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ENHANCING GROWTH AND CHEMICAL CONSTITUENTS OF LETTUCE PLANT GROWN IN SANDY SOIL BY SOME NATURAL PRODUCTS

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ABSTRACT: New natural products for agricultural uses are currently being developed from plants, such as plant extracts and vermicompost-tea, which help crops grow better and high productivity. The present research aims to evaluate the response of the lettuce plant to several biostimulants products. This experiment was carried out during the two successive seasons of 2018/2019 and 2019/2020 at Vegetable Private Farm at Kafr El-Sinhab, Mansoura Distract, Dakahlia Governorate, Egypt, to study the effect of some vermicompost-tea concentrations (5, 10 and 20%) used as soil application and some nature botanical extracts i.e. Rosemary leaf extract (RLE) and Eucalyptus buds extract (EBE) used as foliar spray on lettuce head parameters, as well as physio-biochemical properties. Either vermicompost-tea or plant extracts increased head parameters (fresh weight, dry weight and head diameter), macro nutrient; N, P, K, micro nutrient; Fe, Zn, Mn and carbohydrate faction (total carbohydrate, total sugar and reducing sugar) in lettuce plants compared to those in the untreated control plants. The interactive vermicompost-tea (20%) ×RLE application was the most effective for increasind the abovementioned criteria.

Key words: Lettuce, vermicompost-tea, rosemary and eucalyptus extracts.

INTRODUCTION

Lettuce is an important crop as nutritive source of minerals and vitamins as well as, it is consumed as a fresh green salad (Hanafy *et al.*, 2000). Also, lettuce leaves are consider a rich source of antioxidants, vitamins A and C (Norman, 1992), and phytochemicals which are anti-carcinogenic (Masarirambi *et al.*, 2012). It is contains a lot of cellulose which, is highly important and required for human health and it facilitates digestion. In addition, lettuce consumption improves sleep, because it contains lactocin and lactucopicrin (Chaudhury, 1967). The cultivated area of lettuce in Egypt was about 3450 hectares, which produced about 68644 tons (FAO, 2020). The excessive use of chemical fertilizers raises the major cost in lettuce production and creates degradation for the agricultural environment, as well as affects

the soil fertility; therefore, it has become essential to use untraditional fertilizers and an environmental friendly as supplements or substitutes for chemical fertilizer.

Vermicompost is a product transformed by organic residues using earthworm (Blouin *et al.*, 2019). A previous study showed that vermicompost is an excellent organic fertilizer, with had a physical, chemical, and biological properties that could improve soil fertility and control crop diseases (Patnaik *et al.*, 2020). The study of Fernandez *et al.*, (2011) showed that vermicompost has high microbial functional diversity and the potential to be used for the treatment of pesticide pollution in agricultural production. Jahanbakhshi *et al.* (2019) demonstrated that vermicompost was a good organic fertilizer with an appropriate carbon and nitrogen ratio. Additionally, vermicompost

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promotes better plant growth than thermophilic composts produced from the same organic substrate due to more superior array of biochemical properties in vermicompost that can support plant growth. Recently, vermicompost has expanded its utilization in the form of water extracts commonly referred to as “teas,” aqueous extracts, vermiliquid, or leachates. In this work, vermicompost water extracts and vermicompost teas refer to the same material based on the manner they are prepared and will be used interchangeably throughout the report. Vermicompost water extracts or “teas” are prepared by steeping vermicompost in water with or without aeration to aid in the extraction of nutrients, other metabolites, and microorganisms during vermicomposting. Similar to the original solid form, the use of vermicompost teas has been documented to suppress a number of important pests and diseases on horticultural crops (**Edwards et al. 2010; Radovich and Arancon, 2011**).

Recently, natural botanical extracts can be used as bio-stimulants to improve plant growth and productivity. Biostimulants are biologically active compounds that enhance metabolisms and promote plant development when applied in small quantities. Biostimulants contain microelements, hormones, enzymes, proteins, vitamins, amino acids, flavonoids, gallic acid and other compounds (**Edmeades, 2002**). It is an environmental friendly method of improving plant development that reduces fertilizer and pesticide consumption. The application of biostimulants might be considered as a good production strategy for obtaining high yield of nutritionally valuable vegetables (**Paradikovic et al., 2011**). More recently, Rosemary (*Rosmarinus officinalis*) leaves extract (RLE) and Eucalyptus (*Eucalyptus camaldulensis*) buds extract (EBE) were found to provide benefits for contributing the sustain ability of plant growth and production (**Desoky et al., 2019**).

Therefore, this work was planned to assess the potential ameliorative influences of some vermicompost-tea concentrations and foliar nourishing with RLE and EBE on growth and yield and some physiological and biochemical processes of lettuce (*Lactuca sativa* L.) plants

under sandy soil conditions with drip irrigation system.

MATERIALS AND METHODS

Experiment Layout

Field experiments were carried out during the two successive growing seasons of 2018/2019 and 2019/2020 at Vegetable Private Farm at Kafr El-Sinhab, Mansoura Distract, Dakahlia Governorate (longitude is 31.36535 and latitude is 30.04471), Egypt. The experiments aimed to study the effect of some vermicompost-tea concentrations (5, 10 and 20%) and some nature botanical extracts on growth, yield and some physiological and biochemical processes of lettuce (*Lactuca sativa* L.) plants under sandy soil conditions with drip irrigation system.

Seedlings were transplanted when two leaves were completely expanded 30 days after sowing, seeds of lettuce (*Lactuca sativa* L.) cv Dark Green, were obtained from Vegetable Research Section, Horticulture Research Institute, Agriculture Research Center, Giza. Seedlings were set up in the field on 30th of October and 3rd of November in the first and second seasons, respectively. Seedlings were planted on one side of ridges 25cm apart, ridges were 80 cm in width and 4 m length. Each plot included 4 ridges and the plot area was about 12 m².

The mechanical and chemical analysis of the used soil were given in Table 1 according to **Black (1968)**.

The recommended agricultural practices of growing wheat were applied, All plots received equal amounts of mineral N, P, and K fertilizers which used by lettuce plants in sandy soil at 80, 45 and 70 kg/fed., respectively in the form of ammonium sulphate (20.5% N), calcium superphosphate (15.5% P₂O₅) and potassium sulphate (48% K₂O), respectively. Phosphorus was mixed with the soil before planting, Nitrogen and potassium were added into four portions every 15 days intervals beginning 15 days from transplanting.

A randomized complete block design with three replications was followed in this study, which involved two factors; vermicompost-tea soil application and plant extracts foliar spray.

Table 1. Mechanical and chemical analysis, of the used soil

Mechanical analysis			Chemical analysis										
Coarse Sand %	Silt %	Clay %	Cations mg/100g soil				Anion mg/100g soil				E.C ^{a+} 25 °c dS/m (mmhos/cm)	PH Soil Reaction	W.H.C.
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻			
52.95	27.95	19.3	3.0	1.8	2.5	0.1	0.00	0.5	1.18	5.72	2.96	7.71	34.64

* W.H.C. Water holding capacity

The treatments details were as follows: 1) Control; no vermicompost-tea was applied, 2) vermicompost-tea addition at rate 5% of (50 ml/l); 3) vermicompost-tea addition at rate of 10% (100 ml/l); 4) vermicompost-tea addition at rate of 20% (200 ml/l) as soil application, while plant extracts foliar spray were as follows: 1) control; without foliar spray, 2) Rosemary leaf extract; RLE, 3) Eucalyptus buds extract; EBE.

Vermicompost Teas

Tea was prepared using compost tea system10 (Growing Solutions Incorporated). It is an aerated system equipped with an air pump that produces fine bubble diffusion technology. A gallon of vermicompost was placed in a perforated plastic container suspended from the rim of the main container. Nine gallons of tap water were used to fill the brewer and then left to brew for 24 hrs. The system produced a concentration of 20% and 10%, and 5% vermicompost were made from dilutions. Control (0% vermicompost tea or tap water only) was added to make a total of four treatments: 0% (control), 5%, 10%, and 20% vermicompost teas.

Application of Vermicompost-tea

Vermicompost-tea was applied with irrigation water as a soil application with three concentrations at the rate of 200 L ha⁻¹ in three equal doses with the 2nd, 4th and 6th irrigations during the last 10 minutes of drip irrigation.

Preparation of Rosemary Leaves and Eucalyptus Buds Extracts

Plant materials

Two plant samples [Rosemary leaves (*Rosmarinus officinalis*) and Eucalyptus buds (*Eucalyptus camaldulensis*)] have been obtained from a local market (Zagazig, Egypt).

Chemicals and reagents

Gallic acid, tert-butyl hydroquinone (TBHQ), 1,1-diphenyl 2picrylhydrazyl (DPPH), β-carotene and quercetin were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Determination of total phenolic compounds (TPC) and total flavonoids

The concentrations of TPC in all extracts were measured by UV spectrophotometer (Jenway-UV-VIS Spectrophotometer) as described by Škerget *et al.* (2005). The used reagent was Folin-Ciocalteu reagent (AOAC, 1990). While total flavonoid content was estimated according to Ordon *et al.* (2006)

Applications of Rosemary Leaf Extract (RLE) and Eucalyptus Buds Extract (EBE)

At 15, 30, and 45 days after transplanting, three foliar sprays each of the distilled water (control), RLE, and EBE were performed in the early morning utilizing a 20 L dorsal-sprayer to run-off. RLE, and EBE concentrations. To

optimize the penetration of the spray solution into the plant leaf tissues, the spray solutions received some drops of Tween-20 (0.1%, v/v) as a surfactant.

The Following Data Were Recorded

Head attributes

After 65 days from transplanting, the plants were harvested and head attributes were recorded for each plot. Three plants were randomly chosen from each experimental plot to determine head fresh weight, g plant⁻¹, head dry weight, g plant⁻¹, head diameter, cm.

Determination of nutrient content

Digesting 0.5 g of dried leaf with H₂SO₄ acid in the presence of H₂O₂ (Wolf, 1982). Total nitrogen was determined using a microkjeldahl method according to Chapman and Pratt (1982). Total phosphorus was determined colourmetrically using ascorbic acid method (Watanabe and Olsen, 1965). Total potassium concentrations were measured directly using Flame photometer (Lachica *et al.*, 1973). Zinc, manganese, iron content were determined using atomic absorption spectrophotometer as described by Cottenie *et al.* (1982)

Determination of carbohydrate fractions

Carbohydrate fractions were determined in the dried samples of shoots and grains of all treatments photometrically according to Bernfeld (1955) and Miller (1959) methods with some modifications.

Color reagent preparation

One gram of 3, 5 dinitro salicylic acid was dissolved in 20 ml of 2N NaOH, then 50 ml distilled water and 30 gm of Rochelle salt were added and the mixture was shaken well until dissolving the salt, then the volume was made up to 100 ml with distilled water.

Total carbohydrates

One tenth gram of dry shoot and grains of wheat powder with 20 ml of 6 N HCl were taken in a carbohydrate tube, then the samples were heated for 6 hours in a boiling water bath then filtered using whatman paper No.1.

Twenty ml of 6 N NaOH were added to the filtrate for neutralization, then made up to 100

ml with distilled water. Five ml from the filtrate were added to 2 ml of color reagent in a test tube, shaken well and heated exactly for 10 minutes in a boiling water bath then cooled under running tap water. The color intensities were measured colorimetrically at 550 nm using spectronic-20 spectrophotometer.

Reducing sugars

Half gram dry shoot and wheat grains, as powder was extracted with 50 ml of 70 % ethanol in a carbohydrate tube and incubated in water bath at 70° C for 3 hours, then the mixture was filtered. The filtrate was made up to 100 ml with distilled water. Five ml of the filtrate were taken and 2 ml of the color reagent were added in a test tube to determine reducing sugars as mentioned before in total carbohydrates.

Total sugars

From the filtrate of the above mentioned reducing sugars, 10 ml were taken in a carbohydrate tube, 5 ml 6 N HCl were added and incubated for 2 hours in water bath. After incubation, 5 ml of 6 N NaOH were added for neutralization and 2 ml of the color reagent were mixed as previously mentioned in case of total carbohydrate.

Standard curve

One gram of glucose was dissolved in distilled water and the volume was made up to one liter. Different volumes from glucose solution were taken and raised to 100 ml with distilled water in volumetric flasks.

Five ml of the different glucose concentrations were taken separately in test tube, and 2 ml of the color reagent were added then treated as shown in the determination of total carbohydrates. Finally, the relationship between the readings at 550 nm and the known concentration of glucose were plotted.

Statistical Analysis

Statistically significant variations between means were compared at $P \leq 0.05$ by Duncan's Multiple Range Test. The statistical analysis was done by COSTAT computer software (CoHort Software version 6.303, Berkeley, CA, USA).

RESULTS AND DISCUSSION

Head Parameters

Data present in Table 2 show the effect of soil application of vermicompost-tea in combined with foliar spray with different plant extracts on head fresh and dry weight and head diameter of lettuce plant c.v Dark Green grown under sandy soil conditions with drip irrigation in 2018/2019 and 2019/2020 seasons.

It is obvious that lettuce plant treated with vermicompost-tea showed a significant increases in head fresh and dry weight and head diameter as compared to the untreated control (without application). Treated plants with vermicompost-tea at 20% was the best treatment with increased head fresh by 26.3 and 26.6%, head dry weight by 27.2 and 26.9%, head diameter by 49.3 and 47.9% during the two successive seasons, respectively.

Moreover, it can be also noticed that, treated lettuce plants with bio-stimulants plant extracts i.e., RLE and EBE significantly increased head fresh and dry weight and, head diameter as compared to the untreated control (without foliar spray). The highest values of the above mention parameters were observed under RLE treatment, followed by EBE as compared untreated one.

Data concerning the interaction effect between vermicompost-tea soil application in combined with foliar application of plant extracts significantly enhanced plant growth characters as compared to the control (without soil application + without foliar spray). Among the all-integrative treatments (vermicompost-tea 20%+ RLE) being the most effective treatment in plant growth characters, which increased head fresh weight by 40.1 and 40.8%, head dry weight by 41 and 41.2%, head diameter by 74.1 and 72.1% during the two successive seasons, respectively.

Vermicompost has been verified as promising types of organic fertilizers for the cultivation of lettuce. Most importantly (**Rezaei-Chiyaneh et al., 2021; Ievinsh et al., 2020**), the application of vermicompost increased the yield of plants (**Mostafa, 2018**), but the use of vermicompost also resulted in an increase in soil microbial activity (**Rezaei-Chiyaneh et al., 2021**),

indicating that not only yield and yield quality, but also soil sustainability as well as they, are positively affected by these organic fertilizers. The results of the present study provide additional support to these facts, but also indicate that special attention should be paid to the characteristics of substrates used for the preparation of vermicompost-containing mixes (**Badawy et al., 2009**). **Uma and Malathi (2009)** Pointed out that plants of *Amaranthus* sp. in plots receiving vermicompost had higher values of growth, yield and quality parameters as compared to plants in plots receiving chemical fertilizers. **Suthar (2009)** reported that garlic plants with vermicompost treatment (at 20 t/ha) had higher values of various growth and yield parameters as compared to values of these parameters with chemical fertilizer treatment. The effect of vermicompost and other fertilizers on growth, yield and nutritional status of tomato was studied by **Meenakumari and Shehkar (2012)**, they concluded that the vermicompost was the best treatment of this studies.

The use of vermicompost is thought to be particularly useful in organic farming because vermicompost provides nutrients that would otherwise need to be brought by synthetic mineral fertilizers that are prohibited in organic agriculture. However, to be able to fully assess the upsides and downsides of vermicompost for agriculture, other steps are required. (i) It would be important to compare the increase in plant growth attributable to vermicompost which those attributable to composts produced in the absence of earthworms. (ii) Composting tends to stabilize organic matter, so that composts have long-lasting effects on soil fertility that should be compared between standard composting and vermicomposting. Such a comparison was not possible for us because most vermicomposting studies are shortterm studies. (iii) The economic costs of producing vermicompost and standard compost should be compared.

These results illustrated that application of vermicompost significantly increased head parameters of lettuce plants as compared to the control treatment (without application). Obtained results were confirmed by those reported by **Ali et al. (2007)** and **Meenakumari and Shehkar (2012)**.

Table 2. Effect of interaction between soil application with vermicompost-tea and foliar spray with Rosemary leaf extract (RLE) and or Eucalyptus buds extract (EBE) on head fresh weight (g), head dry weight (g) and head diameter (cm) of lettuce plants cv. Dark Green during 2018/2019 and 2019/2020 seasons

Characters		Head fresh weight (g)		Head dry weight (g)		Head diameter (cm)	
Treatments		2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season
Effect of soil application							
Without		372±3.6 ^d	368±4.1 ^d	53.1±2.2 ^d	52.7±2.5 ^d	15.8±1.1 ^d	14.6±0.8 ^d
Vermicompost 5 %		440±4.2 ^c	437±4.6 ^c	52.9±2.3 ^c	62.4±3.2 ^c	19.7±1.3 ^c	18.2±0.7 ^c
Vermicompost 10 %		455±5.2 ^b	452±4.7 ^b	65.1±2.5 ^b	64.6±2.6 ^b	21.8±1.6 ^b	20.0±0.9 ^b
Vermicompost 20 %		470±4.6 ^a	466±3.6 ^a	67.1±2.6 ^a	66.6±3.9 ^a	23.6±1.7 ^a	21.6±1.1 ^a
Effect of foliar spray							
Without		399±3.9 ^c	396±4.9 ^c	57.1±2.8 ^c	56.6±2.9 ^c	17.3±1.2 ^c	15.7±0.6 ^c
RLE		45±4.5 ^a	453±4.7 ^a	65.3±2.4 ^a	64.8±3.6 ^a	22.3±1.6 ^a	20.7±0.8 ^a
EBE		446±4.8 ^b	443±4.6 ^b	63.8±3.2 ^b	63.3±3.4 ^b	21.1±1.5 ^b	19.4±0.7 ^b
Effect of interaction							
Soil application	Foliar spray						
	Without	349±3.8 ^j	345±4.6 ^j	49.8±2.6 ^j	49.3±2.5 ^j	14.7±1.4 ^j	13.6±1.0 ^j
Without	RLE	388±3.9 ^h	385±5.3 ^h	55.4±2.8 ^h	55.0±3.1 ^h	16.7±1.5 ⁱ	15.6±1.2 ^{hi}
	EBE	379±4.8 ⁱ	376±5.7 ⁱ	54.1±3.1 ⁱ	53.7±3.4 ⁱ	16.1±1.5 ⁱ	14.8±1.1 ⁱ
	Without	410±4.8 ^g	406±5.9 ^g	58.6±3.2 ^g	58.1±3.9 ^g	17.6±1.6 ^h	16.1±1.4 ^{gh}
Vermicompost 5 %	RLE	464±6.3 ^d	461±5.2 ^d	66.4±3.4 ^d	65.8±3.5 ^d	21.6±1.9 ^e	20.0±1.9 ^e
	EBE	447±5.3 ^e	443±5.3 ^e	63.8±3.6 ^e	63.3±3.9 ^e	20.1±1.8 ^f	18.4±1.3 ^f
	Without	414±5.8 ^g	411±5.4 ^g	59.2±3.1 ^g	58.8±2.8 ^g	18.2±1.7 ^{gh}	16.3±1.4 ^g
Vermicompost 10 %	RLE	480±5.4 ^b	477±4.9 ^b	68.6±3.8 ^b	68.1±3.6 ^b	24.2±1.3 ^c	22.5±1.1 ^c
	EBE	471±5.9 ^c	468±4.3 ^c	67.3±2.8 ^c	66.9±3.4 ^c	22.9±1.1 ^d	21.1±1.2 ^d
	Without	425±5.5 ^f	422±4.6 ^f	60.7±2.9 ^f	60.3±3.4 ^f	18.7±1.1 ^g	16.7±0.9 ^g
Vermicompost 20 %	RLE	495±4.3 ^a	491±4.7 ^a	70.8±3.6 ^a	70.2±3.7 ^a	26.6±1.5 ^a	24.6±1.3 ^a
	EBE	489±4.4 ^a	486±4.2 ^a	69.9±3.4 ^a	69.4±3.9 ^a	25.6±1.3 ^b	23.4±1.2 ^b

In addition, treated plants with RLE or EBE as foliar spray play an important role in increasing lettuce yield and head parameters. The composition of RLE or EBE indicates that these extracts can be used as a plant bio-stimulant. It contains phenolic components which play important role in plant growth and reflected on increasing yield (Naikoo *et al.* 2019). Phenolics influence different physiological processes related to growth and development in plants including seed germination, cell division,

and synthesis of photosynthetic pigments (Tanase *et al.*, 2019). Phenolic compounds have been exploited for several application including bioremediation, allelochemical, promotion of plant growth and productivity (Bujor *et al.*, 2015). In plants, phenolic accumulation is usually a consistent feature of plants, which represents a positive mechanism to increase plant yield (Cheynier *et al.*, 2013). Biosynthesis and accumulation of flavonols were also stimulated in plants under many condition and

enhanced yield component (**Ballizany et al., 2012**). Flavonoid accumulation in cytoplasm can efficiently detoxify harmful H_2O_2 molecules generated and at the end oxidation of flavonoids is followed by ascorbic acid mediated re-conversion of flavonoids into primary metabolites (**Hernandez et al., 2009**). However, exogenous Gallic-acid (GLA) treatment resulted in elevation plant growth and productivity (**Ozfidan-Konakci et al., 2015**). It has been suggested that the main reason for growth induction is related to the influence on indole acetic acid concentration of benzoic acid derivatives (like GLA) by stimulating of the cell division or elongation (**Hussain et al. 2011**).

Macro Nutrients

Data present in Table 3 show the effect of soil application of vermicompost-tea in combined with foliar spray with different plant extracts on Nitrogen (N), Phosphorus (P) and Potassium (K) of lettuce plant cv. Dark Green grown under sandy soil conditions with drip irrigation in 2018/2019 and 2019/2020 seasons.

Vermicompost-tea application resulted in increased of N, P and K by increasing the vermicompost-tea concentration as compared to untreated control (without application). Application vermicompost-tea at the rate of 20% gave the best results in increasing macro nutrients, which increased N by 30.1 and 32.2%, P by 36.7 and 36.5%, and K by 35.7 and 37.4 in both growing seasons; 2018/2019 and 2019/2020, respectively.

It is interest to mention that foliar application of plant extracts, i.e. RLE and EBE promoted N, P and K of lettuce plants compared to corresponding untreated plants. In all cases, the increments in macro nutrients were often highly significant in comparison with untreated ones.

Data concerning the interaction effect between vermicompost-tea soil application in combined with foliar application of plant extracts significantly enhanced plant macro nutrients as compared to control treatment (without soil application + without foliar spray). Among the all-integrative treatments (vermicompost-tea 20%+ RLE) was the best treatment. It increased N by 50.6 and 56.6%, P by 56.3 and 57.2% and

K by 56.3 and 58.7% in both growing seasons; 2018/2019 and 2019/2020, respectively.

NPK are an essential elements which play an = 50 important role in plant life cycle. Nitrogen being a major food for plants is an essential constituent of protein (build from amino acids that involves in catalization of chemical responses and transportation of electrons) and chlorophyll (enable the process of photosynthesis) present in many major portions of the plant parts (**Chen, 2019**). Nitrogen plays a most important role in various physiological processes. It imparts dark-green color in plants, promotes leaves, stem and other vegetative part's growth and development. Moreover, it also stimulates root growth. Nitrogen produce rapid early growth, improve fruit quality, enhances the growth of leafy vegetables, increases protein content of fodder crops; It encourages the uptake and utilization of other nutrients including potassium, phosphorous and controls overall growth of plant (**Bloom, 2015**). Also, Phosphorus is an essential macronutrient involved in most growth processes. It is an essential component of most organic compounds in the plant, including nucleic acids, proteins, phospholipids, sugar phosphates, enzymes, and energy-rich phosphate compounds. It has been well reported that P is a necessary component of photosynthetic processes which are systematically implicated in the creation of sugars, oils, and starches and which further helps in the conversion of solar energy into chemical energy, proper plant maturation, and withstanding stress. It helps plants survive in harsh winter conditions, hastens maturity, and increases water use efficiency. It plays an important role in cell division, and in seed and fruit development. It stimulates early root development, leaf size, tillering, flowering, and grain yield and hastens the maturity of crops. It more over, establishes the plant roots and helps them to go deep for getting moisture and nutrients. Deep roots also form the plant in soil and reduce the loss caused by lodging (**Ahmad et al., 2009**). On the other hand, Potassium (K) is an essential plant macronutrient and plays an important role in many physiological processes vital to plant nutrient and water uptake, nutrient transport, and growth, especially under adverse conditions (**Jiang et al., 2018**).

Table 3. Effect of interaction between soil application with vermicompost-tea and foliar spray with Rosemary leaf extract (RLE) and or Eucalyptus buds extract (EBE) on nitrogen (N), phosphorus (P) and potassium (K) of lettuce plants cv. Dark Green during 2018/2019 and 2019/2020 seasons

Characters	N (%)		P (%)		K (%)		
	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	
Effect of soil application							
Without	1.66±0.09 ^d	1.61±0.07 ^c	0.392±0.01 ^d	0.388±0.01 ^d	1.76±0.08 ^d	1.71±0.06 ^d	
Vermicompost 5 %	2.00±0.11 ^c	1.97±0.06 ^b	0.464±0.03 ^c	0.470±0.02 ^c	2.12±0.13 ^c	2.07±0.14 ^c	
Vermicompost 10 %	2.07±0.12 ^b	2.04±0.08 ^b	0.500±0.04 ^b	0.496±0.03 ^b	2.25±0.14 ^b	2.22±0.13 ^b	
Vermicompost 20 %	2.16±0.13 ^a	2.13±0.12 ^a	0.536±0.04 ^a	0.530±0.02 ^a	2.39±0.16 ^a	2.35±0.12 ^a	
Effect of foliar spray							
Without	1.80±0.08 ^c	1.77±0.05 ^c	0.419±0.03 ^c	0.422±0.01 ^b	1.92±0.11 ^c	1.89±0.08 ^c	
RLE	2.08±0.14 ^a	2.07±0.09 ^a	0.507±0.04 ^a	0.505±0.02 ^a	2.27±0.16 ^a	2.22±0.11 ^a	
EBE	2.02±0.11 ^b	1.99±0.08 ^b	0.493±0.03 ^b	0.489±0.03 ^a	2.19±0.18 ^b	2.15±0.15 ^b	
Effect of interaction							
Soil application Foliar spray							
Without	Without	1.52±0.07 ^k	1.50±0.04 ⁱ	0.376±0.01 ^l	0.372±0.03 ^g	1.65±0.09 ^l	1.60±0.07 ^l
	RLE	1.78±0.06 ⁱ	1.72±0.05 ^{gh}	0.404±0.02 ^j	0.400±0.02 ^{fg}	1.87±0.08 ^j	1.80±0.06 ^j
	EBE	1.67±0.08 ^j	1.62±0.04 ^h	0.396±0.01 ^k	0.392±0.01 ^g	1.77±0.06 ^k	1.73±0.05 ^k
Vermicompost 5 %	Without	1.83±0.06 ^h	1.78±0.06 ^{fg}	0.419±0.03 ⁱ	0.429±0.02 ^{ef}	1.93±0.08 ⁱ	1.89±0.09 ⁱ
	RLE	2.10±0.11 ^e	2.08±0.15 ^{bc}	0.495±0.02 ^e	0.489±0.03 ^c	2.25±0.16 ^e	2.19±0.11 ^e
	EBE	2.07±0.13 ^e	2.04±0.13 ^{cd}	0.477±0.03 ^f	0.474±0.03 ^{cd}	2.17±0.18 ^f	2.14±0.15 ^f
Vermicompost 10 %	Without	1.89±0.08 ^g	1.86±0.07 ^{ef}	0.433±0.02 ^h	0.441±0.02 ^e	2.02±0.16 ^g	2.00±0.16 ^h
	RLE	2.18±0.13 ^c	2.15±0.18 ^{bc}	0.543±0.04 ^c	0.539±0.04 ^b	2.41±0.14 ^c	2.37±0.18 ^c
	EBE	2.14±0.15 ^d	2.11±0.17 ^{bc}	0.525±0.02 ^d	0.520±0.03 ^b	2.33±0.12 ^d	2.29±0.12 ^d
Vermicompost 20 %	Without	1.98±0.06 ^f	1.95±0.08 ^{de}	0.447±0.03 ^g	0.448±0.02 ^{de}	2.10±0.13 ^g	2.06±0.16 ^g
	RLE	2.29±0.16 ^a	2.35±0.16 ^a	0.588±0.04 ^a	0.585±0.04 ^a	2.58±0.15 ^a	2.54±0.17 ^a
	EBE	2.21±0.17 ^b	2.18±0.18 ^b	0.574±0.03 ^b	0.569±0.03 ^a	2.50±0.13 ^b	2.45±0.12 ^b

Vermicompost-tea rapidly provides nutrients such as NPK to plants through soil applications (Pathak and Ram, 2004). In this concern, results indicated that application of Vermicompost-tea increased NPK in plant tissue, this results confirmed with those found by (Arancon *et al.*, 2004; Ali *et al.*, 2007; Meenakumari and Shehkar, 2012).

Micro Nutrients

The effect of vermicompost-tea soil application, and plant extract foliar spray on micro nutrients *i.e.*, Iron (Fe), Zink (Zn) and Manganese (Mn) in lettuce leaves are shown in Table 4.

It is conspicuous from the data that application of vermicompost-tea significantly increased micro nutrients as compared to the untreated plants, Application vermicompost-tea at rate 20% gave the best results in increasing of Fe, Zn and Mn. Also, foliar spray with RLE or EBE significantly increased micro nutrients as compared to the untreated plants. The highest values of micro nutrients are observed under RLE treatment.

In addition concerning the interaction effect between vermicompost-tea soil application in combined with foliar application of plant extracts significantly increased micro nutrients

Table 4. Effect of interaction between soil application with vermicompost-tea and foliar spray with Rosemary leaf extract (RLE) or Eucalyptus buds extract (EBE) on iron (Fe), zinc (ZN) and manganese (Mn) of lettuce plants cv. Dark Green during 2018/2019 and 2019/2020 seasons

Characters	Fe (ppm)		Zn (ppm)		Mn (ppm)		
	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	
Effect of soil application							
Without	23.7±1.1 ^d	22.9±1.3 ^d	23.5±1.4 ^d	22.5±1.2 ^d	35.7±2.4 ^d	34.1±2.0 ^d	
Vermicompost 5 %	53.7±1.9 ^c	52.5±1.6 ^c	29.7±1.6 ^c	28.9±2.4 ^c	44.6±3.2 ^c	42.8±2.8 ^c	
Vermicompost 10 %	65.7±2.6 ^b	64.4±2.8 ^b	32.7±1.9 ^b	31.6±2.7 ^b	47.7±3.5 ^b	46.4±3.0 ^b	
Vermicompost 20 %	72.5±2.8 ^a	70±2.4 ^a	35.3±2.1 ^a	34.2±3.0 ^a	50.7±3.8 ^a	49.3±3.5 ^a	
Effect of foliar spray							
Without	39.2±1.4 ^c	37.8±1.5 ^d	26.6±1.2 ^c	25.6±2.1 ^c	39.1±2.7 ^c	37.8±2.1 ^c	
RLE	64.0±2.8 ^a	62.8±3.2 ^a	32.9±1.3 ^a	32.0±2.9 ^a	48.6±3.6 ^a	46.8±3.1 ^a	
EBE	58.5±2.6 ^b	57.1±3.1 ^b	31.4±2.2 ^b	30.3±2.7 ^b	46.3±3.4 ^b	44.8±3.3 ^b	
Effect of interaction							
Soil application Foliar spray							
Without	Without	21.6±1.2 ^j	20.9±1.1 ⁱ	21.0±1.2 ^j	20.0±1.2 ^h	33.4±2.2 ⁱ	32.3±2.6 ^j
	RLE	27.0±1.3 ⁱ	26.3±1.6 ^h	25.7±1.4 ^h	24.8±2.1 ^{fg}	38.2±2.6 ^g	36.3±2.5 ^{hi}
	EBE	22.5±1.5 ^j	21.5±1.9 ⁱ	23.8±1.2 ⁱ	22.7±1.2 ^g	35.4±2.3 ^h	33.9±2.1 ^{ij}
Vermicompost 5 %	Without	38.1±1.6 ^h	37.0±2.9 ^g	27.4±1.6 ^g	26.5±2.3 ^{ef}	39.8±2.7 ^{fg}	37.9±2.2 ^{gh}
	RLE	65.2±2.5 ^d	64.1±3.2 ^d	31.6±2.3 ^d	30.8±2.5 ^{cd}	48.1±3.6 ^c	46.2±2.6 ^{de}
	EBE	57.6±2.4 ^e	56.4±3.1 ^e	30.3±2.1 ^{de}	29.6±2.5 ^{cd}	46.0±3.4 ^d	44.4±2.5 ^e
Vermicompost 10 %	Without	46.4±2.3 ^g	45.0±3.2 ^f	28.5±1.7 ^{fg}	27.6±2.4 ^{def}	40.8±2.8 ^{ef}	39.7±2.7 ^{fg}
	RLE	78.0±3.2 ^b	77.1±3.4 ^b	35.7±2.4 ^b	34.5±3.1 ^{ab}	52.5±3.8 ^a	50.9±2.3 ^a
	EBE	72.8±3.1 ^c	71.2±3.9 ^c	33.9±2.2 ^c	32.8±2.0 ^{bc}	49.9±3.7 ^c	48.4±2.8 ^{cd}
Vermicompost 20 %	Without	50.6±1.6 ^f	48.5±2.5 ^f	29.6±1.8 ^{ef}	28.5±1.6 ^{de}	42.5±3.0 ^e	41.5±2.1 ^f
	RLE	85.8±3.2 ^a	83.9±2.3 ^a	38.8±2.7 ^a	37.8±2.6 ^a	55.8±3.4 ^a	53.9±3.2 ^a
	EBE	81.1±3.8 ^b	79.2±2.6 ^b	37.5±2.6 ^a	36.4±2.5 ^a	53.9±3.6 ^{ab}	52.5±3.5 ^{ab}

as compared to control (without soil application + without foliar spray). Among the all-integrative treatments (vermicompost-tea 20% + RLE) being the most effective and increased Fe by 275 and 278%, Zn by 78.5 and 82%, and Mn by 61.3 and 62.5% in both growing seasons, respectively.

Iron is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments (**Samaranayke *et al.*, 2012**). Iron deficiency is a common nutritional disorder in many crop plants, resulting in poor yields and reduced nutritional quality. In plants, iron is involved in chlorophyll synthesis, and it is essential for the maintenance of chloroplast structure and function. It is still an essential element that is critical for plant life (**Guerinot and Yi, 1994**), as this element is involved in plant metabolism. As a critical component of proteins and enzymes, iron plays a significant role in basic biological processes, such as photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation, and uptake mechanisms (**Kim and Rees, 1992**), and DNA synthesis through the action of the ribonucleotide reductase (**Robinson, 1999**). It is also an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase, and 1- aminocyclopropane acid-1-carboxylic oxidase (**Siedow, 1991**). Also, The Zn plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (**Tisdale *et al.*, 1984**). Plant enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation (**Marschner, 1995**). The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent (**Cakmak, 2000**).

While, manganese plays an important role on oxidation and reduction processes, as electron transport in photosynthesis. Moreover manganese acts as an activator of many enzymes, (more than 35 different enzymes). Manganese has important role on activates several enzymes which involve to oxidation reactions,

carboxylation, carbohydrates metabolism, phosphorus reactions and citric acid cycle. Of the most important these enzymes, protein manganese in photosystem II and superoxide dismutase can be pointed. There is more than 90% of superoxide dismutase in chloroplasts which about 4 to 5 percent of it is in mitochondria (**Millaleo *et al.*, 2010; Jackson *et al.*, 1978**)

Carbohydrate Fractions

The effect of vermicompost-tea soil application, and plant extract foliar spray on carbohydrate fractions *i.e.*, total carbohydrates, total sugar and reducing sugar in lettuce leaves are shown in Table 5. It is conspicuous from the data that application of vermicompost-tea significantly increased carbohydrate fractions as compared untreated plants, Application of vermicompost-tea at the rate 20% gave the best results in increasing in carbohydrate fractions. Also, foliar spray of with RLE or EBE significantly increased carbohydrate fractions as compared to the untreated plants. The highest values of carbohydrate fractions are observed under RLE treatment

Concerning the interaction effect between vermicompost-tea soil application in combined with foliar application of plant extracts significantly increased carbohydrate fractions as compared to control (without soil application + without foliar spray). Among the all-integrative treatments (vermicompost-tea 20%+ RLE) was the best treatment. It increased total carbohydrates by 45.6 and 55%, total sugar by 37.2 and 37.3% and reducing sugar by 105 and 111% in both growing seasons, respectively.

As part of the study, the effects of vermicompost at different volume ratios on rhizosphere environment, quality and yield of lettuce were studied. The results showed that vermicompost significantly improve the soil physical and chemical properties, increase soil nutrients, organic matter and lettuce yield and the contents of carbohydrate fractions in leaves were also enhanced (**Joshi *et al.*, 2015**). In lettuce plants, vermicompost increased the nutrient uptake and leaf-soluble sugars (**Yassen *et al.*, 2020**). In this respect, the obtained results are in agreement with those obtained by (**Zhang *et al.*, 2011; Arancon *et al.*, 2012; Abduli *et al.*, 2013**).

Table 5. Effect of interaction between soil application with vermicompost-tea and foliar spray with Rosemary leaf extract (RLE) and or Eucalyptus buds extract (EBE) on carbohydrate fractions of lettuce plants cv. Dark Green during 2018/2019 and 2019/2020 seasons

Characters	Total carbohydrate (%)		Total sugar (%)		Reducing sugar (%)		
	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	2018/2019 season	2019/2020 season	
Treatments							
Effect of soil application							
Without	7.49±0.21 ^d	7.26±0.22 ^c	5.56±0.22 ^d	5.32±0.29 ^c	2.81±0.15 ^d	2.66±0.12 ^d	
Vermicompost 5 %	8.93±0.22 ^c	8.72±0.25 ^b	6.48±0.32 ^c	6.28±0.34 ^b	4.09±0.21 ^c	3.95±0.13 ^c	
Vermicompost 10 %	9.44±0.23 ^b	9.00±0.31 ^b	6.63±0.35 ^b	6.37±0.37 ^b	4.31±0.22 ^b	4.14±0.19 ^b	
Vermicompost 20 %	10.0±0.25 ^a	9.78±0.32 ^a	6.73±0.36 ^a	6.51±0.33 ^a	4.50±0.23 ^a	4.33±0.21 ^a	
Effect of foliar spray							
Without	8.18±0.25 ^c	7.97±0.25 ^c	5.93±0.24 ^c	5.74±0.42 ^b	3.50±0.13 ^c	3.33±0.11 ^c	
RLE	9.56±0.26 ^a	9.21±0.32 ^a	6.60±0.26 ^a	6.33±0.46 ^a	4.23±0.22 ^a	4.08±0.21 ^a	
EBE	9.19±0.29 ^b	8.89±0.36 ^b	6.51±0.28 ^b	6.28±0.49 ^a	4.05±0.26 ^b	3.90±0.22 ^b	
Effect of interaction							
Soil application Foliar spray							
Without	Without	7.05±0.11 ^l	6.85±0.25 ⁱ	5.07±0.23 ^k	4.90±0.42 ^f	2.33±0.11 ^k	2.19±0.12 ^j
	RLE	7.89±0.21 ^j	7.57±0.26 ^{gh}	5.88±0.25 ⁱ	5.55±0.52 ^e	3.23±0.16 ⁱ	3.08±0.25 ^h
	EBE	7.52±0.16 ^k	7.38±0.28 ^h	5.70±0.32 ^j	5.52±0.52 ^e	2.86±0.17 ^j	2.70±0.12 ⁱ
Vermicompost 5 %	Without	8.23±0.18 ⁱ	7.99±0.27 ^{fg}	6.10±0.32 ^h	5.96±0.56 ^d	3.74±0.16 ^h	3.55±0.16 ^g
	RLE	9.52±0.29 ^e	9.26±0.36 ^{cd}	6.71±0.35 ^d	6.47±0.54 ^{bc}	4.35±0.12 ^d	4.23±0.21 ^d
	EBE	9.03±0.25 ^f	8.83±0.34 ^{de}	6.63±0.39 ^e	6.41±0.59 ^c	4.19±0.13 ^e	4.08±0.22 ^e
Vermicompost 10 %	Without	8.60±0.26 ^h	8.39±0.38 ^{ef}	6.24±0.37 ^g	6.02±0.59 ^d	3.89±0.17 ^g	3.73±0.14 ^f
	RLE	9.95±0.28 ^c	9.35±0.37 ^c	6.86±0.34 ^b	6.58±0.52 ^{ab}	4.55±0.21 ^c	4.37±0.23 ^c
	EBE	9.76±0.23 ^d	9.33±0.36 ^c	6.78±0.36 ^c	6.51±0.55 ^{bc}	4.48±0.22 ^c	4.31±0.25 ^{cd}
Vermicompost 20 %	Without	8.86±0.26 ^g	8.66±0.39 ^e	6.33±0.35 ^f	6.09±0.52 ^d	4.04±0.23 ^f	3.85±0.18 ^f
	RLE	10.9±0.29 ^a	10.6±0.36 ^a	6.96±0.33 ^a	6.73±0.51 ^a	4.79±0.25 ^a	4.64±0.23 ^a
	EBE	10.4±0.32 ^b	10.1±0.31 ^b	6.91±0.37 ^b	6.70±0.55 ^a	4.69±0.26 ^b	4.51±0.27 ^b

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تحسين النمو والمكونات الكيميائية لنبات الخس المزروع في الأراضي الرملية بواسطة بعض المنتجات الطبيعية

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يتم حالياً تطوير منتجات طبيعية جديدة من النباتات للاستخدامات الزراعية، مثل المستخلصات النباتية وشاي الفيرميكومبوست، مما يساعد على نمو المحاصيل وزيادة الإنتاجية. يهدف البحث الحالي إلى تقييم استجابة نبات الخس للعديد من تلك المنشطات الحيوية. أجريت هذه التجربة خلال الموسمين المتعاقبين 2019/2018 و 2020/2019 بمزرعة خضروات خاصه بكفر الشنهاب، المنصورة، محافظة الدقهلية، مصر، لدراسة تأثير بعض تراكيز من شاي الفيرميكومبوست (5، 10، 20%) كأضاه تربة وبعض المستخلصات النباتية (الطبيعية مثل مستخلص أوراق إكليل الجبل (RLE) ومستخلص براعم الأوكالبتوس (EBE) المستخدمة كرش ورقي (مستخلص الرزماري ومستخلص براعم الكافور) وتأثير ذلك على صفات رأس الخس بالإضافة إلى الخصائص الفيزيائية والكيميائية. أدى استخدام شاي الفيرميكومبوست أو المستخلصات النباتية إلى زيادة صفات الرأس (الوزن الطازج، الوزن الجاف، قطر الرأس) والعناصر الكبرى (النيتروجين - الفوسفور - البوتاسيوم) والعناصر الصغرى (الحديد، الزنك، المنجنيز) وصور الكربوهيدرات (الكربوهيدرات الكلية، السكريات الكلية والسكريات المختزله) في نباتات الخس مقارنة بالنباتات الغير. وانت افضل المعاملات هي استخدام شاي الفيرميكومبوست بتركيز 20% مع الرش الورقي بمستخلص الرزماري.

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