit with high carotene content that reduces cancer risk and heart diseases because of its antioxidant properties (Rao and Agarwal 2000). Among carotenes in tomato, lycopene dominates but its content varies significantly depending on variety, stage of ripening, and environment (Brandt et al. 2006; Dorais et al. 2008). Humans obtain 85 % of their dietary lycopene from tomato fruit and tomato-based products. Tomato is grown using chemical as well as organic fertilizers. The nutritional quality of conventionally and organically grown plants has been compared mainly in terms of nutrients, vitamins, and minerals (Yanar et al. 2011). The relationship between farm inputs, soil health, and productivity of tomato is less understood. Thus, a thorough investigation is required to analyze physical properties such as bulk density, capillarity, porosity, and water-holding capacity of soils amended with seaweed, and the impact of soil health on crop yield. Unlike application of seaweed liquid extract as fertilizer and spray which has received world wide acceptance, the potential of seaweed biomass in its natural form as soil conditioner and fertilizer is yet to be evaluated. In the present study, soil health enhancement was carried out with granular and powder forms of Sargassum johnstonii Setchell & Gardener and the growth parameters and fruit quality of *L. esculentum* raised on amended soils were analyzed. The study indicates that seaweeds can be useful components of organic farming.

## Material and methods

Brown seaweed S. johnstonii (Phaeophyceae, Fucales) was collected in January 2008 during low tide periods from Port Okha (22°28.528'N, 069°04.322'E), Gujarat (India). Plants were washed with seawater followed by rinsing with tap water to remove salt and debris. The washed seaweed biomass was then brought to laboratory in airtight plastic bags. Cleaned material was sun-dried for 24 h and later oven-dried for 48 h at 60 °C to facilitate grinding (Kumari et al. 2011). The dried biomass was crushed to prepare two forms of fertilizer. Granular form (800 µm) was obtained by crushing in pestle and mortar, while powder form (100 µm) was obtained by processing the dried seaweed in a mixer and grinder. A vermicompost organic fertilizer (500 µm) obtained from the Golden Mushroom Farm, Haryana (India) was used to compare the efficacy of S. johnstonii as a soil conditioner and fertilizer (positive control).

#### Experimental setup and treatments

Seeds of L. esculentum Mill. (Solanaceae) variety Pusa Ruby were procured from the National Seeds Corporation, Indian Agricultural Research Institute Campus, New Delhi (India). Seeds with uniform size, weight, and color were sown in experimental pots maintained in the Department of Botany, University of Delhi. Three sets of treatments were conducted where garden soil was amended with granular (G) or powder (P) seaweed, and vermicompost (V). In the first set, a series with granular seaweed fertilizer in soil was maintained with concentrations of  $G_1$  (12.5 %),  $G_2$  (25 %), and  $G_3$  (37.5 %) (w/w). In the second and third sets, similar concentrations were made with powder seaweed ( $P_1$ ,  $P_2$ , and P<sub>3</sub>) and vermicompost (V<sub>1</sub>, V<sub>2</sub>, and V<sub>3</sub>), respectively. Controls (C) and three replicates per treatment were maintained. Pots (38-cm rim diameter) were filled with fertilizer (G, P, and V)-amended or unamended (C) soil, and seeds were sown at appropriate spacing of eight seeds per pot in the month of November. Under natural conditions, 15 days after sowing, seedlings were thinned to six per pot. First irrigation in the form of light shower was given immediately after sowing. Thereafter, plants were irrigated depending upon soil condition and crop maturity. Weeding was done periodically, and growth of crop was followed for a period of 24 weeks or 6 months for all treatments.

#### Nutrient status

Concentrations of macro- (Na, K, Mg, and Ca) and micronutrients (Fe, Cu, Zn, and Mn) in amended soil samples (20 days after sowing) were determined using the analytical method given by Allen (1989). To oven-dried (2 g) soil, 50 mL ammonium acetate was added and kept on a rotatory shaker for 45 min. Soil suspensions were filtered through Whatman filter paper no. 44, and volume of filtrate was made to 50 mL with distilled water.

For nutrient analysis of fertilizers, samples (200 mg) were taken in a Kjeldahl tube and digested with conc. HNO<sub>3</sub>, 60 % HClO<sub>4</sub>, and conc. H<sub>2</sub>SO<sub>4</sub>, until a light-colored clear solution was obtained. This solution was then filtered through Whatman filter paper no. 44, and the filtrate was used for the determination of nutrients. For standards of various nutrients, stock solutions (1,000 ppm) were prepared and were diluted to 100 ppm. Further serial dilutions (1, 2, 3, 4, and 5 ppm) were prepared from it and used for preparing the standard curve. A calibration curve was used to determine the nutrients in the sample solution. Atomic absorption spectrophotometry (AAS) was carried out (Shimadzu AA-130, AAS, lamp current 7 mA, slit width of 1.9 cm, and burner height of 7 cm) for Na, K, Ca, Mg, Fe, Cu, Mn, and Zn at wavelengths 589.0, 766.5, 285.2, 433.7, 248.3, 324.8, 213.9, and 279.5 nm, respectively.

#### Physicochemical properties of soil

Soil samples (15 cm depth) were collected (155 days after sowing) and sieved with a  $1.2 \times 1.2$ -cm sieve to remove larger stones. These samples were analyzed for pH, bulk density (Blake and Hartge 1986), porosity (Danielson and Sutherland 1986), water-holding capacity (Kapur and Govil 2000), percolation (EPA 1996), and capillary action (Wells 1968).

#### Morphological parameters

Plants were uprooted 90 and 120 days after sowing for study of vegetative and reproductive parameters, respectively. Various vegetative traits such as shoot length, root length, and plant fresh weight were recorded at 90 days after sowing. At 120 days, the number of flowers and, at 130 days after sowing, the number of fruits and their fresh weight were recorded.

## Biochemical constituents

Estimation of chlorophylls a and b and carotenoids was carried out in dim light according to the method of Hiscox and Israelstam (1979). One hundred milligrams of 80-day-old leaf tissue was placed in a test tube containing 7 mL dimethyl sulfoxide (DMSO) and incubated at 65 °C for 1 h. To this aliquot, 3 mL of DMSO was added. Pigment concentration was calculated using the Arnon (1949) formula. Concentration of proteins in 80-day-old freshly harvested shoots was estimated by the Bradford (1976) method. Fresh shoots (0.5 g) were homogenized in 5 mL of 0.1 M phosphate buffer (pH 7.0), the contents were filtered through four-layered muslin cloth, and the filtrate was then centrifuged at  $6,440 \times g$  at 4 °C for 10 min. To 0.1 mL supernatant, 5 mL Bradford reagent was added, vortexed, and absorbance read at 595 nm. A standard curve was prepared using bovine serum albumin.

Soluble sugar concentration in 80-day-old fresh shoots was estimated by Yemm and Willis (1954). The samples were hydrolyzed in boiling water for 3 h using 5 mL of 2.5 N HCl. The extract was neutralized with sodium carbonate until effervescence ceased and volume made to 100 mL with deionized water. Contents were filtered and the filtrate was centrifuged at  $4,472 \times g$  for 10 min at 4 °C. To the known volume of supernatant, an equal volume of distilled water was added. After adding 4 mL chilled anthrone reagent, the contents were kept in boiling water for 8 min. Upon cooling, the absorbance was read at 595 nm and the amount of soluble sugars was estimated from the standard curve prepared with D-glucose.

To estimate reducing sugars, 100 mg of 80-day-old fresh shoot samples was homogenized in warm 80 % alcohol. Extract was filtered through four-layered muslin cloth and filtrate was evaporated in boiling water bath. To the residue, 10 mL distilled water was added to dissolve sugars. To 0.5 mL of aliquot, 2.5 mL distilled water and 3 mL dinitrosalicyclic acid reagent were added. The tubes were then kept in boiling water for 5 min and then 1 mL of 40 % Rochelle salt solution (sodium potassium tartrate solution) was added. After cooling, absorbance was read at 510 nm and concentration of reducing sugar was estimated from the D-glucose standard curve (Miller 1959).

For estimation of starch concentration in shoot samples, 100 mg of 80-day-old plant biomass was homogenized in 80 % hot ethanol and centrifuged for 10 min at  $4,472 \times g$  at 4 °C. The pellet recovered was repeatedly washed with ethanol and dissolved in 5.0 mL H<sub>2</sub>O and 6.5 mL of 52 % perchloric acid. Dissolved pellet was again centrifuged at  $4,472 \times g$  at 0 °C for 20 min. To the supernatant (0.1 mL), 0.9 mL water and 4 mL chilled anthrone reagent were added. The test tubes with these contents were kept in boiling water bath for 20 min and cooled rapidly (Thimmaiah 1999). Absorbance was read at 630 nm and concentration obtained from the D-glucose standard was multiplied by a factor 0.9 to calculate starch content (Hassid and Neufeld 1964).

For phenol estimation, freshly harvested leaves (100 mg) 80 days after sowing were ground in pestle and mortar with 10 mL of 80 % ethanol and the homogenate was centrifuged at  $4,472 \times g$  for 20 min at 4 °C. Supernatant was collected and residue was again extracted with ethanol and centrifuged. After centrifugation, supernatant from both steps was

pooled, evaporated to dryness, and later dissolved in distilled water. To 0.2 mL of aliquot, 2.8 mL water and 0.5 mL Folin–Ciocalteu reagent were added. After 5 min, 2 mL of 20 % Na<sub>2</sub>CO<sub>3</sub> solution was added and mixed. Tubes were placed in boiling water for 1 min. Absorbance was read at 650 nm and, after estimating concentration (Malick and Singh 1980) from the standard curve prepared with catechol (100  $\mu$ gmL<sup>-1</sup>), expressed as mg phenols 100 g<sup>-1</sup> material.

Estimation of lycopene was based on modified method of Fish et al. (2002). Fruits (harvested 135 days after sowing) were pulped to obtain smooth consistency in a Waring blender. Five grams of pulp was extracted with acetone using mortar and pestle until colorless. Extract was transferred to a separating funnel containing 20 mL petroleum ether and gently mixed. After this, 20 mL of 5 % sodium sulfate was added and the contents were mixed by continuous rotating of funnel (since petroleum ether evaporates fast, 20 mL petroleum ether was added again to achieve separation of two layers). Of the two solvent layers separated, the lower aqueous layer was reextracted with additional petroleum ether until it turned colorless. Petroleum ether extracts were pooled and washed with distilled water in a separating funnel. The upper layer containing carotenoids was kept in a brown bottle having 10 g anhydrous sodium sulfate and left undisturbed for 30 min. It was transferred to a volumetric flask (100 mL) through a funnel containing glass wool. Absorbance was measured at 503 nm with petroleum ether as blank. One mole of lycopene when dissolved in 1 L petroleum ether (40-60 °C) and measured in a spectrophotometer at 503 nm in 1-cm light path gives an absorbance of  $17.2 \times 10^4$ . Therefore, a concentration of 3.12  $\mu$ g lycopene mL<sup>-1</sup> gives a unit absorbance. Amount of lycopene in fruits was estimated by the following formula: lycopene (mg g<sup>-1</sup>)= $(X \times Y^{-1}) \times A_{503} \times 3.12$  where, X=amount of petroleum ether (mL), Y=weight of fruit tissue (g).

Determination of ascorbic acid (vitamin C) concentration in tomato fruits (135 days after sowing) was carried out using the method given by Thimmaiah (1999). Five mL of oxalic acid working standard solution (100 µg mL<sup>-1</sup>) was pipetted into a conical flask. To it, 10 mL of 4 % oxalic acid was added and titrated against 2,6-dichlorophenolindophenol dye ( $V_1$ , mL). Appearance of pink color which persisted for about 5 min indicated the end point. Five grams of tomato pulp (test sample) was extracted in 10 mL of 4 % oxalic acid, volume made to 100 mL with deionized water and centrifuged at 5,000×g for 10 min. To 5 mL of supernatant, 10 mL of 4 % oxalic acid was added and titrated against the dye ( $V_2$ , mL). Amount of ascorbic acid was calculated by the following formula:

 $\label{eq:acorbic acid} \mbox{(mg 100 g}^{-1}\mbox{)} = \frac{0.5 \mbox{ mg} \times V_2 \times 100 \mbox{ mL}}{V_1 \times 15 \mbox{ mL} \times W} \times 100$ 

where W = weight of sample (g).

#### Statistical analysis

Statistical analysis was carried out employing Statistical Package for Social Sciences software for Windows ver. 10.0. All the parameters mentioned were analyzed by means of one-way ANOVAs accompanied by Tukey's post hoc multiple comparison test to determine differences among treatments for each characteristic. Mean and standard deviation were obtained by descriptive statistics. The significance of differences between variables at  $P \le 0.05$  was checked with a multiple comparison least significant difference test.

### Results

#### Physicochemical properties of soil

Dry biomass of *S. johnstonii* when analyzed for nutrients showed presence of both macro- (Na, K, Ca, and Mg) and micronutrients (Fe, Cu, Mn, and Zn). Significant variations were recorded between vermicompost and seaweed fertilizers, with the latter showing higher concentrations of all nutrients except Mg (Table 1). Among all nutrients, maximum concentration was recorded for Cu, Zn, and Ca in seaweed fertilizer. Soil treated with the granular form (G<sub>3</sub>) showed the maximum increase in Na, K, Mg, and Ca (144, 268, 122, and 138 %, respectively). Powdered and vermicompost treatment also resulted in enhanced Mn and Cu content in soil. Concentration of macro- and micronutrients in all amendments showed an increase over control (Table 2).

Seaweed-treated soils showed a near neutral pH when compared with control and vermicompost treatment (Fig. 1a). Bulk density was recorded highest for control

Table 1 Nutrient concentration of seaweed fertilizer and vermicompost

Nutrients	Seaweed fertilizer	Vermicompost
Na (%)	$1.033 {\pm} 0.015^{\mathrm{b}}$	0.964±0.13 <sup>b</sup>
K (%)	$0.0682 \pm 0.001^{c}$	$0.050{\pm}0.005^{b}$
Ca (%)	$2.609 {\pm} 0.067^{b}$	$2.47{\pm}0.176^{b}$
Mg (%)	$0.0628 {\pm} 0.001^{b}$	$0.0752 {\pm} 0.011^{\rm b}$
Fe (%)	$0.5556 {\pm} 0.015^{\circ}$	$0.218 {\pm} 0.0140^{ m b}$
Cu ( $\mu g g^{-1}$ )	$2001.7 \pm 57.3^{b}$	$1771.6{\pm}48.8^{a}$
Mn (%)	$0.036 {\pm} 0.003^{\circ}$	$0.0104 {\pm} 0.0005^{a}$
Zn ( $\mu g g^{-1}$ )	$67.05 \pm 10.06^{\circ}$	$48.69 {\pm} 20.5^{b}$

Values are mean  $\pm$  standard error. Readings with different letters are significantly different (n=18; P=0.05) as analyzed by Tukey's post hoc test

Treatments	Minerals							
	Sodium (mg $g^{-1}$ )	Potassium (mg 100 g <sup>-1</sup> )	Magnesium $(mg \ 100 \ g^{-1})$	Calcium $(mg \ 100 \ g^{-1})$	Iron (mg 100 $g^{-1}$ )	Copper ( $\mu g g^{-1}$ )	Zinc ( $\mu g g^{-1}$ )	Manganese (mg $100 \text{ g}^{-1}$ )
Control	$12.5\pm0.34^{a}$	$10.6 \pm 0.26^{a}$	$7.55 \pm 0.17^{a}$	$125.2\pm 8.50^{a}$	$0.54 \pm 0.06^{a}$	$0.38 {\pm} 0.22^{ m a}$	$3.27 \pm 0.19^{a}$	$0.42 \pm 0.05^{a}$
G1	19.3±2.53 <sup>b, c</sup>	$35.2\pm2.56^{\circ}$	$7.62 \pm 0.41^{a}$	$149.6 \pm 3.06^{b}$	$0.93 \pm 0.18^{a, b}$	$1.66 {\pm} 0.15^{\rm a, \ b}$	7.36±0.30 <sup>b, c</sup>	$0.41 \pm 0.16^{a}$
$G_2$	$24.2 \pm 1.50^{d}$	$35.5\pm0.08^{c}$ , d	$8.79 \pm 0.23^{a, b}$	$171.3 \pm 4.82^{\circ}$	$1.85 \pm 0.23^{b}$ , c	$3.26\pm0.33^{\rm b,\ c}$	$8.18\pm1.02^{b, c}$	$0.85 {\pm} 0.03^{ m b}$
G <sub>3</sub>	$30.6\pm 2.77^{e}$	$39.1 \pm 2.53^{d}$	$16.8 {\pm} 0.88^{ m d}$	$298.6 \pm 7.91^{\rm f}$	$4.41\pm0.16^{\rm d}$	$1.87 {\pm} 0.98^{\rm a, \ b}$	$9.45 \pm 1.16^{\circ}$	$1.27\pm0.10^{c}$ , d
$P_1$	$12.7 {\pm} 0.56^{a}$	$18.56 \pm 1.56^{\rm b}$	$7.56 {\pm} 0.045^{a}$	$145\pm8.56^{\mathrm{b}}$	$0.63 \pm 0.014^{a}$	$1.56 {\pm} 0.24^{\rm a, \ b}$	$4.56{\pm}0.19^{\rm a}$	$0.75 {\pm} 0.03^{ m b}$
$\mathbf{P}_2$	$23.0\pm0.43^{ m c, d}$	$33.2 \pm 1.09^{\circ}$	$8.90{\pm}0.63^{\rm a,\ b}$	$292.9 \pm 3.20^{\rm f}$	$1.99 \pm 0.20^{\circ}$	$1.91 \pm 0.22^{a, b}$	$5.77 \pm 0.88^{a, b}$	$1.42\pm0.03^{c}, d$
$P_3$	25.8±2.15 <sup>d, e</sup>	$32.8 \pm 0.93^{\circ}$	$9.71 \pm 0.46^{b}$	$302.9\pm 5.44^{f}$	$1.34\pm0.45^{a, b, c}$	$1.59 \pm 0.27^{a, b}$	$8.14\pm0.98^{b, c}$	$1.63 \pm 0.04^{ m d}$
$V_1$	$12.6 \pm 1.36^{a}$	$19.1 \pm 0.13^{b}$	$7.65 \pm 0.89^{a}$	$136{\pm}2.56^a$	$0.59 \pm 0.07^{\mathrm{a}}$	$1.43 \pm 0.32^{a, b}$	6.56±0.56 <sup>b, c</sup>	$0.45 \pm 0.015^{a}$
$V_2$	$17.5 \pm 0.15^{b}$	$21.0 \pm 0.23^{b}$	$11.6 \pm 0.36^{\circ}$	$209.8 \pm 2.78^{d}$	$0.74 \pm 0.42^{a}$	$2.31\pm0.03^{b, c}$	$8.23\pm0.39^{b, c}$	$1.07 \pm 1.03^{\circ}$
$V_3$	$17.2 \pm 0.46^{a, b}$	$23.6 \pm 1.23^{b}$	$11.3 \pm 0.17^{c}$	$224.5\pm 2.05^{e}$	$1.42\pm0.64^{a}$ , <sup>b</sup> , <sup>c</sup>	$3.85 \pm 1.02^{c}$	$6.59 \pm 1.59^{b, c}$	$1.23\pm0.04^{\rm c}$ , d
Values are mean	± standard error. Readino	as with different letters	are sionificantly diff	erent $(n=27, P=0.05)$	) as analyzed by Tukev's	nost hoc test		

Table 2 Effect of different concentrations of seaweed and vermicompost treatment on nutrient status of soil

# Morphological growth parameters

Tomato plants showed a linear increase in number of branches when grown in soils with higher concentrations of granular seaweed  $(G_3)$ , powder seaweed  $(P_3)$ and vermicompost fertilizer (V<sub>3</sub>) as compared to C. All three treatments resulted in statistically significant increase in root and shoot length at all concentrations. Minimum root length and shoot length were recorded in control, and maximum in G<sub>3</sub> followed by P<sub>3</sub>-treated plants. Plant fresh weight also showed statistically significant increase with  $G_3$  amendment, followed by  $P_3$ . Vermicompost was less effective in bringing significant increase, as compared to G and P treatments, for the root and shoot growth parameters (Table 3). The overall reproductive growth of seaweed-treated plants was significant over vermicompost and control. Compared to control, a statistically significant increase in the number of flowers/plant was observed in  $G_{3}$  followed by  $P_{3}$ . Early flowering was observed for all the plants raised in amended soils. Total number of fruits produced per plant and their fresh weight was found maximum with G<sub>3</sub>, followed by P<sub>3</sub> when compared to control plants. A significant increase in reproductive growth parameters was observed in vermicompost-treated plants (V<sub>3</sub>) (Table 3). However, even after 120 days of sowing, no flowering and therefore no fruiting was observed in control.

# **Biochemical parameters**

G soil amended with granular seaweed, P soil amended with powdered seaweed, V soil amended with vermicompost

A statistically significant increase was observed for biochemical constituents of tomato plants raised on seaweed-amended soils. Maximum level of chlorophyll (Chl) a was obtained with  $G_3$ , while Chl b and carotenoids showed an increase with  $P_3$  amendment (Fig. 2ac). The rest of the treatments also had improved concentration of photosynthetic pigments over the control. Statistical analysis indicates a significant increase in protein concentration with  $P_3$ , followed by  $G_3$  over control. A statistically similar increase in protein

followed by vermicompost-treated soil at lower concentration (V<sub>1</sub>). All the treatments showed significant difference in bulk density, which decreased with increasing concentration of fertilizers (Fig. 1b), being lowest in G<sub>3</sub>, followed by P<sub>3</sub> and V<sub>3</sub>. The amended soils showed improved percentage porosity with G<sub>3</sub> treatment, followed by P<sub>3</sub> and V<sub>3</sub> as compared to control (Fig. 1c). Water-holding capacity and percolation rate were recorded maximum with G<sub>3</sub>, a significant improvement over control (Fig. 1d, e), while capillarity was observed maximum with P<sub>3</sub>-amended soil (Fig. 1f).



Fig. 1 Effect of organic fertilizer amendments on the physical properties of soil: pH(a), bulk density (b), porosity (c), water-holding capacity (d), percolation (e), and capillarity (f). *G* soil amended with



b

granular seaweed, P powdered seaweed, V vermicompost. Mean values followed by the different letters are significantly different. *Error bars* represent the standard error of replicates (n=27, P=0.05)

concentration was observed with  $G_2$ ,  $P_2$ , and  $V_3$ (Fig. 2d). When compared to control, the concentration of soluble sugar was maximum for  $G_3$ , while  $P_3$  and  $G_2$ resulted in statistically similar results (Fig. 2e).  $G_3$ ,  $P_3$ , and  $V_3$  showed a statistically similar increase in reducing sugars followed by  $G_2$ , while insignificant results were observed with  $P_1$ ,  $V_1$ , and C (Fig. 2f). Starch concentration was maximum with  $G_3$  followed by  $G_2$ , while the rest of the treatments showed statistically insignificant results (Fig. 2g). Phenol was maximum and significant in the leaves of granular seaweed ( $G_3$ )treated plants, followed by  $P_3$  and  $V_3$ . Statistically similar results were obtained with  $G_2$  and  $V_2$  (Fig. 2h). Granular and powder seaweed treatments at higher concentration resulted in maximum and statistically similar increase in lycopene concentration, followed by  $G_2$ . Statistically insignificant results were obtained among  $G_1$ ,  $P_2$ ,  $V_2$ , and  $V_3$  treatments (Fig. 2i). Concentration of vitamin C was recorded maximum with  $G_3$ , followed by  $P_3$ . The amendments  $G_2$  and  $P_2$  also resulted in enhanced vitamin C content of fruits over other treatments and control (Fig. 2j).

## Discussion

Physicochemical properties of soil

Soils treated with *S. johnstonii* (granular and powder) and vermicompost showed significant improvement in

Table 3	Effect of	various	fertilizer	treatments	on	growth	parameters
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Treatments	Growth parameters										
	Vegetative (90	days)		Reproductive							
						Flowering	Fruiting (130 da	ys)			
	Number of branches	Root length (cm)	Shoot length (cm)	Plant fresh weight (g)	Plant height (cm)	(120 days) Number of flowers/plant	Number of fruits/plant	Fresh weight of fruits (g)			
Control	$3.3{\pm}0.33^{a}$	2.7±0.115 <sup>a</sup>	$18.5 {\pm} 0.49^{a}$	$1.26{\pm}0.036^{a}$	$21.3{\pm}0.60^a$	$0.0{\pm}0.0^{\mathrm{a}}$	$0.33{\pm}0.33^a$	$5.1 \pm 1.02^{a}$			
$G_1$	$8.6 {\pm} 0.66^{\circ}$	$8.6 {\pm} 0.152^{e}$	$24.6{\pm}2.18^{b}$	$6.9{\pm}0.37^{d}$	$33.2{\pm}2.25^{c}$	$4.6 \pm 0.33^{c, d}$	$4.0 \pm 0.577^{b, c}$	15.5±1.01 <sup>b, c</sup>			
G <sub>2</sub>	$10.6 {\pm} 0.34^{\circ}$	$9.6{\pm}0.120^{\rm f}$	$30.4{\pm}3.0^{b}$	$9.6 {\pm} 0.38^{e}$	$40.6{\pm}0.42^d$	$6.3{\pm}0.33^d$	$4.6 {\pm} 0.88^{\circ}$	$14.1 \pm 1.80^{b, c}$			
G <sub>3</sub>	$13.0{\pm}0.57^{d}$	$11.2 {\pm} 0.230^{g}$	$44.2 \pm 2.5^{c}$	$17.1\!\pm\!0.28^{\rm f}$	$53.7 {\pm} 0.55^{e}$	$9.0{\pm}0.57^{e}$	$8.6{\pm}0.88^{d}$	$20.2 \pm 1.86^{c}$			
P <sub>1</sub>	$6.3 \pm 0.64^{b, c}$	$3.01 {\pm} 0.56^{a}$	$19.5 \pm 1.24^{a}$	$2.64{\pm}0.35^{b}$	$22.5 {\pm} 0.56^{a}$	$2.0{\pm}0.24^{a, b}$	$1.56 {\pm} 0.64^{a, b}$	$7.3 \pm 1.23^{a}$			
P <sub>2</sub>	$9.6{\pm}0.67^{c}$	$4.53 \!\pm\! 0.656^{b}$	$24.5 \pm 1.5^{b}$	$4.6 {\pm} 0.38^{\circ}$	$28.6{\pm}1.8^{b}$	$3.6 {\pm} 0.33^{\circ}$	$4.0 \pm 0.57^{b, c}$	14.9±1.68 <sup>b, c</sup>			
P <sub>3</sub>	$13.0{\pm}0.58^d$	$6.23 \pm 0.176^{\circ}$	$42.5 \pm 0.4^{c}$	$16.4{\pm}0.29^{\rm f}$	$50.5 {\pm} 0.61^{e}$	$8.6 {\pm} 0.66^{e}$	$7.3 {\pm} 0.89^{d}$	$17.9 \pm 1.44^{c}$			
$V_1$	$3.0{\pm}0.53^{a}$	$2.92{\pm}0.23^{a}$	$17.56{\pm}0.23^{a}$	$1.25{\pm}0.012^{a}$	$21.5 {\pm} 0.026^{a}$	$0.8{\pm}0.45^{\mathrm{a}}$	$0.46{\pm}0.46^{a}$	$6.13 {\pm} 0.56^{a}$			
V <sub>2</sub>	$3.3\!\pm\!0.34^a$	$7.1 {\pm} 0.375^{d}$	$18.6 {\pm} 0.2^{a}$	$1.3 {\pm} 0.098^{a}$	$25.8{\pm}0.51^{b}$	$1.6 {\pm} 0.33^{a, b}$	$1.0{\pm}0.58^{a}$	13.8±1.76 <sup>b, c</sup>			
V <sub>3</sub>	$5.3{\pm}0.36^{b}$	$9.6{\pm}0.871^{\rm f}$	$18.5{\pm}0.3^a$	$2.9{\pm}0.246^{\text{b}}$	$28.1 \!\pm\! 1.24^{b}$	$3.3 \pm 0.33^{b, c}$	$2.0 \pm .0.58^{a, b}$	$12.4 \pm 1.12^{b}$			

Values are mean  $\pm$  standard error. Readings with different letters are significantly different (n=27, P=0.05) as analyzed by Tukey's post hoc test G soil amended with granular seaweed, P soil amended with powdered seaweed, V soil amended with vermicompost

soil health with increasing concentrations of each fertilizer. Seaweeds have a positive effect on soil pH, nutrient availability, and biological activity (respiration and nitrogen mobilization). Soil pH plays an important role as nutrients present in the soil are converted into soluble (dissolved in liquid) form by microorganisms optimally only within the "neutral" pH range of 6.0 to 7.3. The increased pH of seaweed-amended soil may be a function of Na<sup>+</sup> ion present in seaweed (Kantachote et al. 2004). In the present study, application of seaweed in granular or powder form not only brought the soil to a microbe-friendly pH range but also enriched it with both macro- and micronutrients, resulting in an improved overall growth and yield of tomato plant.

The present study shows that *S. johnstonii* can be used to improve the physicochemical properties of soil, which in turn results in increased plant growth and productivity. According to Temple and Bomke (1990), seaweeds are a rich source of polysaccharides which may affect soil aggregation directly or indirectly after decomposition by soil microorganisms. High bulk density creates unfavorable growing conditions for roots and restricts water to upper soil layers, thereby cutting off access to water and nutrients stored deeper in the soil (Abu-Hamdeh 2004). The reduced root length in control plants could be because of this reason. The aim of soil conditioning in the present study was to lower

bulk density of soil with seaweed amendment. Seaweed and seaweed products when added improve waterholding characteristics of soil and help in the formation of crumb-like structure. It is known that the alginic acid in brown seaweeds combines with metallic radicals to form a cross-linked polymer of increased molecular weight with higher moisture retention (Moore 2004). S. johnstonii, with alginic acid in the cell walls, enhances physicochemical properties of soil and leads to improved soil health. Improved soil structure leads to better aeration and capillary action that strengthen the root systems of L. esculentum (present work). Since the granular form resulted in better overall improvement when compared to powder form, it is quite evident that the form of seaweed biomass added to the soil plays an important role.

Capillarity and percolation of water are two other major soil characteristics of prime importance to plants and are extremely vital to microorganisms living in upper layers (Zodape 2001). Percolating water gradually dissolves incompletely decomposed organic matter, organic acids, and free hydrogen ions in the upper layers of soil and leaches them down into deeper strata where, on contact with bases, colloids get precipitated. According to Haslam and Hopkins (1996), addition of kelp increases porosity and water-holding capacity due to the presence of sodium alginate.



**Fig. 2** Effect of organic fertilizer amendments on biochemical properties of *L. esculentum*. Chlorophyll a (a), chlorophyll b (b), carotenoids (c), proteins (d), soluble sugars (e), reducing sugars (f), starch (g), phenols (h), lycopene (i), and vitamin C (j). *G* soil amended with

granular seaweed, P powdered seaweed, V vermicompost. Mean values followed by the different letters are significantly different. *Error bars* represent the standard error of replicates (n=71, P=0.05)

#### Morphological parameters

Though no comprehensive data are available on conditioning of soil with seaweed biomass and its effect on physiology of crop plants, there is ample evidence available for improvement in crop growth and yield with seaweed liquid foliar application (Washington et al. 1999; Kumari et al. 2011). In the present work, seaweed fertilizer in both forms resulted in better vegetative and reproductive growth of the plants. The findings confirm earlier studies carried out in Cajanus cajan, Zea mays, Vigna catajung, and Dolichos biflorus showing that seaweed concentrates from Caulerpa, Gracilaria, and Sargassum enhance branching (Anantharaj and Venkatesalu 2002). S. johnstonii as fertilizer was effective in not only increasing the number of flowers of L. esculentum but it also hastened flowering with maximum increase at higher concentration. Moniem et al. (2008) emphasized that the beneficial effect of algal extract in enhancing the biosynthesis and translocation of carbohydrate may be the reason for improved quality and early fruit maturity in banana. It can be assumed that early flower set in treated plants was elicited by overall improved vegetative plant growth. The seaweed treatment also led to an increase in fresh weight of tomato fruit.

#### **Biochemical parameters**

Application of seaweed liquid extract from Sargassum wightii (Sivasankari et al. 2006), S. johnstonii (Kumari et al. 2011), and Sargassum plagiophyllum (Bai et al. 2011) has been reported to enhance biochemical parameters such as photosynthetic pigments, proteins, amino acids, sugars, and amylases in Vigna spp. and L. esculentum. In the present investigation, concentrations of photosynthetic pigments and protein in leaves were recorded significantly higher with seaweed biomass treatments. One of the possible explanations for increased photosynthetic pigments can be the improved magnesium and copper concentration in soil resulting from application of S. johnstonii. In another study, Baniuniene and Zekaite (2008) concluded that though status of potassium in the soil may not have any direct role in increasing yield, yet it can lead to high starch concentration. Mohammadi et al. (2009) revealed that application of green manure increased protein content in chick pea seed. The present study indicates that seaweed-conditioned soils may support plants with a stronger defense against herbivores. Such plants raised on amended soil showed higher concentrations of phenol, which is known to play important role in providing protection against herbivores, pests, and pathogens.

However, more investigations need to be carried out in this direction to correlate soil conditioning and herbivory.

Lycopene is the principal pigment responsible for the red color of ripe tomatoes. Lycopene counteracts the harmful effects of free radicals which contribute to age-related processes, cell damage, and various types of cancer in humans (Choudhary et al. 2009). Due to their nutritional supplementing nature, scientists are nowadays interested in developing lycopene- and vitamin C-rich plant products. In the present work, it has been observed that there was a statistically significant increase in fruit lycopene content after granular and powder treatments as compared to control and vermicompost. Vitamin C (ascorbic acid) is another important phytochemical constituent of tomato fruit. It is an indicator of fruit ripening and plays an important role in various biochemical processes. High fiber content, high concentration of minerals, vitamins, and phenols such as flavonoids make tomato an excellent fruit vegetable with physiological and nutritional values (Dorais et al. 2008; Kumari et al. 2011). In the present study, all the three treatments, seaweed as well as vermicompost, showed an increase for vitamin C with rise in fertilizer concentrations.

In conclusion, the present study differs from most of the earlier studies carried out with seaweeds because the brown seaweed S. johnstonii was used as a soil conditioner. Many studies have been carried out earlier where seaweed liquid extracts/concentrates were used as potential fertilizer. The present study emphasizes that usage of seaweed in granular/powder form helps to improve soil texture and to condition it which is not possible with liquid extracts/concentrates. The improved physical, chemical, and biological properties of soil in turn influence plant growth and yield. The seaweed not only provides macro- and micronutrients to soil but also allows better aeration, water-holding capacity, and percolation. The present study recommends S. johnstonii as a better option than vermicompost as the former contributes more bioactive constituents, nutrients, and alginic acids. The brown seaweeds which are abundantly available as "drifts" collected from sea coasts or the fresh biomass obtained from mass cultivation can be used as soil supplements to meet the demands of organic farming.

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