COMPARATIVE EFFECTS OF DIFFERENT ORGANIC AND INORGANIC FERTILISERS ON SOIL FERTILITY, PLANT GROWTH, SOIL MICROBIAL COMMUNITY, AND STORAGE ABILITY OF LETTUCE

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The current study was conducted to assess whether organic fertilisation could replace mineral fertilisation in lettuce production without adverse effects on yield, quality, and postharvest behaviour. The effect of either mineral or organic fertiliser on soil fertility or the microbial community was also studied. Control plots were fertilised with recommended rates of mineral fertiliser (150: 45: 65 kg/ha NPK) and the other treatments were 15 t/ha compost, 10 t/ha rabbit manure, and 10 t/ha chicken manure. Our results indicated that all sources of organic fertiliser significantly increased total nitrogen, organic carbon, total fungi, and bacteria contents of soils compared with mineral fertiliser. Rabbit and chicken manure fertilisers resulted in a significant increase in yield. Compared with conventional fertiliser and other organic treatments, plots receiving rabbit manure showed a lower weight loss and microbial load on fresh lettuce head. Moreover, rabbit manure significantly reduced polyphenol oxidase and peroxidase activity. Hence, these results suggest that rabbit manure fertiliser could be an alternative to conventional production without significant reduction in yield and with low enzymatic browning and better storability.

Key words: *Lactuca sativa*, conventional fertiliser, plant performance, enzymatic browning, storage behavior, soil properties

In the Mediterranean region, the overuse of chemical fertiliser application to increase crop productivity caused several soil problems, such as structure deterioration, organic matter reduction, fertility declination, physicochemical plus microbial changes, erosion, and groundwater pollution (Liang *et al.* 2013; Montemurro *et al.* 2010; Meena *et al.* 2016). For this reason, organic fertilisers have been suggested by many researchers as an efficient alternative (Pradeepkumar *et al.* 2017; Cervera-Mata *et al.*

2018). Organic fertilisers are commonly applied instead of mineral fertilisers to enhancing the sustainable agricultural ecosystems without affecting the yield and quality (Hernández *et al.* 2016; Abuarab *et al.* 2019). Furthermore, the demand for organic fertilisers increased due to increasing organic markets of vegetables annually worldwide (Chatterjee *et al.* 2014). Likewise, the organic fertilisers show various benefits including restoring, maintaining, and/or improving physicochemical and biological

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functions of soil, enhancing soil sanitation, improving crop production, and reducing gas emission of greenhouse (Hernández *et al.* 2014; Shehata *et al.* 2017).

In the Mediterranean region, the huge quantity of organic wastes, deposited annually from the agro-food industry (Fernández-Hernández *et al.* 2014), can be exploited as sources for supplying a significant amount of organic matter, macro-, and micro-nutrients. This would improve soil fertility and plant growth (Ayyobi *et al.* 2014; Abdeldaym *et al.* 2019). Besides, these wastes contain bioactive compounds that can improve the suppression capacity against soil-borne diseases and Phyto-parasitic nematode (Abdeldaym *et al.* 2012; Abdeldayem *et al.* 2014; Federica *et al.* 2017; Asses *et al.* 2018; Atia *et al.* 2020).

One of the popular fresh-leafy vegetable crops belonging to the *Asteraceae* family is lettuce (*Lactuca sativa* L.). Lettuce was cultivated intensively in the Mediterranean region. In 2015, lettuce recorded the maximum production of 2.4 million tons in Spain, Italy, France, Germany, and Egypt (FAOSTAT 2015). However, the crop was considered one of the most perishable leafy crops due to high water content and the sensitivity to microbial infections (Martinez-Romero *et al.* 2008).

Several pre-harvest factors affect the postharvest quality of crops such as fertilisation, irrigation, and abiotic stress (Shehata *et al.* 2019; Tudela *et al.* 2013; Viacava *et al.* 2018; El-Mogy *et al.* 2018; Vwioko *et al.* 2019). Mineral and organic fertilisers showed different influences on postharvest quality due to their different characteristics (Mahmoud *et al.* 2019). Mditshwa *et al.* (2017) reported that the nutrients, that release organic fertilisers slower than mineral ones, are the main factor affecting nutrients availability. Thus, the plants' physiological processes affect different crop quality parameters under organic and conventional systems. For instance, Paoletti (2015) recorded that the effect of the source of nitrogen (either organic or mineral) on the organoleptic attributes of crops edible parts. Reganold *et al.* (2010) and Khalil and Hassan (2015) concluded that postharvest quality parameters of many crops grown changed significantly between conventionally and organically managed soils. Many pre- and postharvest factors influenced firmness, soluble solids con-

tent, titratable acidity, nutritional content, pigments, colour, aroma, and microbial content (Oliveira *et al.* 2012; Alam *et al.* 2014; El-Mogy *et al.* 2019a). In this study, we compared composted farmyard wastes, rabbit and chicken manure (the main low-cost organic fertilisers in the Egyptian local environment) with mineral fertilisers. This study also investigated the influence of either organic or conventional fertilisation on soil chemical and microbial properties, yield quality parameters of lettuce heads at harvest time, and storage behaviour during refrigerated storage at 2°C for 28 days.

MATERIAL AND METHODS

Open field experiment setup and treatments

The field experiment was conducted at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Egypt (latitude 30.0131°N, longitude 31.2089°E, and 694 m). Lettuce (Evrancv) was evaluated during two successive winter seasons (from October until December of 2016 and 2017). The soil of the experimental area was ploughed at 30 cm depth. Then, soil samples were collected at the experiment beginning from each plot at 25 cm depth to identify the soil physicochemical and microbial properties (Table 1). The treatments included applications of three organic fertilisers to be compared to mineral ones. The following treatments were evaluated: i) control with mineral fertiliser application equal to 150 kg/ha N, 45 kg/ha P₂O₅, 65 kg/ha K₂O (recommended by the Agricultural Extension Office of Egyptian Agricultural Ministry); ii) 15 t/ha of compost generated from farmyard; iii) 10 t/ha of rabbit manure, and iv) 10 t/ha of chicken manure. The same organic fertilisers amounts were added twice every year (2016 and 2017) according to the chemical analysis (Table 3). The organic fertilisers were added before planting inside the rows. All organic fertilisers were obtained from Agricultural Experimental Station, Cairo University, Giza, Egypt. The chemical analysis and the available elements of all organic fertiliser are presented in Tables 2 and 3. The seed of lettuce was sown by hand in foam trays (209 wells) filled with peat moss and vermiculite (1:1, v:v) in a plastic greenhouse and irrigated daily. Lettuce seedlings were

transplanted in the field at 40 days after sowing. Plot area was 4 m². The space between plants was 20 cm and between rows was 70 cm. The plants were surface drip-irrigated following transplanting and continued through the experiment at a 15-day interval. Lettuce plants were harvested on the 30th of December. After harvesting, soil samples were taken for the determination of changes in soil chemical properties after mineral and organic fertiliser applications.

Soil sampling and soil physicochemical analysis

Soil samples were collected using an auger, following a W scheme to observe any variation in chemical properties, soils were sampled before cultivation (T0 and T2 for 2016 and 2017, respectively) and after harvest (T1 and T3 for 2016 and 2017, respectively). Four samples were taken at 25 cm depth from each plot after removing the weeds and 5 cm of soil

surface. Air-dried samples of soil were sieved 2 mm. For the physicochemical analysis, soil texture, pH (1:2.5, w/v), electrical conductivity (EC 1:2, w/v), total Kjeldahl nitrogen (TKN), mineral Nitrogen (N_{min}), soil organic carbon content (SOC) and available Phosphorus (P) of soil were determined by analytical methods (Abdeldaym *et al.* 2012). The exchangeable Calcium (Ca_{ex}) and Potassium (K) content was determined by atomic absorption and was extracted with an alkaline solution (pH 8.2) containing on BaCl₂ (10%) and triethanolamine (22.5 mL/L). Also, available Zinc (Zn_{av}) and Iron (Fe_{av}) were determined by using atomic absorption (Pye Unicam, model SP-1900, US).2.3.

Chemical analysis of organic fertilisers

Representative samples were taken randomly from plants and organic fertilisers. Both materials

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Physicochemical and biological properties of soils before experiment in 2016 and 2017

Parameter	2016	2017
Clay [%]	29.00	29.00
Sand [%]	45.00	45.00
Silt [%]	26.00	26.00
pH [1:2.5 H ₂ O]	7.55	7.76
EC [dS/m]	0.41	0.37
O.C [%]	0.92	0.82
O.N [%]	0.14	0.13
C/N ratio	6.57	6.31
N _{min} [mg/kg]	43.13	23.37
T.Fe [%]	4.43	4.34
T.Zn [mg/kg]	73.40	56.50
P _{av} [mg/kg]	81.70	68.80
Ca _{ex} [cmol/kg]	16.32	17.50
K _{ex} [cmol/kg]	2.57	2.41
Fe _{av} [mg/kg]	4.38	3.69
Zn _{av} [mg/kg]	2.08	2.31
Total cultivatable bacteria [CFU/g]	7.93	8.87
Total cultivatable fungi [CFU/g]	3.96	4.30

O.C – organic Carbon; O.N – organic Nitrogen; N_{min} – mineral Nitrogen; T.Fe – total Ferric; T.Zn – total Zinc; P_{av} – available Potassium; Ca_{ex} – exchangeable Calcium; K_{ex} – exchangeable Potassium; Fe_{av} – available Iron; Zn_{av} – available Zinc

were air-dried, ground, and analyzed according to Trinchera *et al.* (2006). For the organic fertilisers, the pH and EC were determined using pH and EC meters in aqueous solutions of the fertilisers with 3:50, w/v, and 1:10 w/v, respectively (Italian Official Methods for Fertiliser Analysis, 2002). The Springer–Klee based method (Ciavatta *et al.* 1989) was used for assessing the organic carbon (OC). Total N (TN) and mineral Nitrogen ($N_{\min}$) were determined using the Kjeldahl method. Available P (P_{av}) was assessed using the Olsen method. The available nutrients, like exchangeable Potassium (K_{ex}), exchangeable Calcium (Ca_{ex}), available Ferric (Fe_{av}), and available Zinc (Zn_{av}), were measured on the air-dried samples using atomic absorption spectrophotometer with air-acetylene, fuel.

Chemical analysis of lettuce plants

For the lettuce samples, the total nitrogen content was determined using the modified micro-Kjeldahl method as described by A.O.A.C (1990). Total Phosphorus (T.P) was determined calorimetrically by using the chlorostannous molybdophosphoric blue colour method in sulphuric acid according to Jackson (1973). Total Potassium (T.K) and Calcium (T.Ca) contents were determined using the flame photometer apparatus (CORNING M 410, Germany). Total

Ferric (T.Fe) and Zinc (T.Zn) concentrations were determined using an atomic absorption spectrophotometer with air-acetylene, fuel.

Plant growth and yield traits

Plant growth traits were measured at harvest time. Fifteen lettuce heads were selected randomly from each experimental unit to measure head fresh weight, head diameter, stem diameter, root weight, and SPAD reading (SPAD 502 Minolta Co, Japan).

Post-harvest evaluation

After 75 days from transplanting, the completely formed lettuce heads were harvested randomly from each plot. The outer leaves were trimmed and packed individually in a polypropylene stretch and film and refrigerated stored at 2°C and 95% RH for 28 days. Six heads were used per replicate with three replicates. The following parameters were taken weekly.

Weight loss

Five lettuce heads were used from each treatment to measure the percentage of weight loss. The weight loss was assessed by the detection of weekly variations in the weight of the stored plants until the end of the shelf life. The following equation is a mathematical representation of the weight loss according to El-Mogy *et al.* (2019b).

T a b l e 2

Chemical properties of organic fertilisers used in experiment during growing seasons 2016 and 2017

Analyses	2016			2017		
	Rabbit	Chicken	Compost	Rabbit	Chicken	Compost
pH [3:50 H ₂ O]	7.40	6.30	7.20	7.60	6.42	7.15
EC [dS/m]	1.98	2.58	1.48	1.76	2.44	1.38
O.C [%]	27.40	19.90	16.93	27.30	17.31	18.00
T.N [%]	1.60	1.35	1.22	1.40	1.70	1.08
C/N ratio	17.10	14.70	13.90	19.50	10.20	16.70
T.P [%]	1.40	0.94	0.81	0.72	0.69	0.59
T.K [%]	1.10	0.74	0.62	0.98	0.69	0.58
T.Ca [%]	1.29	1.02	0.97	1.57	1.00	0.94
T.Zn [mg/kg]	188.10	153.70	140.40	177.60	154.50	149.10
T.Fe [mg/kg]	814.20	597.30	645.70	799.70	633.20	731.30

O.C – organic Carbon; T.N – total Nitrogen; T.P – total Phosphor; T.K – total Potassium; T.Ca – total Calcium; T.Zn – total Zinc; T.Fe – total Iron

$$[\text{Weight loss } \%] = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100]$$

Browning index

The browning of stems was targeted by visual assessment and scored according to the following scale: 1 for no browning, 2 for slight browning, 3 for moderate browning, and 4 for severe browning.

Microbiological analysis

From each material (soil, organic fertiliser, and plants), representative samples were collected and kept in the refrigerator at 5°C for 3 days. These samples were used for the microbial analyses of the total fungi and bacteria. Determination of fecal coliform, serial dilution were inoculated into MB medium and incubated for 48 hours at 44.5°C. The results were recorded as positive when the colour of the medium turned into yellow and a notable amount of gas was trapped in the tubes (Rowse 1981). To obtain the total viable bacterial and fungal counts, 5 g from lettuce leaves were transferred to 50 ml falcon tubes in 30 ml of 0.85% NaCl and mixed vigorously for 1 min. Serial dilutions were plated onto nutrient

agar medium to determine the total viable bacterial count. Besides, utilization of the Potato Dextrose Agar (PDA) medium supplemented with 200 µg/ml ampicillin for total fungal counts. Counts of colony-forming units (CFU) were estimated after 3 days of incubation at 28°C. For bulk soil and rhizosphere samples, 5 g of soil or root samples were placed in 50 ml falcon tubes with a 45 ml 0.85% NaCl and mixed using vortex for 1 min.

Browning enzymes of lettuce plants (Polyphenol oxidase and peroxidase)

Browning enzymes were determined in the head tissue of lettuce. The peroxidase extraction methods were performed as described by Bestwick *et al.* (1998) with minor modification. Frozen samples were hydrated overnight with 0.8 mL of sodium phosphate buffer (100 mM, pH 7) at 4°C. The mixture was homogenized, then, centrifuged at 18,000 rpm for 20 min (4°C). The concentration of protein was assessed according to Bradford *et al.* (2019). Peroxidase activity was measured with guaiacol at 470 nm using a spectrophotometer (UV – visible light). Polyphenol oxidase activity (PPO) was

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Amount of elements supplied by different organic fertilisers based on dry weight (DW)

Parameter	First growing season (2016)			Second growing season (2017)		
	Rabbit manure	Chicken manure	Composted farm yard wastes	Rabbit manure	Chicken manure	Composted farm yard wastes
Moisture content [%]	16.20	10.10	36.10	9.30	12.20	43.10
Applied rates [t/ha]	10.00	10.00	15.00	10.00	10.00	15.00
DW of applied rates [t/ha]	8.38	8.99	9.585	9.07	8.78	8.535
EC [dS/m]	7.30	6.00	7.20	8.20	5.70	11.50
O.C [kg/ha]	2,296.10 ^a	1,789.00 ^b	1,622.70 ^c	2,476.10 ^a	1,519.80 ^b	1,536.30 ^b
T.N [kg/ha]	134.10 ^a	121.40 ^b	116.90 ^b	154.20 ^a	122.90 ^b	92.20 ^c
T.P [kg/ha]	87.20 ^a	84.50 ^a	77.60 ^a	65.30 ^a	60.50 ^a	51.01 ^a
T.K [kg/ha]	92.18 ^a	66.17 ^b	59.33 ^b	88.89 ^a	60.58 ^b	49.50 ^b
T.Ca [kg/ha]	108.10 ^a	91.70 ^b	93.00 ^b	142.40 ^a	87.80 ^b	80.20 ^b
T.Zn [g/ha]	1,576.30 ^a	1,381.80 ^b	1,345.70 ^b	1,610.80 ^a	1,356.50 ^b	1,272.60 ^c
T.Fe [g/ha]	6,823.00 ^a	5,369.70 ^b	6,189.00 ^a	7,253.30 ^a	5,559.50 ^c	6,241.60 ^b

DW – dry weight; EC – electrical conductivity; O.C – organic Carbon; T.N – total Nitrogen; T.P – total Phosphor; T.K – total Potassium; T.Ca – total Calcium; T.Zn – total Zinc; T.Fe – total Iron
Values within each row followed by the same letter are not significant according to Tukeytest ($P \leq 0.05\%$)

assessed according to Cao *et al.* (2009). A 1.5 mL aliquot of the enzyme extract was gently mixed with Sodium phosphate buffer (200 mM, pH 6.5) and 1.4 mL of catechol (500 mM) incubated in a water bath at 30°C for 35 min. The spectrophotometer used for this analysis was set at a wavelength equal to 420 nm. The specific activity was expressed as $\mu\text{mol catechol}/\text{min}/\text{mg protein}$.

Statistical analysis

The experiment was designed as a completely randomized block with four replications for each treatment. The analysis of variance (ANOVA) and means were applied by the Tukeytest ($P < 0.05$) using SPSS software (Ver. 19, SPSS Inc., and Chicago, IL). Principal component analysis (PCA) was used to illustrate the associations between observed variables and a new set of non-correlated parameters (variables).

RESULTS AND DISCUSSION

A general overview of the soil and organic fertilisers properties

The soil of the experimental field was characterized by clay-loam (sand 45%, silt 26 and clay 29%), according to texture classification of Indorante *et al.* (1990), low organic carbon, low EC values (Dellavalle 1992), and alkaline pH (pH = 7.9) which was associated by the small number of cations. For the chemical properties of the applied organic fertilisers, Table 2 shows neutral pH (between 6.3 and 7.4) and low EC value (between 1.38 and 2.58 dS/m). The rabbit manure revealed the highest OC (27.4 and 27.3%, first and second year, respectively) and N content (1.6% and 1.4%, first and second year, respectively). The compost and chicken manures contained lower contents of macronutrients in comparison with the rabbit manure.

Table 3 presents the calculated amounts of elements supplied to the soil by organic fertiliser based on the fertiliser dry weight. The rabbit manure supplied the highest amount of organic carbon and total elements in comparison to chicken manure and composted farmyard wastes. However, for the T.P, similar amounts were calculated for the three fertilisers. Chicken manure and composted farmyard

wastes supplied lower amounts of T.N and T.Zn during the second season (2017), while a higher amount of T.Fe was supplied during both seasons.

Effect of different organic fertilisers on soil pH and EC

After two growing seasons, there were no changes in soil pH (~ 7.67 in all plots) resulted from applying organic or mineral fertilisation (Figure 1A). The buffer capacity of the alkaline soil, together with, the relatively short time of the lettuce growth cycle could be the main reason for the pH stability (Hernández *et al.* 2016). This is supported by the findings of Abdeldaym *et al.* (2018) who found stable soil pH under the application of different organic fertilisers. Nonetheless, Abbasi *et al.* (2015) confirmed that the long period of organic fertiliser application changes the soil pH.

The EC values were ranged between 0.37 and 0.42 dS/m at the beginning of the experiment. A significant increase was recorded in the plots fertilised by chicken and rabbit manures at the end of the season (Figure 1B). In particular, EC values ranged between 0.75 and 0.87 dS/m and between 0.72 and 0.90 dS/m at the end of 2016 and 2017, for chicken and rabbit manure, respectively. The detected increase of soil salinity was possibly due to the application rates and EC values of rabbit and chicken fertilisers (Tables 2 and 3), compared to the compost (Abdeldayem *et al.* 2018). Similar results were found by Adande *et al.* (2017), and Azeez and Van Averbek (2012). The later found that poultry and goat manures increased soil EC due to the increase in the ratio of dissolved salts. However, since the EC values of soil fertilised with organic fertilisers were inferior to the critical level (4 dS/m), no prospective threat can be expected to lettuce growth (Tartoura *et al.* 2014).

Effect of different organic fertilisers on SOC and SON contents

Data in Figure 1C and 1D shown that no significant differences were observed in soil organic carbon (SOC) and soil organic nitrogen (SON) contents after applying mineral and organic fertilisers at the beginning of 2016 and 2017 seasons (T0 and T2). The differences were recorded by the end of 2016 and 2017 seasons, (T3 and T4). The treatments of organic fertilisers resulted in higher carbon and ni-

trogen content. The highest values of SOC and SON contents were recorded for plots that received rabbit fertiliser followed by chicken manure and compost compared to mineral fertilisers at T1 and T3 (Figure 1C and D). Figure 1D shows that the addition

of organic fertilisers increased SON content during the sampling times (T1 and T3). However, no significant changes in SON content were recorded for plots fertilised by chicken and composted farmyard wastes during the 2016 season. During the 2017

Figure 1. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – soil pH; B – soil EC; C – soil organic carbon; D – soil organic nitrogen; E – soil total mineral nitrogen; F – soil available phosphorus content (P) at different sampling times (T0 & T2 indicate to initial, T1 & T3 indicate to end of trials). Columns with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Bars are indicating to standard error (SE).

season, the chicken manure significantly increased SON content than composted farmyard manure ($p \leq 0.05$, Figure 1D). Plots fertilised with mineral fertiliser had the lowest SON and SOC contents compared with plots that fertilised with organic fertilisers at T1 and T3 (Figure 1C and 1D). Accumulation of SOC and SON in soil was not only based on C: N ratio of organic matter but also on the quality of the labile source of C and N inputs (Table 3). The highest values of SOC and SON were recorded in treatments fertilised with rabbit manure due to the higher C: N ratio (Table 2). This has resulted in a lower mineralization rate of the organic fertilisers (Burger & Venterea 2008; Apesteguia *et al.* 2018) in contrast to chicken manure and the compost. Similarly, the quality of organic matter of organic fertilisers played a key role in SOC and SON stocks. SOC and SON were largely influenced by exogenous organic C and N as well as the available forms of N. The application of organic fertilisers with recalcitrant organic compounds, such as cellulose, hemicellulose, lignin, and other resistant substrates, could result in higher time for degradation and transformation of organic matter into the soil, and it leads to increasing the SOC and SON (Campitelli *et al.* 2012; Abdeldaym *et al.* 2018; Chen *et al.* 2018). By contrast, the application of available N, as high rates of slurries and chicken manures, can promote the rapid degradation of organic matter, promoting the reduction in contents of SOC and NOC (Angers *et al.* 2011). Thus, it is worth mentioning that incorporation of fresh organic wastes with C: N ratio higher than soil C: N ratio and composted organic manures, i.e. chicken and rabbit manures, improved the SON and SOC contents (Abdeldaym *et al.* 2014; Adande *et al.* 2017).

Effect of different organic fertilisers on soil $N_{\min}$, P_{av} , Ca_{ex} and K_{ex} contents

Statistical analysis showed that the application of different fertilisers had significant effects on $N_{\min}$, P_{av} contents (Figure 1E and 1F) K_{ex} and Ca_{ex} contents (Figure 2A and 2B). The initial values of soil $N_{\min}$ content were low at T0 and T2 and increased in plots fertilised by mineral and rabbit fertilisers at T1 and T3 (2016 and 2017). At T1, the highest value of $N_{\min}$ was recorded in plots fertilised by mineral fertilisers. The concentration of $N_{\min}$ in plots fertilised

by chicken manure was higher than those fertilised by rabbit manure and compost during 2016 (T1). Besides, a negative correlation between the C: N ratio and $N_{\min}$ content of organic fertilisers ($r = 0.87$) were observed. The reduction of soil $N_{\min}$ content in plots fertilised by chicken manure was observed during 2017 as compared to 2016 (Figure 1E). Such reduction could be correlated to the C: N ratio (Table 2) and a higher mineralization rate (Table 3). Several reports confirmed that the lower C: N ratio of organic fertilisers induced nitrogen loss by nitrification and immobilization, as well as ammonia volatilization (Burger & Venterea 2008).

The highest value of soil P_{av} content was observed after the application of mineral fertiliser (100.02 ± 2.8 and 95.5 ± 2.1 mg/kg for T1 and T3, respectively). Similar findings were recorded for soil Ca_{ex} and K_{ex} contents. The maximum values of Ca_{ex} and K_{ex} were observed in plots fertilised with mineral fertilisers and rabbit manures compared to compost and chicken manure. The improvement in soil P and Ca content could be attributed to the low solubility of superphosphate fertiliser and the slow release of nutrients (P and Ca). However, treatments with organic fertilisation added to the soil higher contents of phosphorus concerning mineral fertilisation (Table 3).

Regarding rabbit fertiliser, significant improvement in soil Ca_{ex} and K_{ex} concentrations could be explained by the amount of organic Ca and K supplied from organic fertilisers (Table 3). Also, the organic acids released from the organic fertilisers facilitated dissolving soil nutrients and increasing their availability for the crops (Mondal *et al.* 2015). Cherney *et al.* (2002) indicated that manures of high elements content increased soil extractable K and Ca even after short time application (2 years) in clay soil. Furthermore, the mineralization rate of organic manure and the possibility of soil N, K, and Ca accumulation are based on the manure type and soil properties (Egrinya-Eneji *et al.* 2003; Hernández *et al.* 2016).

In general, Figure 2 (C and D) shows that the organic fertilisers improved Zn_{av} and Fe_{av} contents over the two seasons of the experiment. Particularly, the soil was characterized by lower Zn_{av} and Fe_{av} contents at T0 and T2 than T1 and T3. This increase

was a direct result of applying the organic fertilisers in comparison with mineral fertilisers. However, the highest increase in Zn_{av} and Fe_{av} contents, observed at T1 and T3, was of rabbit manure, followed by chicken manure and compost. For chicken manure and compost, a similar increase in Zn_{av} and Fe_{av} contents was observed over 2016. Nonetheless, chicken manure had higher Zn_{av} and lower Fe_{av} contents, over 2017, than compost.

The different increases in Zn_{av} and Fe_{av} contents, observed for each organic fertiliser, can be contributed to their different composition. Indeed, the investigated correlations between total Zn and Fe contents of the organic fertilisers and Zn_{av} and Fe_{av} contents of the soil showed high correlation coefficients ($r = 0.6$ and in the case of Zn and Fe respectively). Tables 2 and 3 show that rabbit manure contents supplied the highest total Zn and Fe

Figure 2. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – soil exchangeable K; B – soil exchangeable Ca; C – soil available Zn; D – soil available Fe at different sampling times (T0 & T2 indicate to initial, T1 & T3 indicate to end of trials). Columns with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Bars are indicating to standard error (SE).

followed by chicken manure and compost. Organic fertilisers have an important influence on the biological and chemical reactions due to the elements high content in a way increasing the available Zn and Fe (Shahid *et al.* 2016). Also, Rutkowska *et al.* (2014) stated that long-term application of organic fertilisers can alter soil characteristics (e.g. pH and microorganisms) and, therefore, lead to soil richness in available forms of macronutrients regulating the availability of micronutrients to plants.

Effect of different organic fertilisers on soil microbial content

Figure 3A shows soil cultivable fungi and bacteria content over time in various treatments. For all sampling times, soil cultivable fungi and bacteria significantly increased with the addition of organic fertilisers against chemical fertilisers ($p < 0.05$). The microbiological analysis of lettuce plants grown under different fertilisation regimes showed significantly different population densities (Figure 3B and C). A significantly higher bacterial population were detected in the rhizosphere of lettuce plants fertilised by rabbit, compost, and chicken manure (Log CFU counts = 9.03 ± 0.01 , 9.04 ± 0.01 , and 8.99 ± 0.05 , respectively). In contrast, lettuce plants fertilised by compost fertilisers were characterized by the highest

fungal population (Log CFU counts = 4.71 ± 0.03). However, in the second season, the highest bacterial population was detected in the rhizosphere of lettuce plants fertilised by chicken manure (Log CFU counts = 5.72 ± 0.11 and 4.81 ± 0.12 , respectively). Notably, all used organic fertilisers also improved the density of cultivatable bacteria, cultivated fungi in root rhizosphere, and soil microbial content, in comparison to chemical fertilisers. These findings might be associated with amounts of organic carbon in various organic fertilisers (Chakraborty *et al.* 2011; Amalraj *et al.* 2013). This implies that root exudates and organic fertilisers are considered a source of sufficient carbon enriching the micro-organism biomass and its activity (Liu *et al.* 2010; Hernández *et al.* 2016). Similar results were also observed in an open field experiment conducted by Zhen *et al.* (2014), who found that total microbial biomasses significantly increased different organic fertiliser incorporation. It is worth mentioning that high levels of precautions should be considered when applying fertilisers that might contain fecal and urine, such as chicken and rabbit manure. That is because of the high possibility of contaminating vegetable leaves with pathogenic bacteria (e.g. fecal coliforms) which sicken the final consumer (Oliveira *et al.* 2012).

T a b l e 4

Effect of mineral fertiliser (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM) on total endogenous nutrient contents of lettuce head in 2016 and 2017 at harvest time. Data are mean of $4 \pm \text{SE}$ replicates. Values within each row followed by the same letter are not significant according to Tukeytest ($P \leq 0.05\%$)

Fertiliser	T.N [%]	T.P [%]	T.K [%]	T.Ca [%]	T.Fe [ppm]	T.Zn [ppm]
	2016					
MIN	5.45 ± 0.05^a	0.70 ± 0.09^a	6.95 ± 0.08^a	4.25 ± 0.13^a	57.55 ± 0.66^c	162.70 ± 4.09^c
RAB	5.15 ± 0.20^{ab}	0.52 ± 0.08^{ab}	6.60 ± 0.23^{ab}	3.85 ± 0.22^{ab}	78.07 ± 6.78^a	186.60 ± 3.52^a
COM	4.40 ± 0.17^b	0.41 ± 0.01^c	5.50 ± 0.05^c	2.15 ± 0.03^b	71.30 ± 105.83^{ab}	179.75 ± 9.43^{ab}
CHI	4.70 ± 0.11^b	0.61 ± 0.01^b	6.30 ± 0.05^b	2.05 ± 0.12^b	67.30 ± 3.42^b	171.38 ± 3.3^b
	2017					
MIN	5.80 ± 0.40^a	0.55 ± 0.01^a	6.41 ± 0.16^a	5.72 ± 0.02^a	46.60 ± 2.11^c	169.50 ± 1.05^b
RAB	5.60 ± 0.34^a	0.47 ± 0.01^b	6.60 ± 0.17^a	4.85 ± 0.04^{ab}	70.13 ± 2.15^a	192.08 ± 1.17^a
COM	4.05 ± 0.22^b	0.35 ± 0.02^c	5.15 ± 0.14^b	3.00 ± 0.23^b	66.90 ± 1.09^{ab}	163.05 ± 0.77^b
CHI	4.35 ± 0.08^b	0.56 ± 0.01^a	5.98 ± 0.11^b	2.10 ± 0.11^c	56.10 ± 0.17^b	171.75 ± 2.81^b

MIN – mineral fertiliser; RAB – rabbit manure; COM – compost; CHI – chicken manure; T.N – total Nitrogen; T.P – total Phosphor; T.K – total Potassium; T.Ca – total Calcium; T.Fe – total Iron; T.Zn – total Zinc

Effect of organic fertilisers on leaf nutrient content

Table 4 presents the effect of fertiliser types on nutrients content (N, P, K, Ca, Zn, and Fe) in lettuce leaves at the harvest time of 2016 and 2017. Lettuce plants receiving mineral fertiliser and rabbit manure showed higher leaf nutrients content than plots received chicken manure and compost. However, lettuce leaf content of Fe fertilised by organic fertilisers significantly increased more those fertilised by mineral ones. Over the two seasons, the highest

value of leaf Fe content was observed for plots fertilised by rabbit manure and compost, followed by those of chicken manure and mineral fertilisers.

For 2016, the highest Zn content in lettuce leaves was recorded for plots fertilised with rabbit manure, followed by compost and chicken manure. For 2017, rabbit manure application showed the highest value of leaf Zn content, compared to all the other treatments. Thus, insignificant differences in leaf Zn content were observed for treatments of compost,

Figure 3. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – soil bacteria and fungi; B – root cultivatable bacteria; C – root cultivatable fungi at different sampling times (T0 & T2 indicate to initial time, T1 & T3 indicate to end of trial time). Columns with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Bars are indicating to standard error (SE). D – Principle component analysis (PCA) of leaf nutrient content with soil nutrients content and soil organic carbon (SOC).

chicken manure, and mineral fertiliser.

The highest leaf contents of N, K, Ca, Zn and Fe recorded for plants treated with rabbit manure might be attributed to the content of the high element of rabbit manure compared with other organic fertilisers (Table 3). Furthermore, the high element's content of rabbit manure had a positive influence on nutrient availability during plant growth (Masunga *et al.* 2016; Cervera-Mata *et al.* 2019). However, P content showed more increase in lettuce leaves fertilised with chicken manure and mineral fertiliser than P contents in those fertilised with rabbit and compost. No significant difference in the soil

available P content between the applied organic fertilisers (Figure 1F). This can be explained by the high P uptake of lettuce during the growth cycle and the low C: N ratio of chicken manure in the soil. To understand the relationship between leaf nutrient content and soil nutrient content, a PCA has been carried out (Figure 3D). The PCA showed a clear positive relationship between the different available nutrients in the soil and total elements in a lettuce leaf, in particular N, P, K, and Ca. The areas of the leaf and soil nutrient content partially overlapped in the upper quadrants. The area of soil organic carbon was in the lower quadrant. The first component ac-

Figure 4. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – SPAD; B – stem diameter; C – head fresh weight; D – total yield. Columns with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Bars are indicating to standard error (SE).

counted for a large part of soil macronutrient content, while the second component accounted for those nutrients in the leaves of lettuce plants. Soil macronutrient content, supplied by different organic fertilisers, was caused variation of leaf nutrient content of the treated lettuce plants. Soil organic carbon was found as negatively related to available nutrient accumulation in soil and leaf nutrient contents. Furthermore, a positive relation was found between growth parameter and yield, and nutrient contents (N_{min} , P, K, Ca, and Zn) in soil (Figure 4B). A similar correlation was found between SPAD reading (chlorophyll content) and soil – Fe.

Effect of organic fertilisers on lettuce growth and production

Figure 5 shows the influence of the applied three organic fertilisers on lettuce growth and production parameters in comparison to mineral fertilisers. Compost applications showed the lowest SPAD values over the two growing seasons (Figure 4A). In 2016, there were no significant differences in the stem diameters of lettuce heads. However, compost had resulted in a significantly lower stem diameter (Figure 4B). For 2017, there was no difference in stem diameter resulting from applying mineral fertilisers and rabbit manure or applying chicken manure or compost.

Figure 5. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM) on (A) weight loss in 2016 and (B) weight loss in 2017 of lettuce head stored for 28 days at 2°C. Points with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Data are mean of $5 \pm SE$ replicates.

Figure 6. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – browning index (BI) in 2016; B – browning index in 2017 of lettuce head stored for 28 days at 2°C. Points with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Data are mean of $5 \pm SE$ replicates.

nure and compost. Nonetheless, the stem diameter was significantly higher for treatments of mineral fertilisers and rabbit manure increasing the size of lettuce and, consequently, better quality characteristics. Similar results were noted by Ayoola and Ma-

kinde (2014) who observed that the yield of green maize and vegetable cowpea significantly improved with the addition of organic fertilisers.

Figure 4C shows the fresh weight of the lettuce head. In 2016, the fresh weight of lettuce showed

T a b l e 5

Effect of mineral fertiliser (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM) on the Log number of total bacterial CFU counts of lettuce head stored for 28 days at 2°C in 2016 and 2017. Data are mean of 4±SE replicates. Values within each line followed by the same letter are not significant according to Tukeytest ($P \leq 0.05\%$)

Fertiliser	0 d	7 d	14 d	21 d
	2016			
MIN	4.20±0.36 ^a	5.17±0.16 ^b	5.42±0.21 ^a	6.12±0.27 ^b
RAB	3.85±0.23 ^a	5.27±0.23 ^b	5.52±0.09 ^a	6.17±0.37 ^b
COM	3.81±0.26 ^a	4.50±0.26 ^c	5.40±0.29 ^a	6.02±0.28 ^b
CHI	3.81±0.13 ^a	5.90±0.13 ^a	6.26±0.36 ^a	6.76±0.19 ^a
Fertiliser	2017			
MIN	3.68±0.32 ^b	4.76±0.01 ^b	5.36±0.01 ^b	6.05±0.42 ^b
RAB	3.29±0.12 ^c	4.49±0.02 ^c	5.33±0.01 ^b	5.82±0.19 ^b
COM	3.87±0.05 ^a	4.43±0.02 ^c	5.20±0.01 ^c	5.47±0.10 ^c
CHI	3.80±0.08 ^a	5.87±0.04 ^a	5.53±0.04 ^a	6.53±0.04 ^a

MIN – mineral fertiliser; RAB – rabbit manure; COM – compost; CHI – chicken manure

T a b l e 6

Effect of mineral fertiliser (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM) on the Log number of total fungal CFU counts of lettuce head stored for 28 days at 2°C in 2016 and 2017. Data are mean of 4±SE replicates. Values within each line followed by the same letter are not significant according to Tukeytest ($P \leq 0.05\%$).

Fertiliser	0 d	7 d	14 d	21 d
	2016			
MIN	2.17±0.20 ^b	2.33±0.15 ^b	3.10±0.40 ^a	3.18±0.34 ^a
RAB	1.90±0.00 ^b	2.33±0.15 ^b	2.90±0.19 ^a	3.01±0.15 ^a
COM	2.58±0.78 ^a	3.02±0.66 ^a	3.00±0.90 ^a	3.14±0.08 ^a
CHI	2.91±0.41 ^a	2.84±0.16 ^a	2.98±0.50 ^a	3.01±0.18 ^a
Fertiliser	2017			
MIN	2.05±0.21 ^d	2.55±0.07 ^b	3.20±0.13 ^a	3.07±0.10 ^b
RAB	2.34±0.06 ^c	2.64±0.06 ^b	2.50±0.11 ^b	2.64±0.09 ^c
COM	2.59±0.12 ^b	2.75±0.04 ^a	3.10±0.08 ^a	3.09±0.04 ^b
CHI	2.68±0.09 ^a	2.80±0.04 ^a	3.21±0.08 ^a	3.40±0.06 ^a

MIN – mineral fertiliser; RAB – rabbit manure; COM – compost; CHI – chicken manure

insignificant differences in the case of treatments of mineral fertilisers, rabbit, and chicken manure. Compost treatment, however, showed significantly lower weights. This result could be due to the lower concentration of N in compost. In 2017, the highest values of lettuce head fresh weight were recorded for mineral fertilisers and rabbit manure followed by chicken manure and compost (Figure 4C). Lettuce production showed a similar pattern over 2016 and 2017 for each fertiliser used (Figure 4D). The better growth and higher production of lettuce that observed for rabbit and chicken manure treatment could be explained by the regular mineralization of organic

matter by which nutrients were available at any plant time of need (Angin *et al.* 2017). Similar results were reported by Muymas *et al.* (2015) who observed that lettuce fresh weight, dry weight, leaf number, width, and length had increased by applying chitin-rich residues. The less effect of compost on fresh weight of lettuce head and SPAD reading in this study might be due to its lower content of nutrients, as reported by Murakami *et al.* (2011) and Mahmoud *et al.* (2020).

Effect of organic fertilisers on weight loss of head lettuce during storage

The quality of lettuce head during refrigerated storage at 2°C for 28 days was affected by organic

Figure 7. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – polyphenol oxidase (PPO) in 2016; B – polyphenol oxidase in 2017 of lettuce head stored for 28 days at 2°C. Points with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Data are mean of 5 replicates.

Figure 8. Effect of mineral fertilisers (MIN), chicken manure (CHI), rabbit manure (RAB), and compost (COM). A – peroxidase activity (POD) in 2016; B – peroxidase activity in 2017 of lettuce head stored for 28 days at 2°C. Points with the same letters are not significantly different according to Tukeytest ($P \leq 0.05$). Data are mean of 5 replicates.

and inorganic fertiliser applications. The weight loss of stored lettuce head increased with increasing storage duration (Figure 5A and B). Increasing weight loss during storage could be due to the increase of respiration and transpiration during storage (El-Mogy *et al.* 2019c). The weight loss of lettuce fertilised with chicken and compost was higher than lettuce fertilised with rabbit manure and mineral fertiliser over the two seasons. This reduction in weight loss may be due to the increase of dry matter in lettuce heads by organic fertilisers (El-Sayed *et al.* 2015).

There was a negative correlation ($r=0.90$) between weight loss and leaf calcium content. The positive effects of calcium in reducing weight loss of lettuce heads fertilised with organic sources could be due to its importance in maintaining cell wall structure and firmness (El-Mogy *et al.* 2019d).

Effect of organic fertilisers on the browning index and enzymatic browning during storage

Enzymatic browning is considering one of the most important factors affecting the shelf-life of some vegetables (EL-Mogy *et al.* 2020). Data in Figure 6 (A and B) showed the browning colour of the lettuce surface during storage. Browning colour was increased with increasing storage period. There were no significant differences between treatments after 7 and 14 days of storage. After 21 and 28 days, the lowest browning index was

recorded for rabbit manure followed by mineral fertiliser. Chicken manure and compost treatments had the highest browning indexes. Similar tendencies were, also, noted with the activity of polyphenol oxidase (PPO) and peroxidase enzymes (POD) (Figure 7, 8) where the activity of those enzymes increased gradually with storage periods (Shehata *et al.* 2020).

The minimum activity of PPO and POD in lettuce leaves was observed in plants treated with rabbit manure followed by chemical fertiliser and chicken manure. The highest activities of both enzymes were observed in plants fertilised by compost (Degl'Innocenti *et al.* 2005; Santos *et al.* 2016). This might be due to the increment of endogenous nutrients concentrations, particularly the Ca element. Moreover, the results of PCA and simple regression reflected a strong positive relation between browning index (BI) and polyphenol oxidase (PPO) accumulation in leaves ($R^2 = 0.98$, Figure 9). However, BI correlated negatively with Ca accumulation in leaves ($R^2 = 0.90$). Furthermore, the PCA indicated that PPO and POD enzymes were positively correlated to browning. Thus, the increment of endogenous Ca content in plant tissue can reduce the browning appearance (Figure 9A). Khumjing *et al.* (2011) found that Ca, in the form of CaCl_2 , when applied to the soil, significantly reduced the activity of PPO as well as phenolic and

Figure 9. Principle component analysis of leaf calcium content. A – with polyphenol oxidase (PPO); browning index (BI) and peroxidase (POD) additionally simple regression study. B – between polyphenol oxidase (PPO), browning index (BI).

quinone in Grand Rapids lettuce. Also, Ca played a positive role in the reduction of the activity of POD in plant tissue and, consequently, reduced the browning (Rico *et al.* 2006; Luna *et al.* 2012) in lettuce. Other studies reported that Ca preserved cell membrane integrity and reduced the activity of PPO which delayed the browning occurrence (e.g. Brummell *et al.* 2004).

Effect of organic fertilisers on microbiological analysis of lettuce head

Total bacterial and fungal counts during storage of lettuce head were presented in Table 5 and 6. Total bacterial and fungal spoilages on lettuce surfaces increased with increasing the period of storage. The highest bacterial population was recorded in lettuce leaves grown under chicken manure compared to all other treatments. By contrast, the lowest population of bacteria was observed in lettuce plants fertilised by compost at the end of storage during 2017 (Table 5). Similarly, the enumeration of total fungal counts showed the highest population for lettuce plants fertilised by compost and chicken manure during 2017. However, lettuce plants, amended by chemical fertilisers or rabbit manure treatments, significantly reduced the microbial growth on lettuce surfaces during storage times (Table 6). This reduction could be related to the initial account of microbes on lettuce before storage as reported by Bolin *et al.* (1977). Additionally, the inhibitory effect of endogenous Ca might be a result of increased resistance of tissue to bacterial attack rather than to a bactericidal action. Conway and Sams (1984) reported that Ca increased tissue resistance to fungal infection through stabilizing or strengthening cell walls in, a way resisting pectolytic enzymes released by fungi.

CONCLUSIONS

This study indicated that organic fertiliser positively affects soil microbial community and fertility. Lettuce plants can be fertilised with rabbit manure at 10 t/ha without affecting yield and this rate leads to improvement in head quality. Furthermore, the use of rabbit manure reduced weight loss, microbial load, and enzymatic browning during refrigerated storage at 2°C. Compared with conventional fertilis-

er, organic fertiliser, especially with rabbit manure, could be an effective way to reduce the harmful effect of mineral fertiliser on the environment and human health.

Declarations

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