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RESEARCH ARTICLE

Can biochar and chitin as organic soil amendments be alternatives to manures for rocket (*Eruca sativa* Mill.) growth?

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ABSTRACT

Organic amendments are sustainable, and relatively cheap materials derived from plants and animals that are supplemented into the soil for the restoration of the soil's physical and chemical properties, and provide the plants' nutrients before seeding, increasing its productivity. The addition of organic amendments to agricultural soil is recommended to improve soil functions and plant growth. The objective of this study was to evaluate the impact of different organic soil amendments (biochar chitin, horse, sheep manure) on the growth of rocket plants (Eruca sativa Mill.) and answer if biochar and chitin can be alternatives to manures. This study was conducted in pots, under various combinations of organic amendments, using a *typic xerofluvent* agricultural soil, in the spring of 2016. To experiment, four organic amendments (biochar, chitin from shrimp shells, horse and sheep manure) were employed to amend the soil, in a completely randomized design, analyzing the impact of 4% and 8% biochar, 2% and 3% chitin, as well as 2% and 5% horse or sheep manure on the growth of rocket (*Eruca sativa* Mill.). According to the findings, the best growth rate of the rocket was found in the sheep manure amendment at 2% rate, followed by the 5% rate, in the horse manure amendments at 2% and 5% rates, in the biochar amendment at 4% rate, and in chitin at 3% and 2% rates consecutively. Positive effects of organic amendments on plant growth were more evident under the amendments related to sheep manure at a 2% rate.

Keywords: Biochar; Chitin; Growth characteristics; Horse manure; Sheep manure

INTRODUCTION

Organic amendments are sustainable, and relatively cheap materials derived from plants and animals that are supplemented into the soil for the restoration of the soil's physical and chemical properties, and provide the plants' nutrients before seeding, increasing its productivity (Adebayo et al., 2011; Asgharipour & Rafiei 2011). The research concerning the use of organic amendments on soils has mainly focused on agricultural soils. The effect of organic amendments on plant growth is due to the slow mobilization of plant nutrients whereas it depends on the formation of biological succession in the soil food web (Yavuzaslanoğlu 2015).

The application of organic amendments to soils, such as biochar, chitin, and manure can supply it with nutrients and, thus improve the soil structure contributing to plant growth.

Biochar is a carbon-rich product resulting from the pyrolysis of organic materials, and although its application is a very old method of improving soil quality and plant growth (Barbosa Lima et al., 2015), it is considered to have the potential of improving plant growth (Guo, 2020). It could also potentially be used as a soil amendment to improve the quality of agricultural soils, therefore increasing crop production (Chan et al., 2007; Ojeda et al., 2012; Egamberdieva et al., 2016). There were no clear impacts of biochar on plant physiological indicators, though Liu et al. (2016). This is since only a few studies included crop growth (Chan et al., 2007; Tammeorg et al., 2014). For instance, Borchard et al. (2014) reported that in a pot experiment with maize in Germany, not noticeable yield effects were recorded after the application of biochar. The addition of biochar in pot soil at differing amounts of 4, 8, 16, and 32% of char-soil (v/v) has promoted the Asian lotus growth. Still, the impacts of

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biochar on plant physiological indicators were not clear (Liu et al., 2016).

Chitin is a biopolymer abundant in nature, being the second-most common polysaccharide after cellulose. When added to the soil, chitin depolymerizes through chitinase activity in the soil, as there is a particular microflora associated with its decomposition. Consequently, it is a promising organic soil amendment for improving soil quality and plant growth (Debode et al., 2016), while chitin based treatment to a range of crops should not be underestimated since improvements in plant growth have been reported after its application (Liopa Tsakalidi et al., 2010; Sharp 2013).

On the other hand, animal fertilizers, such as horse manure, have long been known as a suitable source of organic matter (Ahmadi & Jafarpour 2015). Composted horse manure can always be used for soil amendment and nutrient supplement in crops (Ancy & Thamaraiselvi 2014) since it is such a valuable resource in terms of plant nutrients that it should be utilized as a fertilizer and soil conditioner on agricultural soils (Parvage et al., 2015). Jannoura et al. (2013) demonstrate that horse manure has a positive effect on the grain yield of the winter wheat.

In the countries of the Mediterranean basin, sheep manure is traditionally used as an organic fertilization source. Ewulo (2005) reported that sheep manure is more effective than cattle manure in improving the yield of millet. It is also a credible alternative to inorganic fertilizers as far as the development of the organic vegetable production business is concerned (Pavlou et al., 2007). Sheep-manure vermicompost is a soil supplement that increases tomato yields, soluble as well as insoluble solids, and carbohydrate concentrations (Gutiérrez-Miceli et al., 2007). Bell pepper from plants grown in soil mixed with sheep manure vermicompost was reported to have given better post-harvest conservation characteristics, as they contained more soluble solids. The pH was lower, and the titratable acidity was higher (Villarreal 1982).

Rocket salad (*Eruca sativa* Mills) is a leafy vegetable crop, belonging to the Brassicaceae family, and being native to the Mediterranean geographic area. Its cultivation area has expanded in the last years in many Mediterranean countries, because of the growing interest of consumers (Pimpini & Enzo 1997). It is served either raw as a salad itself or grilled and mixed with other vegetables.

All things considered, the main objective of this greenhouse study was to evaluate the impact of different organic soil amendments (biochar chitin, horse, sheep manure) on the growth of rocket plants (*Eruca sativa* Mill.) and answer if biochar and chitin can be alternatives to manures.

MATERIALS AND METHODS

Organic amendments

This study was conducted to examine the response of rocket plants (*Eruca sativa* Mill. cv Rucola coltivata, GeoStore, Greece) under various combinations of organic amendments, using local soil, in the spring of 2016. The soil was collected from a site in Amaliada, Ilias, in Western Greece, and it constitutes a *typic xerofluvent* agricultural soil of this area (Soil Survey Staff, 2014) since the site had had a long history of cropping. A composite sample was collected from the surface layer (0-30cm) according to the LUCAS sampling methodology (Toth et al., 2013). The soil samples were air-dried and grounded to pass through a 2 mm sieve for laboratory analysis. The soil physical and chemical characteristics (Table 1) were then determined according to Page et al. (1982).

To carry out the greenhouse pot experiment, four organic amendments, namely biochar (Carbon Terra GmbH) (Table 2), Chitin from shrimp shells (Sigma Aldrich, CAS Number 1398-61-4), horse, and sheep manure, were employed to amend the soil. The horse and sheep manure were collected from fermentation heaps from local farms. Each amendment was added to the soil at two rates (4% and 8% biochar, 2% and 3% chitin, 2% and 5% horse or sheep manure). The concentrations of soil amendments were chosen considering the literature (Major, 2010; Guo et al., 2016). Each category of organic amendments was mixed with soil at volume per volume (v/v) percent ratios. Non-amended soil was used as a control. The experiment was laid out in a completely randomized design.

Greenhouse pot experiment

Initially, ten rocket seeds were planted in 2-liter pots, and after germination, the plants were thinned to five per pot. The treatments were arranged in a randomized design with five replicates and five plants per pot. During the growing period (56 days), the pots were kept in a naturally

Table 1: Characteristics of used agricultural soil and organic amendments

Soil Type	Agricultural Soil	Horse Manure	Sheep Manure
Texture	Clay Loam		
WHC (g/100g)	41.6		
pH	7.4	8.7	7.3
pH-value ($CaCl_2$)	7.2	8.4	7.2
EC (μS cm^{-1})	264	3400	1790
CEC (mmolc kg^{-1})	17.2	49.7	40.5
N_{tot} (%)	0.5	1.6	1.2
C_{inorg} (%)	0.42	0.95	1.19
C_{org} (%)	0.7	54.6	53.4
ρ_B (g cm-^{3})	1.35	0.23	0.19

WHC = Water Holding Capacity. EC = Electrical Conductivity. CEC = Catio Exchange Capacity. C_{inorg} = inorganic carbon. C_{org} = inorganic carbon. ρ_B = bulk density

Table 2: Characteristics of the biochar used in the experiments.

Parameter of biochar					
Water, 4h, 103°C (%)	15.6	Cr (g/t)	10.6	K (g/kg)	7.01
Ash (%)	9.9	Ni (g/t)	7.71	Mg (g/kg)	2.5
Volatile matter (%)	77.2	F (mg/kg)	<80	Ca (g/kg)	22.9
H (%)	1.05	Cu (g/t)	10	P (g/kg)	0.57
C (%)	76.8	Zn (g/t)	34.5		
N (%)	1.4	Mn (mg/kg)	626		
TOC (%)	71.3	Pb (g/t)	1.1		
ρB (g/cm3)	0.14	Cd (g/t)	0.1		
pH (H2O)	9.98	Hg (g/t)	<0.02		

illuminated greenhouse and a temperature range between 22 and 28°C. The vegetative data of the rocket plants were being collected every 7 days, starting approximately on the 27th day after sowing and finishing on the 56th day. Plants were being watered daily throughout the growth period keeping the soil moisture content to field capacity.

Growth characteristics

The growth characteristics that were measured were: plant height (cm), length of the upper part of the plant (cm), No 1, 2, 3, and 4 leaf length (cm), fresh and dry root weight (g), No 1, 2, 3 and 4 fresh and dry leaf weight, and the number of leaves per plant. The length was measured using a meter scale in cm. Leaf length was measured on different days after sowing. The plant leaf positions were determined from base to apex and were numbered from No 1 to No 4 (base to apex), indicating their relative positions. Leaf ages were not determined at all. Concerning the leaves, four leaves from each plant were first measured followed by the measurement of their fresh and dry average weight, and finally, their mean was calculated. As for the fresh and dry leaves weight, fresh leaves weight was recorded and dry leaves weight was determined after drying the samples in an oven at 70°C until achieving constant weight. Weight measurements were taken with an Electronic Precision Balance in grams (g).

Statistical analysis

The data analysis for the vegetative growth data of the rocket (*Eruca sativa* Mill.) in different rates of soil, enriched with chitin, biochar, sheep, and horse manure was carried out according to the randomized complete block design. The outcome of each examined trait was ranked according to Duncan's multiple range test, and the Post-Hoc comparison was used alternatively with the Student-Newman-Keuls (SNK) and the Tukey methods.

RESULTS AND DISCUSSION

Plant height

The plant growth observation of the rocket (*Eruca sativa* Mill.) lasted 56 days under a greenhouse pot experiment. The plant height of the rocket was low (9.5cm) in the control, and high when grown with organic soil amendments (10.3cm in biochar, 10.6cm in chitin, 12.2cm in horse manure, and 13.7cm in sheep manure amendments). The rocket plant height with the sheep manure amendment evoked the best response as compared to the other organic soil amendments (Fig. 1).

Data in Fig. 1 showed that the most significant increase of plant height (40.3 and 36.4cm) was found with the sheep manure amendment at a 2% rate, followed by the 5% amendment rate, respectively. Moreover, the horse manure amendment at 5 and 2% rates enhanced favorable effects on plant height, when compared to the control treatment. As the data in Fig.1 depict that chitin amendment at 2 and 3% rates increased significantly the plant height at 32.0cm, and 32.8cm and biochar amendment at 4% rate provoked a plant height increase of 33.3cm.

Length of the upper part

The upper part length of the rocket reached its highest in the sheep manure amendment, which is 24.6cm higher than the control (18.2cm). Similarly, horse manure (22.5cm) was also on par with chitin (21.9cm), which was significantly more than the control.

The minimum upper part increase of the rocket in organic soil amendments was recorded under chitin treatment (20.2cm) (Fig. 2).

The sheep manure amendment at a 2% rate caused the tallest upper part length of rocket plants (26.7cm) among the treatments, followed by the horse manure amendment at a 5% rate. An upper part length of 18.2cm was recorded in the control, where non-amended soil was employed. During the growth experiment, it was observed that with most treatments, the increase in the upper part length of the rocket only continued up to the 49th day after the sowing. The exception was at the sheep and horse manure amendments at 2% rates, where the growth continued until the end of the experiment.

Root length

The root length of the rocket was highest in the sheep manure amendment, where it was 13.7cm higher than in the control (9.5cm). Similarly, the biochar amendment was also on a par with the sheep manure amendment, which was significantly less than in the control (Fig. 3).

Root length of the rocket grown in sheep manure amendment at 5 and 2% rates, and in horse manure amendment at 2% rate was 13.9cm, 13.2cm, and 13.0cm more than in the control, respectively. Chitin amendment rate at 3% was on a par with the horse manure amendment

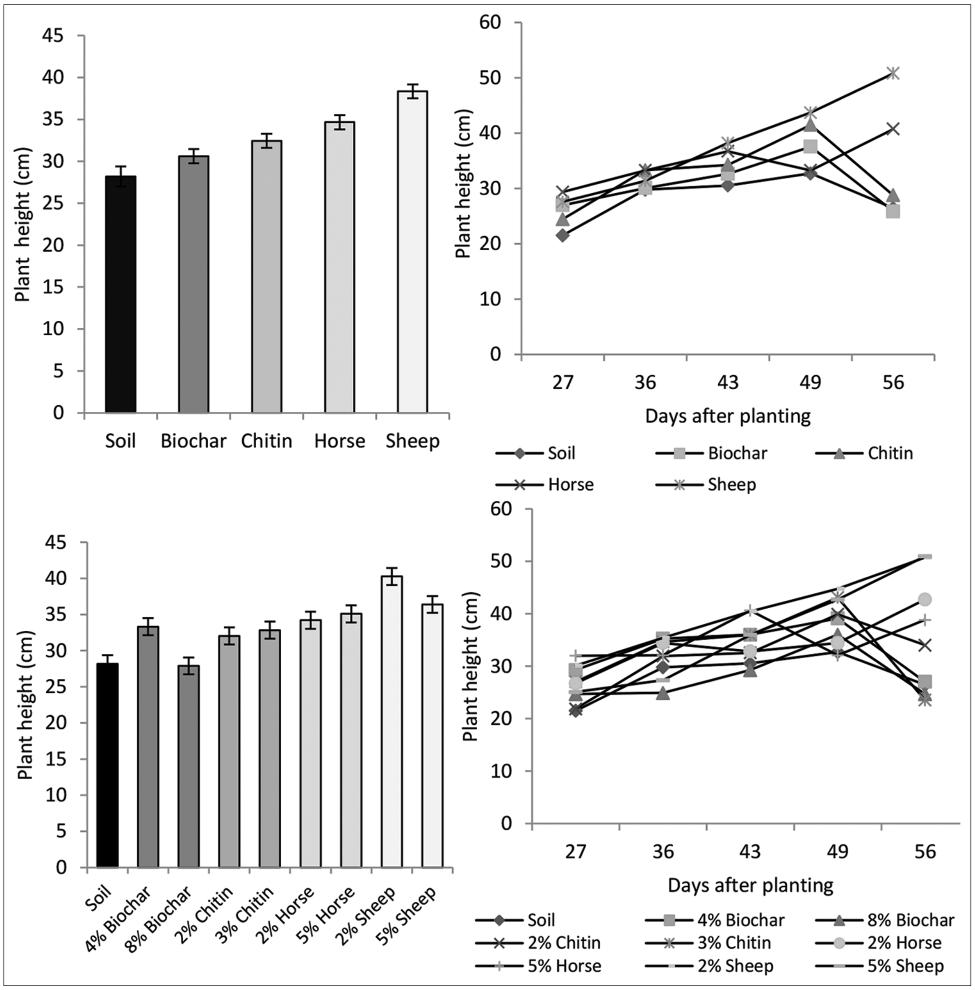

Fig 1. Effect of organic soil amendments on the plant height of rocket (*Eruca sativa* Mill.) during the growth period.

at 5% rate, and biochar amendment at 4% rate, which in turn was significantly higher than in the control. During plant growth in the greenhouse, the sheep manure amendment at a 5% rate was followed by the sheep manure amendment at a 2% rate in the soil, where it primarily increased the root length of the rocket (Fig. 3).

Leaf length

The length of No 1, 2, 3, and 4 leaves of the rocket was low in the control and high in the organic soil amendments, while their length was longer in the horse manure amendment at a 5% rate. Similarly, sheep manure amendment at 2 and 5% rates were also on par with the biochar manure amendment at a 4% rate, which still was significantly higher than the control (Fig. 4).

Leaf and root weight

Both the fresh and dry leaves of the rocket were heavier when grown in horse manure amendment at a 5% rate, followed by sheep manure amendment at 2% rate, biochar amendment at 4% rate, and sheep manure amendment at 5% rate, respectively. The minimum weight of fresh and dry leaves of the rocket in organic soil amendments (20.2cm) was recorded in the horse manure amendment at a 2% rate (Table. 3). Moreover, as compared to the control, the fresh and dry leaves of the rocket were observed to be lighter in biochar amendment at an 8% rate, and chitin amendment of soil at both 2 and 3% rates (Table. 3)

The weight of the dry rocket leaves was the highest in the sheep manure amendment at a 2% rate, followed by the 5% rate, and the horse manure amendment at a 5% rate respectively (Table. 3).

The weight of the fresh rocket root was highest in the sheep manure amendment at a 5% rate, followed by the sheep manure amendment at a 2% rate, biochar amendment at 4 and 8% rates, and horse manure amendment at 5% rate, respectively. Furthermore, in comparison to the control, less weight of the fresh rocket leaves was observed in the horse manure amendment at a 2% rate, and biochar and chitin soil amendments at both 2 and 3% rates (Table 3).

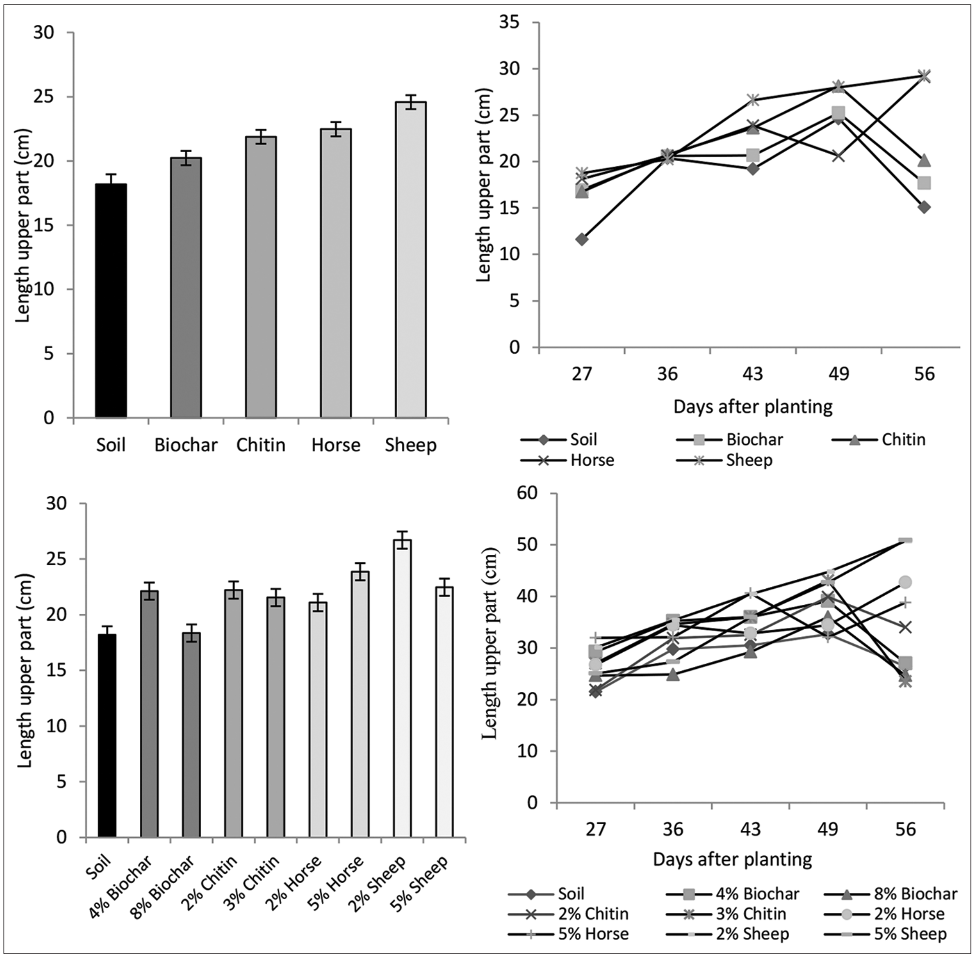

Fig 2. Effect of organic soil amendments on the upper part length of rocket (*Eruca sativa* Mill.) during the growth period.

Table 3: Effect of organic soil amendments on the weight of both the fresh and dry leaf and root of the rocket (*Eruca sativa* Mill.).

Soil types	Leaf		Root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Soil	$7.69^{a} \pm 1.72$	$0.87^{a} \pm 0.20$	$0.57^{a} \pm 0.20$	$0.31^{a} \pm 0.08$
4% Biochar	$9.00^{b} \pm 1.72$	$0.92^{b} \pm 0.20$	$0.65^{b} \pm 0.20$	$0.21^{b} \pm 0.08$
8% Biochar	$6.02^{c} \pm 1.72$	$0.68^{c} \pm 0.20$	$0.60^{c} \pm 0.20$	$0.28^{a} \pm 0.08$
2% Chitin	$6.55^{c} \pm 1.72$	$0.65^{c} \pm 0.20$	$0.55^{a} \pm 0.20$	$0.20^{c} \pm 0.08$
3% Chitin	$6.15^{c} \pm 1.72$	$0.65^{c} \pm 0.20$	$0.50^{d} \pm 0.20$	$0.20^{c} \pm 0.08$
2% Horse	$5.80^{d} \pm 1.72$	$0.65^{c} \pm 0.20$	$0.45^{e} \pm 0.20$	$0.10^{d} \pm 0.08$
5% Horse	$9.80^{b} \pm 1.72$	$1.00^{d} \pm 0.20$	$0.60^{c} \pm 0.20$	$0.20^{c} \pm 0.08$
2% Sheep	$9.60^{b} \pm 1.72$	$1.10^{d} \pm 0.20$	$0.65^{b} \pm 0.20$	$0.20^{c} \pm 0.08$
5% Sheep	$8.70^{e} \pm 1.72$	$1.00^{d} \pm 0.20$	$0.75^{e} \pm 0.20$	$0.30^{a} \pm 0.08$

The dry weight of the rocket root was the highest in the control (Table 3).

The four organic amendments used in these experiments did expose conspicuous differences in the growth of the rocket since the growth characteristics were higher than in the control. Positive effects of organic amendments on plant growth were more evident under the amendments related to sheep manure. The results of the study indicated the usefulness of sheep manure on plant height. The longest and shortest plant height measurements were recorded in the sheep manure amendment at a 2% rate and in the control, respectively. Azarmi et al., (2009) demonstrated that vermicompost produced from sheep manure had increased plant height when compared to the control for cucumber 30, 60, and 90 days after transplanting. Moreover, the weight of the dry rocket root was higher in the control, which also agrees with Mahmoodabadi et al. (2011). The high biochar amendment at an 8% rate to soil resulted in a decrease of the upper part of the plant height when compared to the control. On the contrary, the low biochar amendment at a 4% rate increased the upper part of the plant and the plant height. Gravel et al. (2013) have shown that in unamended potting soil, the growth effect of biochar amendment to potted plants depends on the plant species when biochar is provided in large proportions. Specifically, Song et al. (2014) showed that garlic grew faster when planted in biochar-amended soil and had a higher average plant height than that of those planted in the reference soil. Similarly, Viger et al. (2015) in the Arabidopsis and the crop plant lettuce, observed that

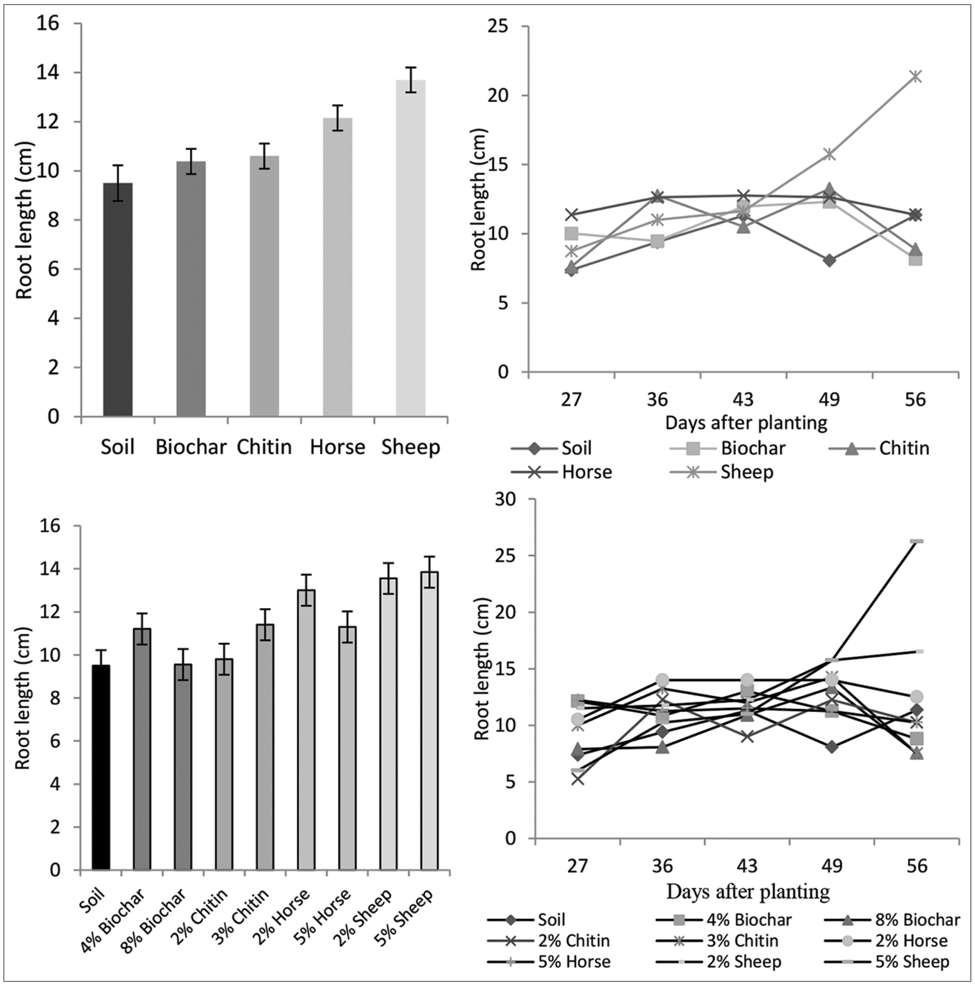

Fig 3. Effect of organic soil amendments on the root length of rocket (*Eruca sativa* Mill.) during the growth period.

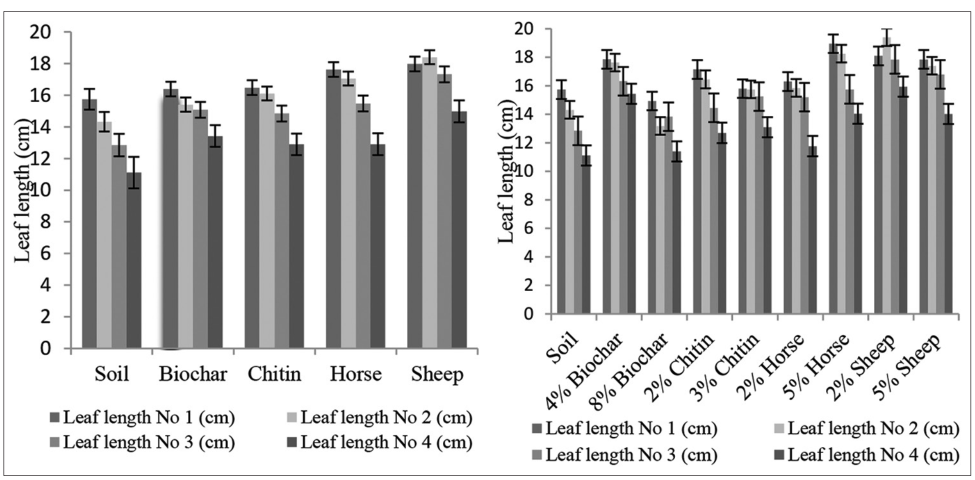

Fig 4. Effect of organic soil amendments on the leaf length of rocket (*Eruca sativa* Mill.) during the growth period.

the height was maximized following biochar application. The results of this study showed that the amendment of chitin at both 2 and 3% rates on soil had a positive effect on the plant height and the upper part length of the rocket, while the 2 and 3% rates of chitin amendment application on soil decreased the weight of the leaves and the root of the rocket. Liopa-Tsakalidi et al. (2010) showed that chitin affected tarragon leaves, increasing the total chlorophyll content. D' Addabbo (1995) mentions that if chitin concentration in the soil exceeds 1%, it has a phytotoxic effect. The relations between the chitin addition and the growth features of the plants are complex and hard to estimate. The present study indicated that the horse manure amendment at 5 and 2% rates revealed favorable effects on the plant height, length of the upper part, and root length when compared to the control treatment. Similarly, Levy et al. (2003) reported that horse manure compost stimulates root and shoots growth of tomato seedlings. Moreover, Ahmadi and Jafarpour (2015) reported that treatment of horse manure and vermicompost at an 8% rate resulted in longer spinach leaves. Furthermore, Duggan (2015) reported increased lettuce shoot and root growth when horse manure compost was used as a growing medium amendment for lettuce.

CONCLUSIONS

The high biochar amendment at an 8% rate to soil resulted in a decrease of the upper part of the plant height. On the contrary, the low biochar amendment at a 4% rate increased the upper part of the plant and the plant height. The amendment of chitin at both 2 and 3% rates on soil had a positive effect on the plant height and the upper part length of the rocket, while the 2 and 3% rates of chitin amendment application on soil decreased the weight of the leaves and the root of the rocket. The horse manure amendment at 5 and 2% rates revealed favorable effects on the plant height, length of the upper part, and root length when compared to the control treatment. Positive effects of organic amendments on plant growth were more evident under the amendments related to sheep manure at a 2% rate. More research is needed to confirm some of the observations that were made after mixing with the soil different organic soil amendments. The reproducibility of our findings in different environmental conditions either in the field or the greenhouse is a crucial requisite for meaningful and robust generalization of our observations.

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Authors' contributions

The authors of the paper equally contributed to the writing of the paper and were involved in the overall planning and supervision of the work.

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