Physiological, Biochemical, Growth, and Yield Responses of Radish (*Raphanus sativus* L.) Plants Grown on Different Sewage Sludge–Fly Ash Mixture (SLASH) Ratios

Bhavisha Sharma and Rajeev Pratap Singh

1 Introduction

Sustainable environmental management of solid wastes has become a significant issue of global concern. Rapidly growing rates of global population and urbanization coupled with ongoing industrial development have further escalated the generation of various types of solid wastes like sewage sludge (SS) and fly ash (FA) [23, 33]. SS is also referred to as biosolids that originate as a waste by-product of sewage treatment process [27], whereas coal FA is mainly an industrial waste produced as a result of coal combustion in thermal power plants and different industrial processes [13]. Both of these waste products are generated in enormous amounts and are disposed off openly at dumpsites occupying large area of land and also deteriorating the air, water, and soil quality of adjacent areas due to accumulation, leaching, and increased bioavailability of heavy metals, and other contaminants posing a threat to human health and environment. Rising solid waste generation has also been a key factor in increasing environmental pollution worldwide [34]. Hence, safe disposal of such wastes is not only a major challenge but also a deepening environmental concern these days.

Over the time, using SS and FA in agriculture has become quite popular around the world. SS is an abundant source of organic matter, and many essential micro- and macro-plant nutrients (N, P, and K); however, presence of toxic heavy metals, pathogens, and other organic micro-pollutants has caused apprehension about its use [2, 32]. FA although lacking macronutrients viz. nitrogen and organic carbon, contains many micronutrients like Ca, Si, Fe, Mg, Na, K, S, Mo, Ni, Cu, Co, Cd,

B. Sharma · R. P. Singh (✉)
Department of Environment & Sustainable Development, Institute of Environment & Sustainable Development, Banaras Hindu University, Varanasi, India
e-mail: rajeevprataps@gmail.com

B. Sharma
e-mail: bhavisha.sharma@gmail.com

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Pb, Zn, etc., and improves soil structure and water holding capacity [33]. Land application of these wastes has multiple benefits like improvement in soil's physico-chemical properties, recycling of beneficial plant nutrients, substitute to inorganic fertilizers, alkaline stabilization of SS using FA in place of lime to ameliorate acidity, reduce bioavailability of heavy metals and pathogenic microbes [12], along with agronomic and economic benefits. Apart from these benefits, combining SS and FA as mixture (SLASH) can be evaluated as an approach to integrated solid waste management, wherein wastes from two different waste streams (i.e., municipal and industrial) are utilized simultaneously to produce a mixture which is more stable and less likely to cause environmental pollution than their individual application [20]. This approach enables to tap potential benefits of SS in boosting plant growth and FA which serves as a composting ingredient to neutralize acidic SS thereby minimizing the bioavailability of heavy metals and the attendant injury to plants and crops. It also helps in treating both SS and FA as a valuable resource and not as waste being in conformity with the principles of integrated waste management hierarchy, which states recycling of wastes to be more environmentally preferred than disposal methods like landfilling, open and ocean dumping, and incineration [22].

The present study is an attempt to investigate the potential of SLASH mixtures as a plant growth medium through assessment of physiological, biochemical, growth, and yield responses of test plant Radish (*Raphanus sativus* L.) to determine the best mixture(s) which can be used further at different amendment rates/doses in soil to derive maximum fertilizing benefits for plants and minimum environmental risk.

2 Materials and Methods

2.1 Study Area

The experiment was conducted at the agricultural field of Institute of Environment and Sustainable Development, Banaras Hindu University, a suburban area of Varanasi, Uttar Pradesh, India between December 2016 and February 2017. This period of the year is characterized by mean monthly maximum temperature between 20.5 and 27.39 °C and mean monthly minimum temperature between 11.74 and 12.67 °C. The total rainfall during the experimental period was 5.08 mm. The mean maximum relative humidity ranged between 95.64 and 84.96% and mean minimum relative humidity between 76.09 and 38.10%.

2.2 Experimental Design and Raising of Plants

SS collected from Bhagwanpur sewage treatment plant, Varanasi was air dried and ground properly to make it homogeneous and FA was collected from Hindalco Indus-

Table 1 Description of different SLASH (sewage sludge–fly ash) mixture ratios

1	Control	Agricultural farm soil (AFS)
2	**SLASH A** (4:1)	4 parts sewage sludge + 1 part fly ash
3	**SLASH B** (4:2)	4 parts sewage sludge + 2 parts fly ash
4	**SLASH C** (4:3)	4 parts sewage sludge + 3 parts fly ash
5	**SLASH D** (4:4)	4 parts sewage sludge + 4 parts fly ash

tries Ltd., Renukoot, Sonbhadra, Uttar Pradesh, India. Pot experiment was carried out using Radish (*Raphanus sativus* L.) as the test plant grown at different SS and FA mixture ratios (SLASH). The treatments used in experiment comprised of SS and FA mixed uniformly in four different ratios viz. 4:1, 4:2, 4:3, and 4:4 designated as A, B, C, and D respectively. The SLASH mixture ratios were prepared in heaps as follows: A [4(SS): 1(FA)], B [4(SS): 2(FA)], C [4(SS): 3(FA)], and D [4(SS): 4(FA)] (Table 1) and left in the field for 10 days. Agricultural field soil was dug up to a depth of 30 cm, air dried and thereafter mixed uniformly, served as control. There were three pots of each treatment along with control. The pots were filled with the prepared SLASH mixtures uniformly and left for 5 days to stabilize. Necessary moisture levels were maintained and then six seeds were sown manually in each pot at equal distances. Thinning was done after germination of seeds to keep three plants in each pot. The test plants were grown upto maturity in the soil and different treatments were given using standardized agronomic practices. Identical light, water, and temperature conditions were maintained for all the treatments during the growth period of plants. Climatic variables like minimum and maximum temperature, rainfall, humidity, etc., were measured throughout the growing period of the plant. Selected physiological and biochemical parameters were quantified during vegetative and reproductive phases of plants for assessing the impact of different SLASH mixtures on pigments, metabolite contents, and antioxidants. Upon maturity, plants were harvested to examine the crop responses with respect to following parameters: morphological characteristics, biomass accumulated, and yield.

2.3 Analyses of Morphological or Growth Parameters, Biomass, and Yield

For the analysis of morphological or growth parameters and biomass determination, Radish plants were harvested in triplicate from different pots of each treatment at 45 and 65 DAS. Morphological parameters viz. root and shoot lengths, number of leaves ($plant^{-1}$), leaf area, and component-wise biomass were assessed. Leaf area measurements were done using portable leaf area meter (Model Systronics 211). For determination of biomass, after initial washing of plants to remove adhering soil particles, oven drying of separated parts (root and shoot) at 80 °C was done to achieve constant weight. Thereafter, separated plant parts were weighed individually to assess biomass accumulation expressed as g plant^{-1}. Yield was expressed in g pot^{-1} by calculating fresh weight of root (belowground part) per pot at the time of harvest.

2.4 Estimation of Physiological and Biochemical Parameters

Physiological and biochemical activities of test plant Radish were measured by estimation of photosynthetic pigments, antioxidants, and different metabolites in fully expanded fresh leaves of Radish plants sampled manually at 40 and 60 DAS and stored in deep freezer. These estimations were done through methods given by Machlachlan and Zalik [19] and Duxbury and Yentsch [9] for chlorophyll and carotenoid contents, respectively, Lowry et al. [18] for foliar protein content, Bray and Thorpe [6] for total phenol, Bates et al. [4] for proline, Britton and Mehley [7] to estimate peroxidase activity, Fahey et al. [10] for thiol content, and Heath and Packer [14] for lipid peroxidation.

2.5 Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 20 software. Duncan's multiple range test was performed as post hoc to test the significance of difference between the treatments.

3 Results and Discussion

3.1 Physiological Response

Raphanus sativus (Radish) plants grown at all the SLASH mixture ratios (viz. A, B, C, and D) showed significant increase in photosynthetic pigments reported as total chlorophyll as compared to the control plants (grown in agricultural farm soil) at 45 DAS (Fig. 1). However, at 65 DAS total chlorophyll content in Radish plants decreased, even though the content was higher in plants grown in all the SLASH mixtures as compared to control plants (Fig. 2). Increase in photosynthetic pigments (total chlorophyll) initially at 45 DAS can be ascribed to higher nutrient availability to plants provided by SS which is an ample source of essential plant nutrients (N, P, K, Mg S, Ca, Mn, Zn, etc.) [28], and its decrease at 65 DAS may be linked to increased degradation or lowered biosynthesis of chlorophyll molecules due to heavy metal induced oxidative stress [12, 26]. Alvarenga et al. [2] also reported increased chlorophyll content in *Lolium multiflorum* L. due to SS amendment which resulted in higher readily available form of total nitrogen ($N\text{-}NH_4^+$) content. Significant rise in accessory pigment carotenoid was also observed in Radish plants grown in different SLASH mixtures as compared to control plants at both ages of observations, i.e., 45 and 65 DAS (Figs. 1 and 2). Gupta and Sinha [11] reported an increase in carotenoid content in mung bean plants (*Vigna radiata* L. var. PDM 54) grown at 10 and 25% FA amendment. Increased carotenoid content which is a non-enzymatic antioxidant clearly indicates toward the active defense mechanism of Radish plants to protect the chlorophyll pigment against oxidative stress owing to heavy metals [15, 25]. Chl a/b ratio decreased due to different SLASH mixtures at both ages of observation; however, significant decrease was observed only in plants grown in SLASH mixture D at 45 DAS (Fig. 1). Chl a/b ratio is a good indicator of environmental stress and an increase in chl a/b ratio is the tolerance mechanism adopted by plants in presence of heavy metals [17]. Total chl/carotenoids ratio increased in plants grown across all the SLASH amendment mixtures at 45 DAS (Fig. 1); however, at 65 DAS total chl/carotenoids ratio decreased significantly in SLASH mixture B, C, and D plants (Fig. 2). Higher total chl/car ratio indicates greater production of chlorophyll as compared to carotenoids in Radish plants grown on different SLASH mixture ratios and reduction may be caused by heavy metals.

3.2 Biochemical Response

Lipid peroxidation quantified as malondialdehyde (MDA) concentration increased significantly in Radish plants grown on different SLASH mixture ratios as compared to those grown on control soil at both the ages, i.e., 45 and 65 DAS (Figs. 3 and 4). Increased lipid peroxidation (LPO) in plants grown on SS and FA mixture, quantified as malondialdehyde (MDA) content is a reliable indication of oxidative damage or peroxidation of membrane lipids caused by reactive oxygen species (ROS) generated due to heavy metal stress [17]. Proline content showed significant rise in plants grown at different SLASH mixtures as compared to respective control plants at both ages of observation (Figs. 3 and 4). Minimum increment in proline content was seen in SLASH mixture B followed by mixture A at 65 DAS (Fig. 4). Proline increment in plants is an indication of environmental stress and provides stress tolerance through scavenging of hydroxyl radicals, protection against dehydration of enzymes, and osmoregulation [24]. Peroxidases are important enzymatic antioxidant in plants involved in free radical detoxification. Peroxidase activity in Radish plants was reported to increase significantly in all the SLASH mixtures at 45 and 65 DAS (Figs. 3 and 4) as compared to plants grown in control soil except for SLASH mixture D which significantly decreased at 65 DAS (Fig. 4) indicating heavy metal induced oxidative stress. Protein content in Radish increased signifi-

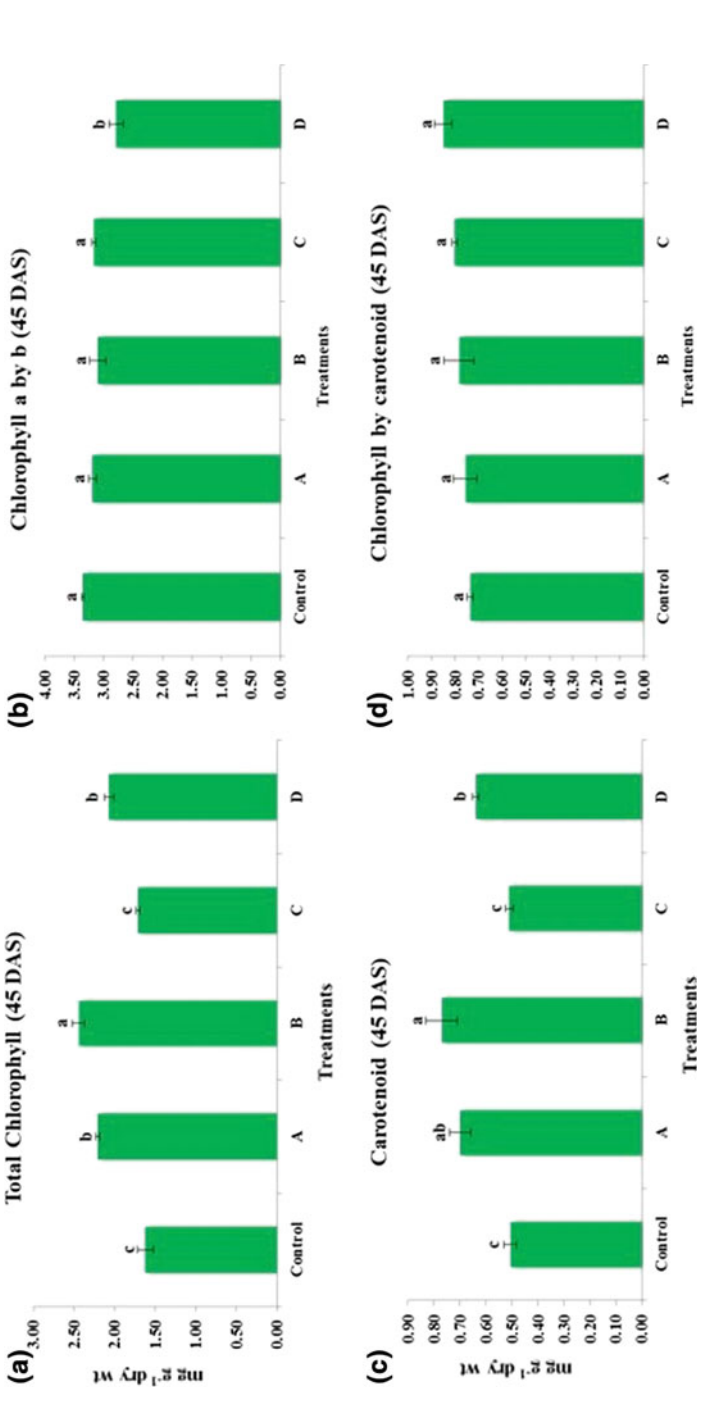

Fig. 1 Variations in physiological parameters **a** Total chlorophyll, **b** Chlorophyll a by b, **c** Carotenoids, and **d** Total chlorophyll by carotenoids of *Raphanus sativus* plants grown in control soil and SLASH mixture ratios at 45 DAS (Mean ± 1SE). Bars with different letters in each group show significant difference at $p < 0.05$

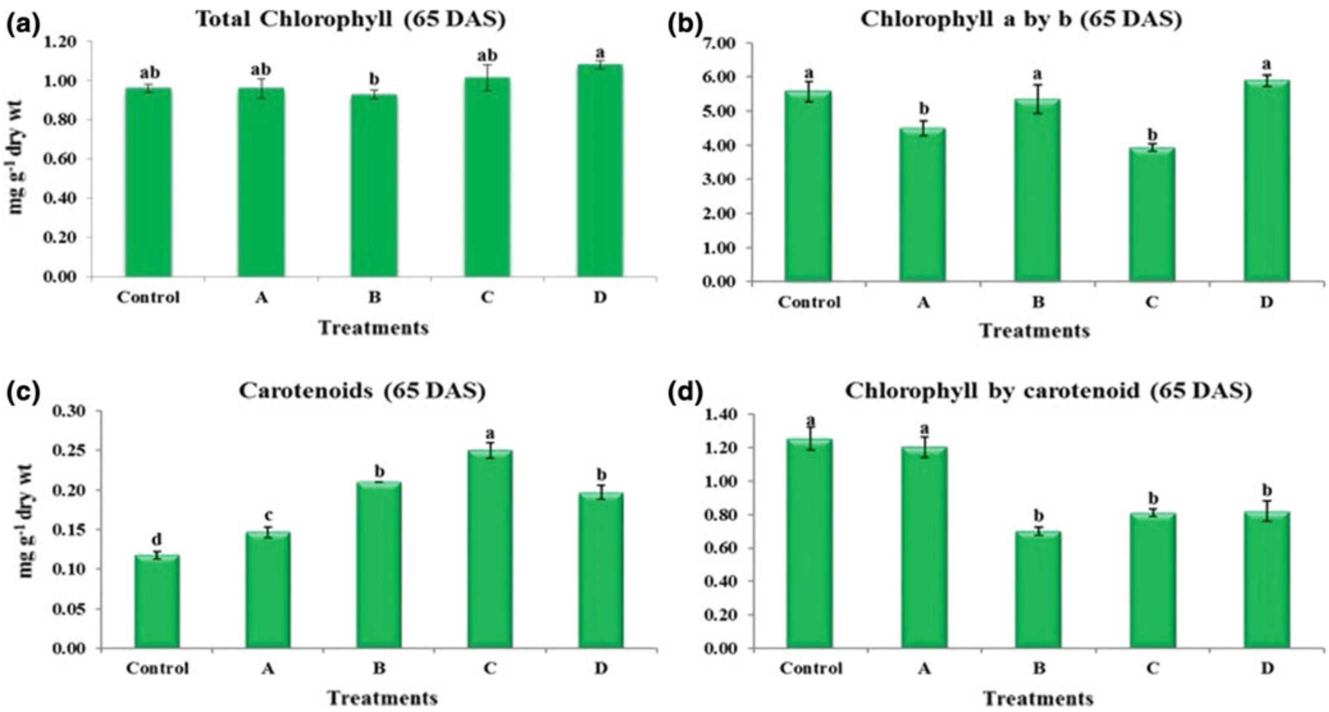

Fig. 2 Variations in physiological parameters **a** Total chlorophyll, **b** Chlorophyll a by b, **c** Carotenoids, and **d** Total chlorophyll by carotenoids of *Raphanus sativus* plants grown in control soil and SLASH mixture ratios at 65 DAS (Mean ± 1SE). Bars with different letters in each group show significant difference at $p < 0.05$

cantly under all the SLASH mixtures as compared to those grown in control soil; however, the increase was higher at 45 DAS than 65 DAS (Figs. 3 and 4). Maximum increment in protein content was shown by plants grown in SLASH mixture B and A at 45 DAS (Fig. 3). Similar findings of increased lipid peroxidation, proline content, peroxidase activity, and protein content in plants (*B. vulgaris, A. esculentus* and *O. sativa, and V. radiata*) grown on SS and FA amendments have been reported by many researchers [11, 23, 26, 28, 29]. The results are also in agreement with the findings of Gupta et al. [12] who also reported increased foliar protein content in *Brassica campestris* L. (cv. Pusa Jaikisan) grown in mixtures of tannery sludge and FA in the ratio of 4:1 (A) and 4:2 (B) in comparison to control plants at different amendment rates. Higher protein content in Radish plants under different SLASH ratios may be credited to the higher availability of organic matter and nitrogen contents due to SS [22, 27]. It could also be due to heavy metal stress [26] as both SS and FA contain heavy metals. Thiol content in Radish increased significantly across all the SLASH mixtures, in comparison to control plants at 45 DAS; however, at 65 DAS significant increase was seen only in plants grown at SLASH mixture B (Figs. 3 and 4). Thiols help in fighting oxidative stress induced by heavy metals as an important part of non-enzymatic defense adopted by plant cells through detoxification of metals and phytochelatin synthesis [8, 11]. Significant increase in thiol content was also reported in nodulated alfalfa plants due to application of SS [3]. Phenol, a secondary metabolite, also plays an important role in providing resistance against many stresses in plants and protects the plants against oxidative stress through scavenging of free radicals [25, 29]. Phenol content increased significantly in Radish plants grown in different SLASH mixtures as compared to respective control at both 45 DAS and 65 DAS (Figs. 3 and 4). Both Singh and Agrawal [26] and Singh et al. [25] reported a decrease in foliar phenol content in *Beta vulgaris* grown at different SS and FA amendment rates, respectively.

3.3 Morphological or Growth and Yield Response

In the present study, growth parameters of Radish such as root length, shoot length, number of leaves, leaf area, root biomass, shoot biomass, and total biomass increased significantly in plants grown in different SLASH mixtures as compared to control plants at both ages of observation (45 and 65 DAS) except for a decrease shown by parameter root length in SLASH mixture D (at 45 DAS) (Table 2). Maximum increment in growth parameters at 45 DAS was shown by Radish plants grown in SLASH mixture A followed by C; and at 65 DAS it was seen in plants grown on SLASH mixture A followed by B (Table 2). Similarly, yield of Radish increased significantly in all the SLASH mixtures; however, maximum increase was shown by plants grown in mixture A (87.8%) followed by C (87.5%) and B (84.8%), respectively (Table 2). Positive morphological response or increase in growth parameters and yield in plants due to SS and/or FA amendments have been reported in many studies such as Singh and Agrawal [28, 30, 31] (lady's finger [*Abelmoschus esculentus* L. var Varsha uphar],

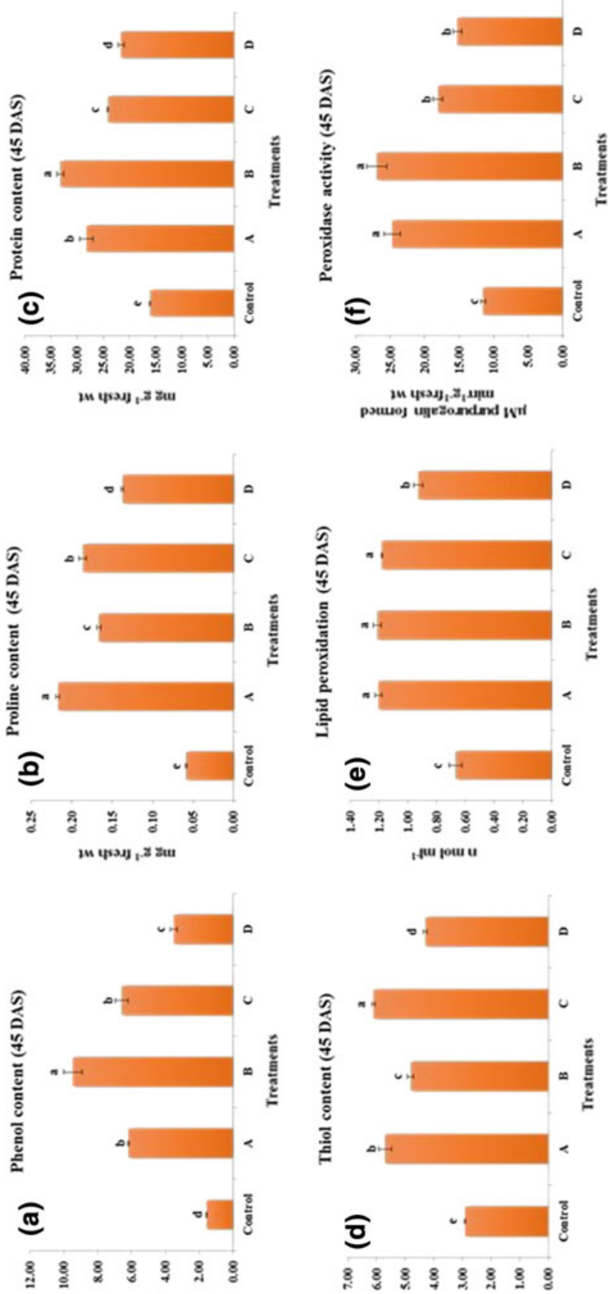

Fig. 3 Variations in biochemical parameters **a** Phenol content, **b** Proline content, **c** Protein content, **d** Thiol content, **e** Lipid peroxidation, and **f** Peroxidase activity of *Raphanus sativus* plants grown in control soil and SLASH mixture ratios at 45 DAS (Mean ± 1SE). Bars with different letters in each group show significant difference at $p < 0.05$

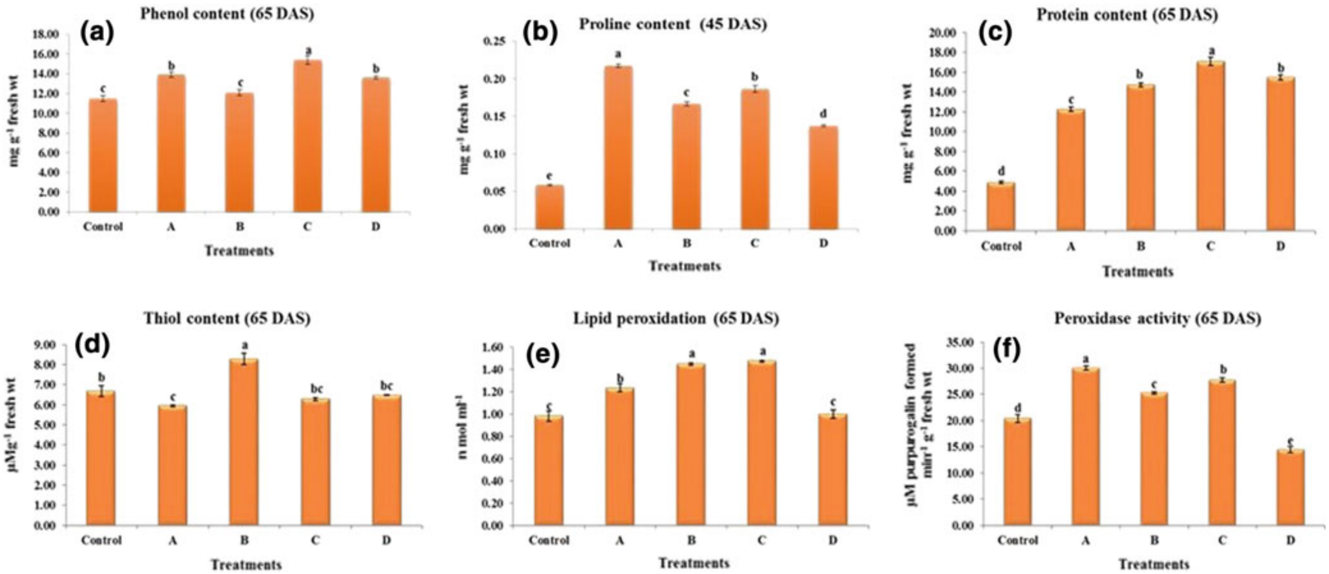

Fig. 4 Variations in biochemical parameters **a** Phenol content, **b** Proline content, **c** Protein content, **d** Thiol content, **e** Lipid peroxidation, and **f** Peroxidase activity of *Raphanus sativus* plants grown in control soil and SLASH mixture ratios at 65 DAS (Mean ± 1SE). Bars with different letters in each group show significant difference at $p < 0.05$

Table 2 Variations in different morphological characteristics of *Raphanus sativus* plants grown in control soil and different SLASH ratios at 45 and 65 DAS (Mean ± 1SE)

	Treatments				
Parameters	Control	SLASH A	SLASH B	SLASH C	SLASH D
45 DAS					
Root length (cm)	12.8 ± 0.9^{bc}	16.17 ± 1.09^{a}	12.93 ± 1.11^{bc}	14.7 ± 1.01^{ab}	11.47 ± 0.59^{c}
Shoot length (cm)	19.7 ± 0.86^{b}	37.33 ± 1.09^{a}	39.20 ± 0.56^{a}	39.60 ± 1.00^{a}	37.70 ± 0.61^{a}
Leaf number ($plant^{-1}$)	5.00 ± 0.0^{d}	9.67 ± 0.33^{a}	7.67 ± 0.33^{bc}	8.33 ± 0.67^{b}	7 ± 0.00^{c}
Leaf area (cm^2)	51.1 ± 3.3^{d}	910.0 ± 44.95^{a}	523.73 ± 31.3^{b}	628.9 ± 53.9^{b}	400.23 ± 11.48^{c}
Root biomass (g $plant^{-1}$)	0.03 ± 0.00^{e}	0.40 ± 0.00^{a}	0.10 ± 0.01^{d}	0.25 ± 0.00^{b}	0.12 ± 0.00^{c}
Shoot biomass (g $plant^{-1}$)	0.21 ± 0.01^{d}	3.97 ± 0.24^{a}	1.73 ± 0.09^{c}	2.70 ± 0.15^{b}	1.44 ± 0.07^{c}
Total Biomass (g $plant^{-1}$)	0.24 ± 0.0^{d}	4.36 ± 0.24^{a}	1.83 ± 0.09^{c}	2.95 ± 0.15^{b}	1.56 ± 0.07^{c}
65 DAS					
Root length (cm)	11.97 ± 0.4^{c}	22.47 ± 1.74^{a}	18.47 ± 1.41^{b}	18.23 ± 1.09^{b}	19.73 ± 0.90^{ab}
Shoot length (cm)	17.5 ± 0.76^{b}	41.33 ± 2.01^{a}	38.67 ± 2.13^{a}	38.10 ± 1.35^{a}	37.93 ± 1.85^{a}
Leaf number ($plant^{-1}$)	5.00 ± 0.0^{d}	13.67 ± 0.33^{a}	13.33 ± 0.88^{a}	11.00 ± 0.58^{b}	9.00 ± 0.58^{c}
Leaf area (cm^2)	77.5 ± 5.76^{c}	1257.3 ± 103.3^{a}	1339.03 ± 126^{a}	680.70 ± 47.3^{b}	682.17 ± 28.77^{b}
Root biomass (g $plant^{-1}$)	0.10 ± 0.01^{d}	1.39 ± 0.08^{a}	0.80 ± 0.03^{b}	0.43 ± 0.02^{c}	0.42 ± 0.02^{c}
Shoot biomass (g $plant^{-1}$)	0.39 ± 0.02^{d}	6.24 ± 0.21^{a}	5.33 ± 0.44^{b}	2.42 ± 0.19^{c}	2.40 ± 0.13^{c}
Total Biomass (g $plant^{-1}$)	0.48 ± 0.02^{d}	7.63 ± 0.14^{a}	6.13 ± 0.47^{b}	2.85 ± 0.21^{c}	2.81 ± 0.15^{c}
Yield (g pot^{-1})	1.82 ± 0.04^{d}	14.93 ± 0.14^{a}	12.01 ± 0.18^{b}	14.63 ± 0.21^{a}	10.03 ± 0.44^{c}

Different letters in each group show significant difference at $p < 0.05$.
A= 4:1 Sewage sludge: Fly Ash;
B= 4:2 Sewage sludge: Fly Ash;
C= 4:3 Sewage sludge: Fly Ash;
D= 4:4 Sewage sludge: Fly Ash

rice [*Oryza sativa* L. cv. Pusa sugandha 3], and mung bean [*Vigna radiata* L.]), Bhat et al. [5] (Radish [*Raphanus sativus* L.]), Latare et al. [16] (wheat [*Triticum aestivum* L. cv. Malviya 234]), Ajaz and Tyagi [1] (cucumber [*Cucumis sativus*]), Gupta and Sinha [11] (mung bean [*Vigna radiata* L. var PDM 54]), Sajwan et al. [21] (sorghum sudangrass [*Sorghum vulgaris*]), and Wong and Selvam [35] (*Brassica chinensis*). However, significant reductions in growth parameters in palak plants (*Beta vulgaris* L. var. Allgreen H-1) grown in different SS and FA amendments were reported by Singh and Agrawal [26] and Singh et al. [25], respectively. Significant increase in growth parameters and yield in Radish plants in the present study can be ascribed to higher availability of plant growth promoting macro- and micronutrients present in SS and FA, whereas reduction in these parameters occurs due to higher concentration of heavy metals in plant tissues causing phytotoxicity or due to variable tolerance of different plant species to heavy metals and salt stress [13, 22, 35].

4 Conclusions

The present study clearly showed significant rise in biochemical parameters (viz. proline, protein, carotenoids, phenol, thiol, and peroxidase activity) and plant productivity (chlorophyll content, total biomass, leaf area, and yield) of Radish plants grown on SLASH mixtures A, B, and C indicating toward strong antioxidant defense of Radish plants against heavy metal stress to maintain increments in their growth parameters, biomass accumulation, and yield. Hence, SLASH mixtures A, B, and C may be a good option to utilize as a fertilizer supplement (at different amendment rates/doses) or plant growth medium for Radish plants, but the amendment rates/doses in soil should be strictly regulated to avoid food chain contamination and risk to human health due to heavy metals.

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