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Differentiating Biological Roles of Some Organic Amendments on Some Parameters for Sandy and Clay Soils Cultivated with Lettuce (*Lactuca sativa L.*) and Final Yield

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lettuce (*Lactuca sativa* L.) was planted in pot tests during 2020–2021 and 2021–2022 years in a randomized complete block design (RCBD) including nine treatments using soils sampled collected from Ismailia and Giza for cultivation. Treatments included usage of potassium humate (KH), vinasse (Vin), and olive mill waste (OMW) as replacements for the recommended mineral fertilizers. Effects of these alternatives on chemical, biological and physical characteristics for both lettuce and

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soil were studied at harvest. The combination of mineral fertilizer (NPK) with either OMW, Vin or KH in equal quantities (50%) produced the highest yields. As for Giza soil, compared with 100% NPK, the yield increased by 1.7, 1.6 and 1.6 folds using 50% NPK combined with either 50% OMW, Vin or KH, respectively. On the other hand, Ismailia soil either 50% Vin or 50% OMW with 50% NPK achieved yield of 1.8 and 1.65 folds that of 100 % NPK. Addition of 50% OMW or Vin recorded the best mean values of all tested, all parameters and improved plants growth and increased the total yield of curds besides enhancing yield and its components compared to the other treatments or the control and at the same time saved and reduced mineral fertilizer recommendation of Lettuce.

Keywords: Organic fertilizer; olive mill waste wastes; vinasse; k-humate; oxidoreductase enzyme; lettuce (Lactuca sativa L); yield.

1. INTRODUCTION

The Global Environment Facility (GEF, 2008) reported that soil nutrient reserves were reduced due to soil organic matter deficiency, of which the Egyptian soil had experienced significant depletion due to mineralization and fertilizer use. In Egypt, two types of agricultural lands were reported; clay soil found in the Delta and the Nile Valley and sandy soils as reclaimed lands in the desert. Both poor farming practices and changing climatic scenarios affected more than 75% of Earth's land surface that resulted in salinized, eroded and low organic matter soils (Talukder et al., 2021).

The effective management of chemical fertilizer can solve the environmental issue of how to dispose of agro-industrial residue and significantly contribute to boosting and maintaining national food security (De Chaves et al., 2020; Ebaid et al., 2024).

Organic fertilization was studied in many researches as an indispensable alternative to inorganic forms and proved its reliability. One potential solution to decrease the usage of chemical fertilizers is to use organic additions derived from recycling organic waste. Due of the low clay content, sandy soils exhibited little shrinkage or expansion characteristics (Khan, 2018). The use of organic supplements in sandy soils was found to be hindered by the issue of frequent turnover, since the decomposition rate was fast and the additional organic materials were often mineralized within short cropping seasons (Baiamonte et al., 2019). Also, using organic amendments exceeded availability of nutrients it contained as by increasing soil porosity, aeration and maintaining soil moisture, as mentioned by (Ismail & Obeid, 2023).

Olive oil production is an essential global agroindustry, especially in Mediterranean countries. Around 750 million productive olive trees cover approximately 10 million hectares and produce about 2.5 million tons of olive oil. More than 90 % of them were produced in the Mediterranean region (Fraga et al., 2021). The processes of olive oil extraction produce 20 % of olive oil as a product, beside both 30 % of moist solid waste (65 % moisture) called Pomace or Jift (Ochando-Pulido et al., 2018) and 50 % liquid waste called olive mill wastewater (OMW), or Zibar, as a byproduct. Pomace is a mixture of olive paste and stones that remains after olive pressing (Sygouni et al., 2019). According to (Khdair & Abu-Rumman, 2020), Most of the produced olive pomace is regarded as an energy source. Pomace is a key part of organic manures, which provide nutrients, enhance soil aggregation, and promote microbial diversity (Seleem et al., 2022).

Vinasse is regarded as a byproduct of the production of sugar. It stands for the residues from the fermentation of molasses. Vinasse is now regarded as an ecologically friendly source of organic amendments for the agriculture industry (Hoarau et al., 2018). The composition of liquid vinasse is characterized by a high content of major ions and organic compounds, along with significant amounts of plant macronutrients like iron, copper, magnesium, manganese, phosphorus, and potassium (Oldroyd & Dixon, 2014; Katakojwala et al., 2019). Benefits of using vinasse in fertigation systems include increased crop productivity and soil fertility (Cerri et al., 2020; Vadivel et al., 2014) stated that the use of vinasse in agriculture resulted in a substantial increase in nutrient content, improved the soil quality of degraded land and boosted crop yields.

According to (Tejada & Gonzalez, 2005), humic acids positively affected the growth and metabolic activity of plants. (Kumar et al., 2013) clarified the role of using K-Humate as soil conditioner and growth promoter for plants, where it reduced leaching of macro and micronutrients while improving soil structure and increase its water retention. Humic acid (HA) has a substantial impact on the physical, chemical, and biological properties of soil, including its texture, structure, ability to hold water, ability to exchange ions, pH, soil carbon content, enzyme activity, nitrogen cycle, and nutrient availability. The impacts of HA on crop development, plant hormone production, nutrient intake and absorption, yield, and protein synthesis were highlighted in many researches in the review done by (Kwame et al., 2022).

Humic acid had changed the composition of the soil by raising its electrical conductivity, porosity, and density while decreasing its acidity and increasing its density which improved its hydraulic properties (Abou Tahoun et al., 2022).

The effectiveness of applying soil amendments including OMW, vinasse, and potassium humate varied widely depending on the land use, soil type, and ecosystems globally. Extra or intracellular enzymes played an important role in maintaining clay and sandy ecosystem quality functional diversity, and nutrient cycling (Sinsabaugh et al., 2002). Enzyme activities are a promising measure of soil quality because they respond swiftly to soil management and fertilization strategies. Soil microorganisms, as main sources for enzymatic activity in soil, are essential to the decomposition of organic matter, nutrient cycling, soil fertilization and development of soil structure because they share in production of soil organic carbon, which boosts soil fertility and water-retaining capacity (Christopher, 2017; Mkhinini et al., 2020). Soil enzymes are an important parameter in carbon sequestration and soil nutrient dynamics (Lemanowicz et al., 2023). Soil dehydrogenases represent oxidoreductase enzymes that do not accumulate extracellularly in soil but occur inside microbial cells linked to their oxidation-reduction processes and indicate overall soil microbial activity, as they do share in biological oxidation of soil organic matter (OM) upon which their activities are found proportional to microbial biomass in soil (Wolińska & Stępniewska, 2012).

On the other hand, plants developed many adaptation mechanisms, including antioxidant defense systems like Peroxidase (POD) and Catalase (CAT). In order to protect the roots and support the plants during stress, the POD and CAT reduce reactive oxygen species by employing a variety of substrates as electron donors (Hafez, 2021).

Lettuce (*Lactuca sativa L.*) is grown all over the world and is considered a winter cash crop for Egyptian farmers (Chen et al., 2019). Organic fertilizer did mitigate heavy metals in soil that did benefit lettuce yield over chemical fertilizers (Hossain & Ryu, 2017).

The current work aims to investigate the potentiality of replacing mineral fertilizers with the three previously stated organic amendments as additives on the growth and yield of lettuce grown in a clayey and sandy soils, as well as some of their chemical, physical and biological properties.

2. MATERIALS AND METHODS

The current research was conducted at the Agriculture Research Center over two consecutive seasons (2020/2021 and 2021/2022) to examine the impact of olive mill waste (OMW), vinasse (Vin), and potassium humate (KH) as soil nutrients on vegetative development**,** fresh weight, head diameter, number of lettuce leaves, Photosynthesis pigments and chemical components of Lettuce *(Lactuca sativa L.)* as well as enzyme activity.

Surface soil samples: (0-30 cm) were collected from both Agricultural Research Center - Giza governorate (clay soil) and Ismailia agricultural stations - Agricultural Research Center (sandy soil) to represent two different textured soils.

Organic fertilizer: KH was obtained from the Agricultural Research Center (ARC) at Giza governorate, Egypt. OMW was obtained from Olive Oil Production unit (OOP) at the Horticultural Research Institute, Agricultural Research Center (ARC), Giza governorate, Egypt. Vin was obtained from Hawamdia Sugar and Distillation Company. Egypt.

Experimental Design: The current pot experiment was randomized complete block design. The pot experiments included Giza (Clay) and Ismailia (Sandy) soils that had been distributed individually in pots with a size capacity of 10 kg soil where only one lettuce transplant was cultivated individually in each pot. Uniform size seedlings of Lettuce seedlings *(Lactuca sativa L.)* of 40 days old were brought from Horticulture Research Institute at ARC-Giza governorate. They were transplanted during the second week of September in the two growing seasons into the aforementioned pots at a rate of one seedling/pot.

The NPK fertilization was followed after the recommendation of Agricultural Extension Office – Ministry of Agriculture – Egypt, at rates of 150: 45: 65 kg/ha as N: P2O5: K2O, respectively, as mentioned by (El-Mogy et al., 2020). NPK was applied as ammonium nitrate (33%N), calcium supper phosphate (15.5% P2O5) and potassium sulfate (48% K2O) equivalent to 265, 34 and 97 Kg/ha of NPK, respectively. Calcium supper phosphate was added once to soil combinations in each pot before transplantation, while either of ammonium nitrate and potassium sulfate was added as half dose at a time after 15 and 30 days of plantation. Harvested lettuce and soil samples were collected for determination of yield parameters, chemical and biochemical properties. The remaining agricultural procedures were executed in accordance with the prescribed techniques for lettuce cultivation (El-Ghinbihi & Mahmoud, 2007)]. The three types of organic water soluble amendments were applied individually in irrigation water (fertigation) either as 100% or 50% of recommended dose ($5m^3fed^{-1}$each of OMW, Vin and kH), However, the control was added at 100% of the recommended NPK dose According to the recommendations of Egyptian Ministry of Agriculture, plants were fertilized with no added organic fertilizer and were planned as shown in Table 1.

The other agricultural activities were implemented in accordance with the prescribed procedures for cultivating lettuce (El-Ghinbihi & Mahmoud, 2007). After harvest.

Chemical Characteristics of Soil: Random soil samples were obtained from the experimental soils, air-drying, crushed and sieved using a 2.0 mm sieve. Following that, the soil samples underwent chemical and physical analysis, according to the defined methodologies specified by (Page et al., 1982) and (Klute, 1986). The objective of these investigations was to ascertain the many chemical and physical characteristics of the soil. An electrical conductivity meter was used to test the electrical conductivity (EC) of the soil paste extract (Rhoades et al., 1989). The soil pH was determined by measuring the pH of a 1:2.5 soil to water suspension using a pH meter, as per the specified method of (Thomas & Devi, 2018). In a soil paste extract the presence of soluble cations [Ca^{++}, Mg^{+}, Na^{+}, K^{+}] and anions [CO_3^{-}-, HCO_3^{-}, Cl^{-}] were determined according to (Jackson, 1973). Sulphate was calculated by subtracting the total summation of total determined soluble anions from the summation of total soluble cations. The organic matter was determined according to (Walkley & Black, 1934).

Organic amendments Analysis: Three fertilizers (OMW, Vin, and KH) were analyzed as follows: pH, electrical conductivity (EC), and the contents of organic matter. Potassium (K) was determined using flame photometer, and phosphorus (P) was determined using spectrophotometer according to (Cottenie et al., 1982). The Kjeldahl method was used to calculate total nitrogen (T.N).

Vegetative Growth Characteristics: Various traits were assessed by randomly selecting sets of three plants from each experimental pot: vegetative growth, fresh weight, head diameter, number of lettuce leaves, chlorophyl II, carotenoids, TSS% and NPK of lettuce leaves. The plant leaf was then dried at 70°C until it reached a consistent weight, from which the dry weight per plant was calculated.

Plant Enzyme activities: Assessment of soil and plant biological activities pertaining to enzyme activity in the soil rhizosphere and fresh plant. Biological activity was assessed in soil samples collected from the rhizospheres of lettuce plants. 105 days post-planting.

A 0.5g sample was well mixed with 10 ml of cold phosphate buffer (50 mM, pH 7). The homogenates were subjected to centrifugation at a speed of 4000 revolutions per minute (rpm) at a temperature of 20°C for a duration of 20 minutes. The liquid portion, known as the supernatant, was used as a raw extract for the enzymatic test. Reagents used in the following assays were prepared in the same previously indicated buffer.

Peroxidase enzyme activity was determined using methylene blue assay (Magalhães et al., 1996). A single unit of peroxidase was determined as the quantity of enzyme necessary to oxidize 1.0 µmol of methylene blue for one minute.

Catalase enzyme activity included measuring the degradation of H_2O_2 using UV detection at 240nm, as stated by (Beers & Sizer, 1952). The method stages were executed (Pine et al., 1984). The units of catalase were determined by using a molar absorbance coefficient of 43.6 for H_20_2. A single unit of catalase was determined as the quantity of enzyme needed to break down 1.0 µmol of H_2O_2 per minute.

Table 1. The layout of the experimental design

Treatment	NPK%		Organic Fertilizer %	Treatment symbol
Control	100	+	0	T1
Olive mill waste	0	+	100	T2
	50	+	50	T3
Vinasse	0	+	100	T4
	50	+	50	T5
K-humate	0	+	100	T6
	50	+	50	T7

Soil Enzymes Activities: The activity of dehydrogenase (DeH-ase) was measured using the tri-phenyl tetra-zolium chloride (TTC) technique as described in (Casida et al., 1964). A single unit of dehydrogenase was determined as the quantity of enzyme needed to catalyze the hydrolysis of TTC, resulting in the formation of 1.0 µmol of tri-phenyl formazan (TPF) per hour (µmol g-1h-1).

Para nitro phenyl phosphate was used to evaluate phosphatase activity (P-ase) (Tabatabai & Bremner, 1969). Phosphatase activity of one unit was defined as the amount of enzyme required to release 1µg of p-nitro phenol hydrolyze per hour.

Nitrogenase activity was measured by acetylene reduction assay as described by (Johnsen & Apsley, 1990).

Statistical Analysis Procedure: The tests and analytical results were reproduced a minimum of three times, and the data reported represents the average values. The collected findings underwent a one-way analysis of variance (ANOVA) to establish the significance between treatments. The kind of analysis used depends on the variables that impacted the experiment. CoStat software was used for this study (Stern, 1989).

3. RESULTS AND DISCUSSION

Analytical results represented in Table 2 characterized soil samples gathered from Giza (A) and Ismailia (B) soils. Based on their physical analysis, texture classifications of soils (A) and (B) were found to be clay and sandy soils, respectively. Both soils had alkaline pH of 8±0.2, while EC value of soil (A) was nearly double that of (B). Organic matter, $CaCO_3$, soluble anions (HCO_3, Cl, SO_4), soluble cations (Ca, Mg, Na), macronutrients (N, P), and micronutrients (Fe, Mn) in soil (A) surpassed that of soil (B).

Organic wastes gathered from different agro-industrial activities were analyzed mainly for their NPK contents which were expected to partially substitute NPK mineral fertilization in lettuce cultivation. The three types of organic wastes used as organic amendments had considerable amount of organic matter (OM%), while with insufficient NPK contents compared to required doses for plantation, as shown in Table 3. Vin and OMW were found to be acidic but KH was alkaline, while Vin had the highest EC value among organic wastes.

Substituting mineral NPK with organic fertilizers diminish the share of mineral NPK forms which were easily available for plant. Add to that, NPK contents in organic amendments were mostly in complex organic forms that would be released slowly depending on soil rhizosphere conditions including microbial activities. Low pH characterizing Vin and OMW used as soil amendments resulted in a slight increment in the insoluble acid fraction (Arafat & Abd-Elazim, 2002; Christofoletti et al., 2013).

The application of vinasse often resulted in pH values decreasing due to the acidic influence of vinasse (Seddik et al., 2016). Noticeably, all chemical, biological and physical analysis carried out for plant and soil samples were attributed only to their residual characterization at harvest.

3.1 Leaf Enzymes

Enzymatic activities measured in lettuce leaves at harvest, as illustrated in Fig. 1, elucidated residual oxidation reduction activities that might had been affected by their nutritional states.

Among all treatments, both leaf catalase and peroxidase activities in lettuce harvested from clay soil were mostly higher than those from sandy soil. Added to that, enzymatic activities were higher in second season than in first season from lettuce cultivated in both types of soils, that might be attributed to the accumulation of organic amendments used and consequently to any changes that had happened in the rhizosphere biological contents. In both types of soils and within most treatment cases, both peroxidase and catalase activities increased in the second season compared to the first.

Catalase and peroxidase antioxidant enzymes increased in lettuce at early stage of growth while they decreased at the latter stage (harvest) in response to stress (Zhao et al., 2022). Consequently, in the present work, the level of catalase and peroxidase activities at harvest time would be much less than that at the early stage of growth. Nevertheless, the variations in catalase and peroxidase activities correlated to applying different organic amendments could be considered as crucial evaluation for lettuce status in response.

Variations in catalase activities measured in lettuce sampled from clay soil treated with organic amendments compared to control in the first and second seasons were difficult to describe as a fixed trait.

Using a higher concentration of OMW (100%) caused decrease in plant catalase from clay soil while increasing catalase plant from sandy soil, but in both cases catalase in the first season was lower than in the second season might be due to accumulation of OMW in soil. On the contrary, catalase activities from Vin (50 &100%) and KH (100%) were higher than those in control in the first season and all Vin and KH (50 & 100%) were lower than those in control in the second season.

In lettuce sampled from sandy soil, catalase activities in all organic fertilization treatments were lower than that in control in both seasons, being the minimum in vinasse treatment. Catalase in case of OMW and KH treatments increased in the second season and with less treatment sharing (50%).

Peroxidase and catalase had an adequate role in quenching ROS resulting from oxidative stress (Leitão et al., 2021). The formation of reactive oxygen species ROS needed to be removed boosted the plant to form those complex antioxidant enzymes (Zhao et al., 2022).

3.2 Plant Analysis

In Tables 4 and 5 the photosynthesis pigments, NPK, fresh weight (Yield), head diameter and number of lettuce leaves results in lettuce leaves at harvest were shown, while in Table 5 their correlations were calculated.

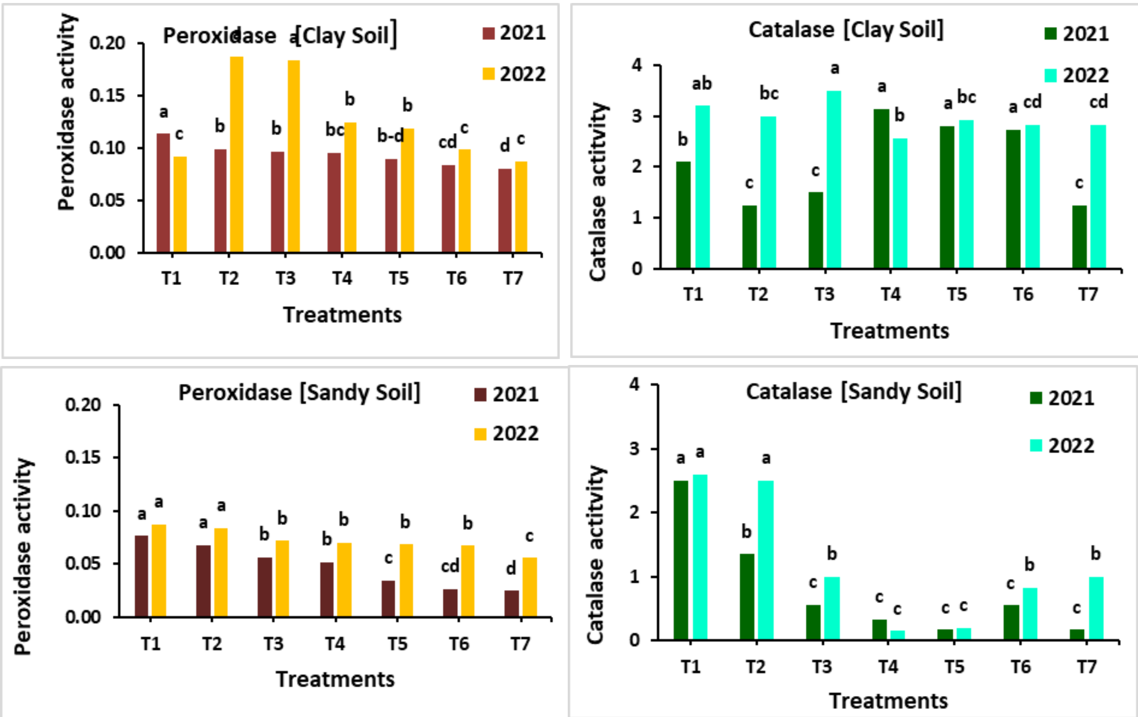

Fig. 1. Catalase and Peroxidase activities in lettuce plant at harvest, where $LSD_{0.05}$ for Clay soil peroxidase [0.011/0.015] and catalase [0.466/0.305], while for sandy soil peroxidase [0.009/0.009] and catalase [0.362/0.496] in 2021/2022 seasons, respectively. [T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%

Table 2. Chemical and physical properties of soil before plantation

Analyte	O.M (%)	$CaCO_3$ (mg. Kg^{-1})	pH	EC (dS. M^{-1})	Coarse sand %	Fine sand %	Silt %	Clay %
Clay soil	7.50	29.0	8.03	2.75	14.2	8.32	26.5	51
Sandy Soil	5.2	18.5	7.95	1.25	12.80	73.20	8.30	5.70
Analyte	Soluble Anions (mmol. l^{-1})				Soluble Cations (mmol. l^{-1})			
	$(HCO_3)^{-1}$	$(Cl)^{-1}$	$(SO_4)^{-2}$		$(K)^{+1}$	$(Ca)^{+2}$	$(Mg)^{+2}$	$(Na)^{+1}$
Clay soil	5.65	12.33	9.55		0.87	10.90	5.66	10.07
Sandy Soil	1.54	3.22	7.74		0.88	3.85	2.90	4.87
Analyte	Available Macronutrients (mg. Kg^{-1})				Available Micronutrients (mg. Kg^{-1})			
	N	P	K		Cu	Fe	Mn	Zn
Clay soil	53.3	4.20	60.4		0.40	3.40	2.33	0.65
Sandy Soil	33.50	2.96	40.3		0.38	1.49	0.89	0.55

Clay Soil form Giza (A), Sandy Soil from Ismailia (B)

Table 3. Analysis of agro-industrial wastes used as organic fertilizer

Organic fertilizer	pH	EC	O.M%	N%	P%	K%
Olive mill water	5.9	2.1	60.3	0.95	0.28	1.28
vinasse	4.3	12	35	0.23	0.35	5.46
K-humate	8.1	4	21.5	0.52	0.00	6.25

As shown in Table 4, the effect of lowering the share of organic amendments on analytic level in leaves at harvest varied according to organic fertilizer and soil types. TSS contents in lettuce increased parallel to higher Vin and KH fertilizer shares in clay soil, while increased OMW and Vin shares in sandy soil led to decrease in TSS content.

Worthy to notice that, the increase of chlorophyll and carotenoids in cultivated lettuce could be recognized in the second season than in the first among all treatments in both soil types exceeding that of control in most cases.

On the other hand, catalase activities in leaves were negatively correlated to chlorophyll contents while positively correlated to carotenoid contents in most cases of lettuce harvested from both soil types, as shown in Table 6.

Putting into consideration that the level of chlorophyll contents in any plant leaves are negative signs for their health states when facing any type of stress while carotenoids are positive signs for those states. So, whenever the catalase increases as a positive response to stress the carotenoids are positively correlated, while vice versa with chlorophyll. Lettuce chlorophyll content the use of several organic amendments, including molasses and vinasse, enhanced the crop (dos Santos et al., 2010; Liu et al., 2014). The increase in pigments responsible for photosynthesis could be attributed to the successful tolerance mechanism in lettuce (Deuner et al., 2020).

3.3 Measurements of Nitrogen, Phosphorous, and Potassium in plants (N P K)

Vinasse is beneficial because it contains chelated organic micro and macro nutrients, which are essential for plant growth, and because it may increase the bioavailability of NPK, which is essential for photosynthesis pigment synthesis (as mentioned by (Parnaudeau et al., 2008). Previous studies reported that leaf nutrient concentrations had increased when agro-industrial waste was applied to soil cultivated with several crops due to increased availability of nutrients in their rhizosphere (Carvalho et al., 2014; Mansour, 2018). Nitrogen decreased in the leaf when treated by Vin and OMW than control, which could be clarified by N consumption in the formation of head lettuce (Mirdad, 2016).

In clay soil, the lower share of both KH and OMW used increased chlorophyll and nitrogen while decreasing the potassium contents, while they were all increased by decreasing shares in all organic fertilizer types used in sandy soil.

Table 4. Photosynthesis pigments (µg/g FW), TSS% and NPK (%) in harvested lettuce leaves

Soil / year	Treat.	TSS%				Chlorophyll				Carotenoids				N				P				K			
Clay Soil		mean	±	se	rank	mean	±	se	rank	mean	±	se	rank	mean	±	se	rank	mean	±	se	rank	mean	±	se	rank
2021	T1	0.10	±	0.01	b	0.43	±	0.02	de	0.47	±	0.01	c	3.87	±	0.13	a	0.12	±	0.02	b	12.67	±	0.17	a
	T2	0.13	±	0.00	a	0.53	±	0.04	cd	0.39	±	0.03	cd	1.39	±	0.06	d	0.15	±	0.01	a	10.13	±	0.13	b
	T3	0.12	±	0.01	a	0.93	±	0.04	b	0.72	±	0.02	b	1.87	±	0.13	c	0.15	±	0.01	a	9.33	±	0.09	c
	T4	0.12	±	0.00	a	0.55	±	0.03	c	0.31	±	0.04	de	1.47	±	0.03	d	0.15	±	0.01	a	8.63	±	0.19	d
	T5	0.11	±	0.01	ab	0.33	±	0.02	e	0.25	±	0.03	e	1.12	±	0.01	d	0.15	±	0.00	a	8.40	±	0.10	d
	T6	0.09	±	0.00	b	0.87	±	0.04	b	0.87	±	0.04	a	2.17	±	0.17	c	0.14	±	0.00	ab	7.33	±	0.08	e
	T7	0.07	±	0.01	c	1.09	±	0.06	a	0.34	±	0.03	de	3.13	±	0.12	b	0.13	±	0.00	ab	6.00	±	0.18	f
LSD			0.02			0.12				0.09				0.32				0.03				0.42			
Clay Soil	T1	0.08	±	0.01	b	0.55	±	0.03	c	0.61	±	0.05	c	3.10	±	0.05	ab	0.09	±	0.00	d	12.83	±	0.17	a
2022	T2	0.12	±	0.01	ab	0.59	±	0.06	c	0.42	±	0.04	d	1.55	±	0.05	c	0.12	±	0.01	c	10.67	±	0.17	b
	T3	0.13	±	0.01	ab	1.10	±	0.07	ab	0.77	±	0.02	b	1.93	±	0.07	bc	0.13	±	0.01	bc	9.96	±	0.04	c
	T4	0.10	±	0.03	ab	0.59	±	0.05	c	0.39	±	0.03	d	1.86	±	0.07	bc	0.14	±	0.00	b	8.93	±	0.18	d
	T5	0.14	±	0.03	a	0.47	±	0.04	c	0.38	±	0.01	d	2.73	±	0.15	a-c	0.14	±	0.00	bc	8.77	±	0.06	d
	T6	0.09	±	0.02	ab	0.93	±	0.04	b	0.90	±	0.03	a	2.98	±	0.03	ab	0.15	±	0.00	a	7.77	±	0.04	e
	T7	0.08	±	0.01	b	1.23	±	0.13	a	0.42	±	0.02	d	3.65	±	0.17	a	0.16	±	0.00	a	6.23	±	0.04	f
LSD		0.06				0.20				0.09				0.93				0.01				0.35			
Sandy Soil	T1	2.65	±	0.49	ab	0.41	±	0.06	c	0.56	±	0.02	a	2.68	±	0.17	ab	0.10	±	0.01	b	5.90	±	0.10	a
2021	T2	2.11	±	0.45	b	0.17	±	0.00	d	0.23	±	0.02	e	2.07	±	0.07	a-c	0.13	±	0.00	a	5.73	±	0.15	ab
	T3	3.80	±	0.52	a	0.66	±	0.05	b	0.43	±	0.04	b	2.25	±	0.14	a-c	0.12	±	0.01	a	5.50	±	0.29	a-c
	T4	2.34	±	0.37	b	0.43	±	0.04	c	0.30	±	0.02	de	1.61	±	0.10	c	0.12	±	0.00	a	5.10	±	0.10	cd
	T5	3.39	±	0.48	ab	0.47	±	0.02	c	0.32	±	0.01	cd	2.94	±	0.07	a	0.11	±	0.01	ab	4.83	±	0.17	d
	T6	3.87	±	0.50	a	0.76	±	0.02	b	0.40	±	0.03	bc	2.60	±	0.05	ab	0.09	±	0.00	b	4.13	±	0.13	e
	T7	2.96	±	0.40	ab	0.93	±	0.02	a	0.33	±	0.01	cd	2.00	±	0.29	bc	0.07	±	0.01	c	5.33	±	0.17	bc
LSD		1.40				0.11				0.07				0.60				0.02				0.39			
Sandy Soil	T1	3.12	±	0.35	bc	0.49	±	0.04	c	0.60	±	0.02	a	2.93	±	0.07	a	0.08	±	0.01	b	5.97	±	0.03	a
2022	T2	3.29	±	0.68	bc	0.21	±	0.01	d	0.31	±	0.02	e	2.67	±	0.17	a	0.12	±	0.01	ab	5.93	±	0.07	a
	T3	3.19	±	0.40	bc	0.73	±	0.04	b	0.51	±	0.01	b	2.87	±	0.13	a	0.13	±	0.01	ab	5.67	±	0.17	ab
	T4	1.97	±	0.25	c	0.47	±	0.02	c	0.34	±	0.04	de	1.83	±	0.09	b	0.10	±	0.03	ab	5.57	±	0.07	b
	T5	3.86	±	0.26	b	0.49	±	0.03	c	0.39	±	0.01	cd	3.13	±	0.13	a	0.14	±	0.03	a	4.97	±	0.03	c
	T6	3.92	±	0.52	b	0.85	±	0.04	b	0.41	±	0.01	c	2.88	±	0.12	a	0.09	±	0.02	ab	4.97	±	0.03	c
	T7	5.87	±	0.49	a	1.13	±	0.08	a	0.42	±	0.02	c	1.88	±	0.09	b	0.08	±	0.01	b	4.93	±	0.10	a
LSD		1.35				0.13				0.06				0.46				0.06				0.26			

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%
The colored horizontal graph columns express the magnitude of results individually under each parameter

On the other hand, substituting chemicals with organic fertilization did increase not only soil organic matter but also the total nitrogen and decreased EC which was positively correlated to increase in final lettuce yield. In the present study (Hossain & Ryu, 2017), addition of OMW might have shared in decreasing EC of clay soil Increased OMW and vinasse did increase their effect of acidity and OM content in both soil types.

3.4 Correlations between Leaf Analysis and Yield

In correlations described in Table 6, based on results in Tables 4 and 5, data were held to emphasize if head diameter or leave number were well correlated with final fresh weight. In sandy soil, both head diameter and leave number were positively correlated to final fresh weight (yield), while in clay soil the leaf number was the main contributor to fresh weight yield.

The positive correlation of leave no. with chlorophyll and carotenoid contents in lettuce from both types of soil and seasons was similarly noted by work of (Zhao et al., 2022). Worthy to mentioning that the positive effect of both chlorophyll and carotenoids on fresh weight (yield) at harvest appeared as positive correlations, obviously in which the chlorophyll had the upper hand than carotenoids beside it had more impact on yield in sandy soil than in clay soil (more positively correlated).

In case we were comparing organic amendments effect depending on their [NPK and OM] contents, we could get correlations shown in Fig. 2, putting in regard that the more fertilizer we add the less mineral we put. In clay soil, the head diameters were positively correlated with increments in all organic fertilizer additions from 50 to 100%, based on their organic matter, N, and P contents, while this relation was absent in sandy soil. As in clay soil, important nutritional contents were said to be captured and slowly released for the benefit of planted lettuce, while on the contrary, the sandy soil loses so much nutrients (Garbowski et al., 2023).

The unusual increase in lettuce yield and analytical results may be attributed to undetermined analytical characteristics. Beneficial characteristics include low pH, Cu, Zn, Fe, K, and low pH in OMW, while negative characteristics include high EC, phenols, heavy metals, phenols and high pH in vinasse. The addition of more fertilizer may positively affect yield due to beneficial characteristics rather than its NPK or OM content (El-Etr & Hassan, 2017).

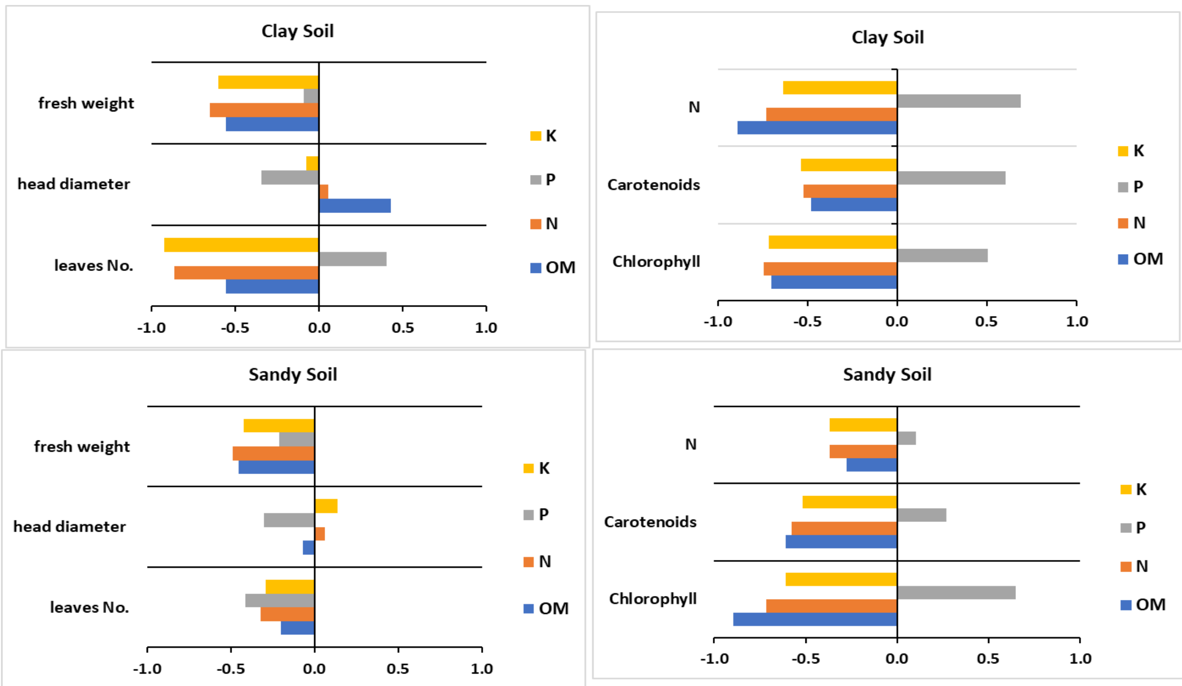

Fig. 2. Correlating analytical contents in fertilizers used with those in lettuce and yield parameters measured

Table 5. Fresh weight (Yield), head diameter and number of harvested lettuce leaves

Soil / year	Treat.	leaves No.				head diameter (cm)				fresh weight (g/plant)			
		mean	±	se	rank	mean	±	se	rank	mean	±	se	rank
Clay Soil 2021	T1	25.0	±	0.29	d	1.50	±	0.29	b	483	±	4	c
	T2	27.7	±	0.15	b	2.20	±	0.12	a	327	±	8	e
	T3	27.0	±	0.29	c	1.40	±	0.06	b	851	±	5	a
	T4	19.7	±	0.15	f	1.40	±	0.12	b	228	±	10	f
	T5	23.7	±	0.15	e	1.73	±	0.15	ab	764	±	7	b
	T6	27.7	±	0.15	b	1.50	±	0.12	b	422	±	4	d
	T7	29.3	±	0.15	a	1.50	±	0.06	b	765	±	9	b
LSD			0.37				0.55				25.5		
Clay Soil 2022	T1	28.0	±	0.29	c	1.80	±	0.06	bc	510	±	6	c
	T2	29.0	±	0.29	b	2.93	±	0.07	a	381	±	10	e
	T3	30.0	±	0.29	a	1.63	±	0.07	cd	857	±	12	a
	T4	24.5	±	0.29	d	1.33	±	0.09	e	228	±	10	f
	T5	24.3	±	0.20	d	1.90	±	0.10	b	775	±	8	b
	T6	28.4	±	0.23	bc	1.50	±	0.00	de	438	±	10	d
	T7	29.8	±	0.17	a	1.60	±	0.06	cd	784	±	8	b
LSD			0.77				0.21				28.4		
Sandy Soil 2021	T1	23.7	±	3.34	ab	1.24	±	0.12	bc	467	±	3	c
	T2	15.7	±	0.15	c	1.00	±	0.24	c	384	±	8	d
	T3	23.0	±	0.29	ab	2.00	±	0.19	a	784	±	12	b
	T4	15.0	±	0.29	c	1.50	±	0.24	ac	354	±	7	e
	T5	20.0	±	0.58	b	1.73	±	0.12	ab	823	±	12	a
	T6	10.0	±	0.29	d	1.50	±	0.09	ac	395	±	4	d
	T7	25.0	±	0.29	a	1.50	±	0.05	ac	782	±	9	b
LSD			3.85				0.61				31.8		
Sandy Soil 2022	T1	17.5	±	0.29	d	1.83	±	0.09	bc	475	±	9	c
	T2	17.5	±	0.29	d	1.10	±	0.10	e	388	±	8	de
	T3	23.8	±	0.15	b	2.50	±	0.12	a	793	±	6	b
	T4	16.5	±	0.29	e	2.23	±	0.15	ab	365	±	6	e
	T5	21.6	±	0.30	c	1.72	±	0.12	cd	832	±	13	a
	T6	11.8	±	0.17	f	1.33	±	0.09	de	406	±	7	d
	T7	26.4	±	0.10	a	1.73	±	0.34	cd	794	±	14	e
LSD			0.72				0.50				37.1		

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%
The colored horizontal graph columns are expressing the magnitude of results individually under each parameter

Table 6. Correlations between analyzed parameters in lettuce leaves

	Analyte	Catalase	TSS%	Chlorophyll	Carotenoids	N	P	K
Clay Soil 2021	Peroxidase	0.0	0.5	-0.6	-0.1	0.3	-0.3	1.0
	Catalase		0.1	-0.5	0.0	-0.3	0.1	0.0
	TSS%			-0.5	-0.1	-0.7	0.6	0.5
	Chlorophyll				0.5	0.3	0.0	-0.6
	Carotenoids					0.1	-0.1	0.0
	N						-0.9	0.3
	P							-0.3
Clay Soil 2022	Peroxidase	0.5	0.7	-0.1	0.0	-0.9	-0.1	0.3
	Catalase		0.3	0.2	0.4	-0.1	-0.4	0.5
	TSS%			-0.3	-0.2	-0.6	0.0	0.1
	Chlorophyll				0.4	0.4	0.6	-0.6
	Carotenoids					0.1	0.1	0.1
	N						0.2	-0.4
	P							-1.0
Sandy Soil 2021	Peroxidase	0.8	-0.5	-0.8	0.3	-0.1	0.6	0.8
	Catalase		-0.4	-0.5	0.6	0.2	0.1	0.6
	TSS%			0.6	0.4	0.5	-0.3	-0.6
	Chlorophyll				0.2	0.0	-0.8	-0.4
	Carotenoids					0.5	-0.3	0.2
	N						-0.2	-0.2
	P							0.3
Sandy Soil 2022	Peroxidase	0.7	-0.6	-0.8	0.3	0.5	0.1	0.9
	Catalase		0.0	-0.4	0.3	0.3	-0.3	0.7
	TSS%			0.7	0.0	-0.1	-0.3	-0.6
	Chlorophyll				0.3	-0.3	-0.5	-0.7
	Carotenoids					0.4	-0.3	0.3
	N						0.5	0.1
	P							0.0

POD: Peroxidase, CAT: Catalase, TSS: total soluble salts, Chlor.: Chlorophyll, Carot.: Carotenoids, HD: Head diameter, negative and positive correlations are expressed in yellow and green columns, respectively.

Table 7. Soil enzymes activity

Soil / year	Treat.	DeHase				N-ase				P-ase			
		mean original	±	se	rank	mean original	±	se	rank	mean original	±	se	rank
Clay Soil 2021	T1	2.81	±	0.12	e	40.17	±	0.31	e	14.53	±	2.12	b
	T2	33.56	±	0.30	b	67.83	±	0.38	b	23.83	±	2.67	a
	T3	22.83	±	0.51	c	60.73	±	0.45	c	20.73	±	3.22	ab
	T4	33.45	±	0.46	b	33.83	±	0.11	f	24.73	±	1.88	a
	T5	35.00	±	0.29	a	82.67	±	0.38	a	24.38	±	2.55	a
	T6	12.52	±	0.24	d	46.33	±	0.34	d	21.93	±	2.95	ab
	T7	21.86	±	0.07	c	68.17	±	0.56	b	24.90	±	1.21	a
LSD		0.97				1.16				7.44			
Clay Soil 2022	T1	3.93	±	0.23	f	40.33	±	0.24	e	13.50	±	1.68	b
	T2	34.18	±	0.29	b	67.83	±	0.46	b	22.87	±	3.90	a
	T3	23.90	±	0.08	c	60.67	±	0.41	c	21.43	±	3.70	ab
	T4	36.47	±	0.29	a	33.73	±	0.41	f	25.23	±	2.26	a
	T5	35.83	±	0.27	a	82.33	±	0.24	a	23.90	±	1.82	a
	T6	13.83	±	0.17	e	46.17	±	0.44	d	22.57	±	2.42	a
	T7	22.80	±	0.20	d	68.10	±	0.17	b	25.13	±	2.68	a
LSD		0.70				1.08				8.35			
Sandy Soil 2021	T1	1.79	±	0.12	d	16.90	±	9.77	e	8.67	±	0.76	c
	T2	16.90	±	0.49	b	23.23	±	13.43	b	15.17	±	1.36	a
	T3	20.37	±	0.88	a	21.17	±	12.24	c	13.63	±	1.25	ab
	T4	12.57	±	0.90	c	24.95	±	14.42	a	11.63	±	1.59	bc
	T5	18.43	±	0.54	ab	20.17	±	11.66	d	13.90	±	0.96	ab
	T6	12.63	±	0.43	c	21.67	±	12.52	c	12.00	±	1.10	abc
	T7	18.53	±	0.40	ab	22.50	±	13.01	b	12.87	±	0.67	ab
LSD		1.80				0.74				3.43			
Sandy Soil 2022	T1	3.07	±	0.56	e	16.90	±	0.37	f	7.90	±	0.74	e
	T2	18.30	±	0.55	b	23.17	±	0.20	b	16.00	±	0.64	a
	T3	20.67	±	0.44	a	21.10	±	0.15	d	14.17	±	0.61	bc
	T4	13.83	±	0.46	c	24.90	±	0.19	a	12.57	±	0.67	cd
	T5	19.67	±	0.63	ab	20.20	±	0.21	e	14.73	±	0.47	ab
	T6	11.17	±	0.62	d	21.73	±	0.39	cd	12.11	±	0.52	d
	T7	14.33	±	0.63	c	22.53	±	0.29	bc	13.17	±	0.46	cd
LSD		1.70				0.83				1.79			

T1: control, T2 & T3: OMW 100 & 50%, T4 & T5: Vinasse 100 & 50%, T6 & T7: K-Humate 100 & 50%
The colored horizontal graph columns are expressing the magnitude
of results individually under each paramete

Studies have shown a positive relationship between nitrogen in leaves and chlorophyll concentration, with 50% vinasse having the strongest impact on growth and yield. The notable disparity in chlorophyll content in sandy soil may be due to accelerated nitrogen mineralization of fertilizer derived from olive mill water and the elevated nitrogen needs during rapid growth (Diacono & Montemurro, 2015). Multiple investigations have shown a positive relationship between nitrogen in leaves and chlorophyll concentration (Ding et al., 2005). Using 50% vinasse seemed to have the strongest impact on growth and yield (Roig et al., 2006).

Furthermore, the notable disparity in chlorophyll content in sandy soil may be attributed to the accelerated nitrogen mineralization of fertilizer derived from OMW, as compared to other treatments, and the elevated nitrogen need of plants during the rapid growth period (Lawlor et al., 2004). Application of vinasse along with chemical fertilizers before lettuce plantation provides nitrogen with higher potassium and phosphorus (Gómez-Muñoz et al., 2011; Ali et al., 2021). Plant nutrient content was enhanced by applying vinasse (Schiavon et al., 2010).

3.5 Soil Analysis

The residual enzyme activities in soil at harvest in response to soil and organic fertilizer types used and their cultivation seasons shown in Table 7 and as correlated to fertilizer analytical contents were well represented in Fig. 3. These enzyme activities were representing part of soil biological characters after harvest, which gave considerable expectations for future impacts of using organic amendments in soil ready conditions for the next plantation season.

For instance, both phosphatase and nitrogenase were positively responding to nitrogen, phosphorous and organic matter contents in organic amendments while potassium was reversely correlated to phosphatase and dehydrogenase activities in sandy soil. On the other hand, the organic matter and phosphorous contents were beneficial for dehydrogenase activities, while nitrogenase activities were negatively correlated to potassium, phosphorous and organic matter contents in organic amendments used in clay soil. Phosphatase activities in clay soil was positively affected to potassium and phosphorous contents in organic amendments .

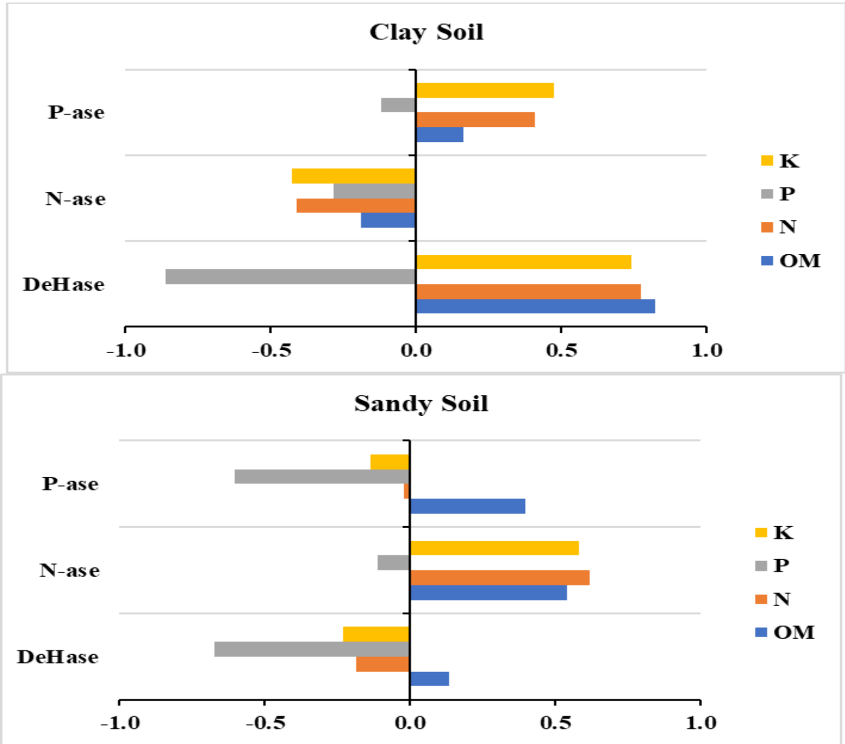

Fig. 3. Correlation between soil residual enzymes activities and analytical contents of fertilizers used

Phosphorous form in organic amendments were stated to be mostly in an organic form that needs increased microorganism phosphatase activities to be released for plant benefit in both soil types (Rawat et al., 2021).

Soil enzyme activity is a sensitive indicator of changes in soil quality due to management and land use changes. Soil additives decompose, releasing phenolic compounds, reducing biological activity. Organic matter treatment, like food waste compost, increases enzyme activity in lettuce growing, (Bowles et al., 2014). Soil enzyme activity is a sensitive indicator of changes in soil quality due to management and land use changes. Soil additives decompose, releasing phenolic compounds, reducing biological activity. Organic matter treatment, like food waste compost, increases enzyme activity in lettuce growing (Lopez-Piñeiro et al., 2011; Natywa et al., 2014). Soil organic matter treatment, such as food waste compost, increased soil enzyme activity in lettuce growing, according to another research (Wojewódzki et al., 2022). Potassium humate as an organic matter promoted microorganisms in soil (Khaled & Fawy, 2011).

4. CONCLUSION

This study imposed valuable insights about impact of partial substituting NPK mineral fertilization with diverse organic sources individually or in combination and permutation on the growth and yield of lettuce seedlings (*Lactuca sativa* L.). Applying OMW, Vin and KH to soil presented a viable way to improve organic farming practices while promoting resource and residue recycling in agricultural ecosystems. The utilization of OMW, Vin and KH along with reduced NPK fertilization by 50% was found to be the most effective in achieving desired growth while minimizing reliance on the mineral fertilizer by 50%. Besides, the chosen treatments were beneficial for enzymatic activities relying on microbial community in soil rhizosphere. Intended researches in future could focus more on the synergistic impacts of using those organic sources on physical, chemical and biological characteristics of soil. Added to that, comprehensive understanding of their agricultural implications in enhancing water and nutrient use efficiently.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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