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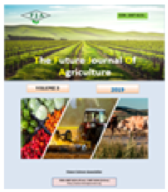

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IMPROVING PRODUCTIVITY AND QUALITY OF ROSELLE PLANT (*Hibiscus sabdariffa* L.) BY ADOPT ENVIRONMENTALLY FRIENDLY ORGANIC FERTILIZATION PRACTICES

Abeer M.O. Shoeip[1,*] and Samia M. Abd-El Hameed[2]

[1]Central Laboratory of Organic Agriculture, Agricultural Research Center, Giza, **Egypt**.
[2]Medicinal and Aromatic Plants Department, Horticulture Research Institute, Agricultural Research Center, Giza, **Egypt.**

[*]**Corresponding author:** shoeipabeer@yahoo.com **Received:** 20 Aug. 2022 **; Accepted:** 25 Oct. 2022

ABSTRACT: Hibiscus plant (*Hibiscus sabdariffa* L.) belongs to the family *Malvaceae*. Plant is a valuable medicinal crop rich in phytochemicals compounds with nutritional and medicinal properties cultivated in arid and semi-arid regions. In Egypt, roselle plant has unparalleled health and economic benefits. Roselle plants cultivated under upper Egypt conditions with low water and nutrients requirements. A field experiment was conducted at the experimental station of Elkanater Elkhairia, Qalyubia, Governorate, Egypt, during two successive growing seasons of 2017 and 2018. To study the effects of using some organic fertilizers as compost, compost-tea, potassium humat with a mixture of bio-fertilizers containing non-symbiotic nitrogen fixers (*Azotobacter chrococcum* and *Azospirillum lipoferum*) and phosphate dissolving bacteria (*Bacillus megaterium*) as an alternative to chemical fertilizers as they increase sustainable soil fertility by adopt environmentally friendly organic fertilization practices. Obtained results showed that, the organic - bio fertilizer treatments were significantly increase the vegetative growth parameters (number of branches/ plant, number of fruits/plant, sepals fresh weight/plant and sepals dry weight /plant.) and quality parameters such as chemical compositions (total chlorophylls, total carbohydrates contents, total phenol compounds, flavonoids, antioxidant activity %, ascorbic acid g./100g and anthocyanin content and acidity% as well as N, P and K%) compared to the ordinary mineral fertilizers are not environmental friendly. The highest values were recorded by the treatment of biofertilizers plus potassium humat and compost-tea, while the control treatments were giving the lowest values during the two growing seasons.

Key words: Hibiscus, Compost, Compost tea, Potassium humat, Biofertilizers, Quality.

INTRODUCTION

The hibiscus (*Hibiscus sabdariffa* L.) is a member of the *Malvaceae* family. Roselle is a medicinal plant rich in phytochemicals with nutritional and medicinal properties. They include organic acids, minerals, carotene, amino acids, vitamin C, total sugars, flavonoids, anthocyanin, micronutrients and protein **(Abdou *et al*., 2022)**. The economical part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules) **(El-Naim *et al*., 2017).** Medicinal plants are among the most promising crops that can increase Egypt's foreign exchange income **(Al- Sayed *et al*., 2020)**.

Soil nutrient deficiency is one of the major issue for agricultural production worldwide that affect the quantity and quality of crops. In order to improve crop yields, farmers have commonly used chemical fertilizers for agricultural production causing negative effect on indigenous organisms and deteriorating the quality of agro-ecosystems and aquatic resources **(Ghabour *et al*., 2021).** The extensive uses of mineral fertilizers have given rise to a variety of economic, environmental, ecological and social problems. in addition, the rising costs of agrochemicals includes fertilizers, soil conditioners and pesticides have left farmers helpless and lowered commodity prices, thus lowering the farmer income (**Shehata, 2019).** Also, the excessive use of mineral fertilizers have a negative impact on the quantity and quality of the active ingredients in medicinal plants, so it is preferable to use the raw materials produced by agrochemical-free farming as organic farming practices which, increase the economic value for the medicinal plants, improving soil quality, agricultural products and mitigates pollutants **(Moradzadeh *et al*., 2021).**

Resently attention has been focused on the use of organic and biofertilizers that contain beneficial microorganisms which have improved plant growth by providing plant nutrients and helping to maintain the environmental health and soil productivity instead of the mineral fertilizers **(Ghabour *et al*., 2019).**

Organic fertilizers are used to reduce the amount of toxic compounds (such as nitrate) produced by mineral fertilizers, improve physical, biological and chemical properties of the soil which leads to an improvement in the quality of vegetables and their safety for human health **(El-Mogy *et al*., 2021).**

In addition, compost and compost tea are organic soil amendments that increase plant nutrient availability as well as plants uptake. Compost tea enhances soil quality by improving its chemical, physical and biological properties, increasing organic matter content, soil water holding capacity and microbial diversity. It also provides essential nutrients to plants and suppressing diseases, which promote plant growth **(Abou Hussien and Elbaalawy, 2020)**.

Potassium-humate (KH) is a part of the humus compounds, which contains most of known trace minerals and plays an important role in plant nutritional balances **(Fallahi *et al.*, 2016)**. Potassium- humate fertilizer is effective organic potash that is uses as fertilizer or a growth promoter quality on the crops. Humic substances are hormones like substances, which improve plant nutrient uptake, root growth, enhance enzyme activity and increase yield **(Mohaseb *et al*., 2018).** Potassium-humate (KH) is a necessary natural substance that affects plant performance and soil fertility by increasing the numbers of microbes in the soil, stimulates the soil properties and increase the cation exchange capacity, also, increases the rate of nutrient uptake, improves plant biomass and decreases soil compaction. In addition, improve membrane permeability, enzyme activities, hormonal activity and increase water-holding capacity **(Mohaseb *et al*., 2018).**

Biofertilizers contains beneficial microorganisms which improve plant growth and protect plants from pests and diseases, they have the key role in productivity and sustainability of soil, also protect the environment as eco-friendly and cost-effective inputs for the farmers. Biofertilizers keep the soil environment rich in all kinds of macro and micro- nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil **(Yimer and Abena, 2019).** *Azotobacter* spp. fixes about 20- 40 Kg N/h/year **(Thomas and Singh, 2019),** while *Azospirillum* spp. alone fixes nitrogen up to 20- 40 Kg/ha/year (**kumar *et al*., 2022).** Phosphorous is a major macronutrient required for plant growth and development (**Bamagoos *et al*., 2021).** Soil contains a reasonable quantity of phosphorus up to 400–1200 mg/Kg of soil, but it is not available, which reduces crop yield **(Wang *et al*., 2017).** Phosphate- solubilizing bacteria are able to convert the organic form of phosphate into an inorganic form **(Tandon *et al*., 2020),** while **Santoyo *et al*. (2021b)** explained that *Baillus* spp. have been reported to solubilize phosphorous in soil**.**

Bacillus, *Azospirillum* and *Azotobacter* spps. produce Indole Acetic Acid (IAA), Cytokinins (CK), Gibberellins (GA) and inhibitors of ethylene, which takes up the great responsibilities of nutrients and water uptake required for plant growth **(Dhayalan and Karuppasamy, 2021).** Soil enzymes have been effectively used as indicators of soil quality. Improving the action of soil enzymes and the factors that affecting their activity is vital to enhancing soil management, quality, and food production (**Sulewska *et al*., 2020)**. Soil enzyme activities can provide an early picture of improved soil health during biological fertilization practices. The enzymatic activities are critical indicator of soil fertility because enzymes play an important role in nutrient cycles (**Ahmed and Zaki, 2021).**

This study was conducted to evaluate the effect of compost, compost tea, potassium-humate with biofertilizers in single or mixed combinations compared to the full dose of mineral fertilizer on vegetative growth, yield quantity and quality (chemical compositions) of hibiscus *(Hibiscus sabdariffa* L.) plants.

MATERIALS AND METHODS

A Field experiment was carried out at the experimental farm of agriculture research station of Medicinal and Aromatic Plants Research Department, El Kanater El Khairia, Horticulture Research Institute, Qalyubia Governorate, Agriculture Research Centre (ARC), Egypt. In two successive growing seasons of 2017 and 2018. Roselle (*Hibiscus sabdariffa* L.) seeds (Sabhia 17 dark red variety) were sown on 15th April, 2017 and 2018 (in the first and second season) under surface irrigation system. A randomized complete block design with 9 treatments and 3 replications was used. The experimental plot area was 9.6 m^2, which contains 4 rows. Each row was 4 m. length and 0.6 m. wide. Seeds were sown in the upper third of the row at spacing of 50 cm apart. When plants achieved adequate growth about 15 cm height, thinning was carried out leaving one plant/hole (about 14.000 plants/ feddan).

This experiment aiming to investigate the effect of compost, compost tea, potassium humate with mixture of biofertilizers using *Azospirillum*, *Azotobacter* and *Bacillus* spps as well as their combination, comparing full dose of mineral fertilizer on vegetative growth and yield of hibiscus.

Chemical and physical properties of the soil:

Three soil samples were randomly taken and mixed into one homogeneous sample before planting. Then, soil sample was subjected to physical and chemical analysis according to the standard method described by **Jackson (1973)** in Table (A) as well as the irrigation water was analyzed.

Table (A). Some physical and chemical characteristics of the experimental soil and the irrigation water before planting

physical characteristics of the experimental soil					Available nutrients (mg/kg)				
Coarse Sand	Fine sand	Silt	Clay	Texture class	N	P	K		
3.19	17.32	19.85	59.64	Clay	39.13	8.62	396.52		
Chemical characteristics of the experimental soil (mq/ L.)									
pH (1: 2.5)	EC (dS/m^3) (1:5)	Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.8	1.44	7.27	1.63	4.30	1.20	--	1.35	2.32	10.73
The irrigation water (mq/ L.)									
7.6	0.45	2.53	1.17	0.69	0.11	--	1.80	0.91	1.79

Microbial analysis as:

A- Total microbial count: Total microbial count in soil were determined before planting (x10^6 cfu/g dry soil), as total bacteria **(Difco, 1985)**, Total fungi **(Allen, 1959)** and Total actinomycetes **(Jensen, 1930)** counts.

B- The most probable number MPN technique: For free-living nitrogen fixers and phosphate solubilizing bacteria in hibiscus rhizosphere soil were carried out using N-deficient medium for *Azospirillum* spp. **(Dobereiner and Day, 1976),** Modified Ashby's broth medium for *Azotobacter* spp**. (Abd El-Malek and Ishac, 1968)** while, the Pikovskaya's agar medium for phosphate solubilizers bacteria **(Pikovskaya, 1948).**

C-Identifications: The nitrogen fixer's which isolated locally from the cultivated hibiscus soil were identified according to cultural morphological and physiological characteristics described as Bergy's Manual **(George *et al*., 2005)** as *Azotobacter chroococcum, Azospirillum lipoferum.*

Bacillus megaterium var. phosphaticum was kindly obtained from Department of Microbiology, Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt.

D-Soil enzymatic activity: Nitrogenase enzyme activity (µ mole C_2H_4/g soil/h) in the rhizosphere was measured as acetylene reduction assay (ARA) by GC analysis at 60, 120 and 150 days according to **Somasegaran and Hoben (1994),** Dehydrogenases enzyme activity (µg TPF/ soil) was also determined according to **Skujins (1976)** while, total phosphatase enzyme (Acid and Alkaline) was determined according to **Tabatabai (1982).**

Preparing of bacterial inocula: The inocula of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus megaterium* were prepared in mixture forms.

The strains were grown to maximum growth to 10^8 cfu /ml., on their specific medium for each bacterial strain, incubated at 30º C for 48 hours on a rotary shaker at 120 rpm. The cultures were centrifuged for 10 min at 5000 rpm at 4º C. Equal volumes of each bacterial strains were mixed to make an inoculum mixture and added to the soil with the surface irrigation after 2, 4 and 6 weeks, etc. (1ml contains 10^8 cell) after being diluted with water at 1: 20 according to **Mashhoor *et al*. (2002),** at a rate of 5 litter/ fed. **Afifi *et al*. (2014)**.

Mineral fertilizer: Full-recommended dose of NPK was added according to the recommended dose by Ministry of Agriculture and Land reclamation, Egypt, as follows: 300 Kg/fed. Ammonium nitrate (33.5% N), 150 kg/ fed. calcium superphosphate (15.5% P_2O_5) and 50 kg/fed. potassium sulphate (48% K_2O). Ammonium nitrate was added after sowing in two equal doses: the first dose was added after one month from sowing and the second was added after one month later. Calcium superphosphate and potassium sulphate were added before sowing **(Matter, 2009).**

Organic fertilizers: Compost was added to the soil as 5 ton fed^{-1} before planting. Some physical and chemical properties of compost were determination and shown in Table (B) according to **Page *et al*. (1982)**.

Table (B). Some physical and chemical characteristics of the compost used

Physical and Chemical properties		Values
Bulk density	kg m^3	593
Moisture content	%	27.9
Dry matter	%	72.1
pH (1: 10)		8.10
EC (1: 10)	(ds/m)	5.98
Organic matter	%	38.98
Organic Carbon	%	22.61
Ash	%	61.02
Ammonia	ppm	38.00
Nitrate	ppm	402.60
Total nitrogen	%	1.67
C/ N ratio		14: 1
Total phosphorus	%	0.56
Total potassium	%	0.77
Seed Weed		Not detected
Nematodes		Not detected

Compost tea

Compost was immersed in water at a dilution ratio of 1:10 (W/V) dilution ratio, then homogenized using a pump for 48 h until extract turned brown then the extract was filtered according to **Mohaseb, *et al*. (2018**). Compost tea was added as soil drench during the two seasons after a month of planting, then applied monthly at rate of 20 L. / fed. according to **Ezz El-Din and Hendawy (2010)**. The properties of the compost- tea are shown in Table C.

Potassium humat substance: Potassium humate was kindly obtained from Department of Microbiology, Soils, Water and Environ. Res. Inst., (ARC), Giza, Egypt. The potassium humate was added as soil drench during irrigation water at rate of 7.5 L fed^{-1} **(Afifi *et al*., 2014)** after one month from planting and repeated monthly later **(El-Mogy *et al*., 2021).**

Characteristic of humic substances are shown in Table C. Total phosphorus was determined by **Murbhy and Riley (1962)**. Total potassium was determined by **Chapman and pratt (1961).** Total nitrogen was determined according to **Jackson (1973).**

Table (C). Some chemical characteristics of the compost- tea and humic acid used

	pH	EC (dS/m)	Total N %	Total P %	Total K %
Compost tea	7.5	2.2	1.7	0.96	1.28
Potassium humate	8.78	60.4	1.75	1.1	9

The treatments were arranged as following

(T_1) Recommended dose of chemical fertilizers as control, (T_2) Compost + Compost- tea, (T_3) Compost + Potassium humate, (T_4) Compost + Biofertilizer, (T_5) Compost + Compost- tea + Potassium humate, (T_6) Compost + Compost- tea + Bieofertilizer, (T_7) Compost + Potassium humate + Biofertilizer and (T_8) Compost + Compost- tea + Potassium humate+ Biofertilizer

Data recorded

A- Vegetative growth parameter: The plants were collected from each plot after 180 days from planting (in the first and second season). At harvest, three plants from each plot were randomly taken to evaluate vegetative growth parameters, i.e., (plant height, number of branches/plant, number of fruits /plant, fruits fresh weight/plant, sepals fresh weight/plant and sepals dry weight /plant.

B- Chemical constituents: Total chlorophyll during vegetative growth stage were determined according to **A.O.A.C. (1990)**, total Carbohydrates were determined in the dried leaves as described by **Herbert *et al*. (1971),** total soluble solid **(A.O.A.C., 2000)** in the sepals, total phenol compounds **(Shahidi and Naczk, 1995)**, flavonoids **(Marinova *et al*., 2005),** Scavenging activity of DPPH radical (antioxidant activity %) **(Brand-william *et al*., 1995)**, ascorbic acid (g/100g) **(Kapour *et al*., 2012)**, anthocyanin content mg/100g **(DU and Francis, 1973)**,. Total titratable acidity (g/100g) **(A.O.A.C., 2000)**, Total nitrogen, phosphorus and potassium percentages as reported by **Black *et al.* (1965)** and **Wilde *et al*. (1985)** and total protein% was calculated by multiplied N value by 6.25 according to **Pirie (1955)**.

Statistical analysis

The obtained data were statically using complete randomized blocks design (simple experiment) with three replicates, separation between means was performed by the L.S.D method at 5% level. **(Snedecor and Cochran, 1980).**

RESULTS AND DISCUSSION

1-The microbiological analysis

Microbial population in the soil before planting

Soil microbial populations were enumerated before plantation where bacteria constitute the most abundant group of soil microorganisms 13.10 x 10^6 – 13.70 x 10^6 CFU per gram of soil, followed by actinomycetes 2.21 x 10^5 – 2.5 x 10^5 CFU/ g. soil and fungi 4.1 x 10^4 – 4.5 x 10^4 CFU/ g. soil in the first and second season, respectively, as showed in Figure 1. These results are in agreement with **(Bhattarai *et al*., 2015)** who said that, there is a large number of bacteria in the soil of small size, so they have a smaller biomass, actinomycetes are 10 times smaller in number but in larger in size so they are similar in biomass to bacteria in soil while, fungi have a smaller number but it dominates the soil biomass**.**

The most probable number (MPN) of biofertilizer isolates such as *Azotobacter* spp. recorded 8.9 X10^6/ml. specific cultural medium and *Azospirillum* spp. recorded 7.6 X10^6/ml. specific cultural media, while *Bacillus* spp. recorded 8.5 X10^6/ml. specific cultural media as shown in Figure (2).

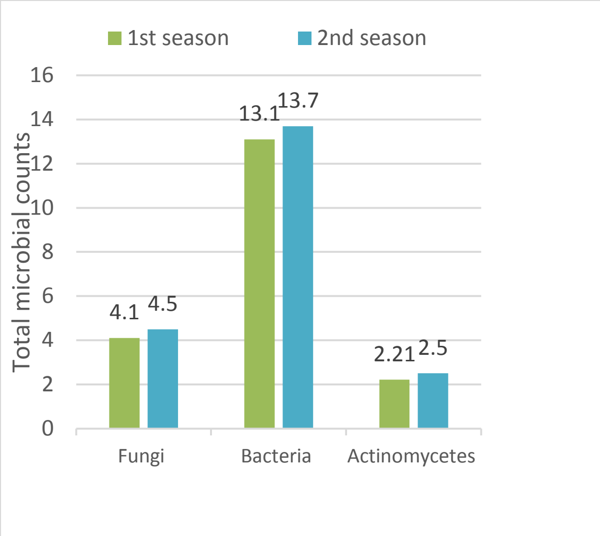


Fig 1. Soil microbial population in the hibiscus rhizosphere before planting

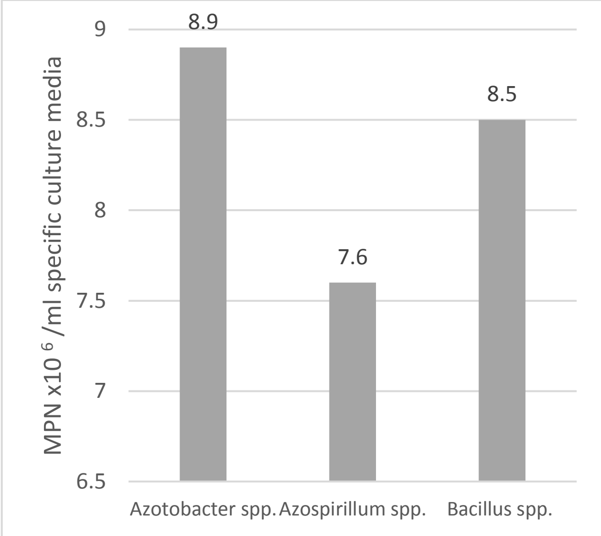


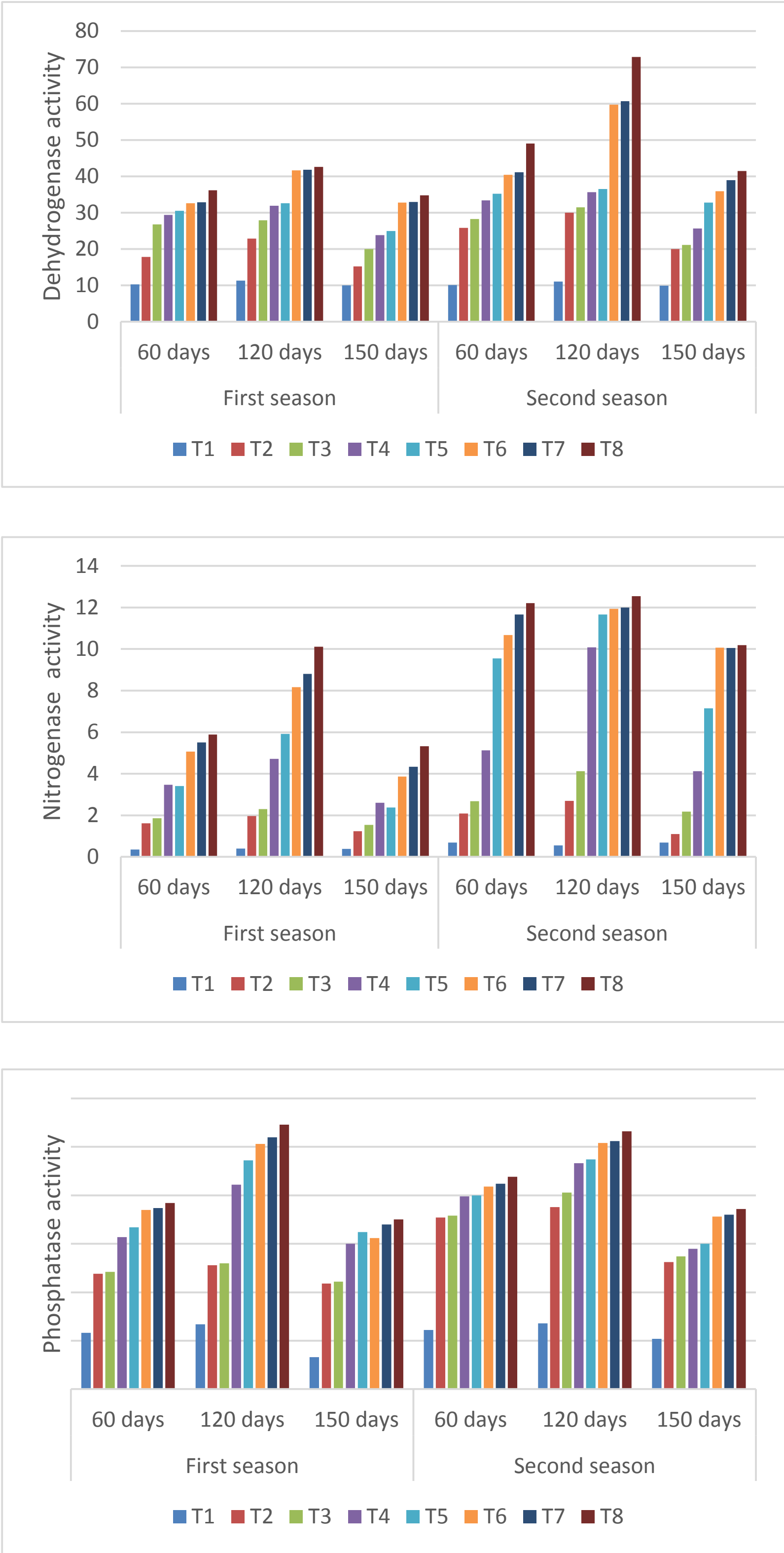
Fig 2. The most probable number (MPN) of the biofertilizer isolates in the hibiscus rhizosphere before planting

Enzymes activities

Soil enzyme activities provide an early impression of soil health due to biofertilization practices. The current results indicated an increase in the activity of dehydrogenase, phosphatase and nitrogenase enzymes in the rhizosphere of the inoculated treatments. Figure (3) showed the determination of enzymatic activity in rhizosphere area of hibiscus plants.

The activities of enzymes in hibiscus rhizosphere plants are influenced by the activity of beneficial microorganisms that colonize the roots of plant. According to Figure (3), there was an increase in observed enzymes of dehydrogenase, nitrogenase and total phosphatase (acid and alkaline) activities at 120 days more than at 60 days after planting, then it recorded a decrease after150 days of planting. Increases in the enzymes activities were achieved with the plants that received compost, biofertilizer, compost tea and potassium humate whether in a single form or in a mixture compared to the control. T_8 (compost + compost tea + K- humate + biofertilizer) recorded the highest activity at 60, 90 and 120 days after planting of dehydrogenase as 36.19, 42.59 and 34.77 in the first season and 49, 72.82 and 41.52 in the second season and nitrogenase as 5.89, 10.11 and 5.33 in the first season and 12.21, 12.54 and 10.19 in the second season while, in the total phosphatase recorded 1.92, 2.73 and 1.75 in the first season and 2.19, 2.66 and 1.68 in the second season, respectively.

The current study recorded an increase in the activity of nitrogenase, total phosphatase and dehydrogenase in the rhizosphere in plants that received organic and biofertilizer compared to plants that received mineral fertilizer, which recorded a deficit in the activities of the enzymes. this means that the excessive use of mineral fertilizers causes a detrimental effect on the biological activity in the soil. The enzymes showed a higher activity in the flowering phase (120 DAT) and then the activity decreased towards 150 DAT. These results are in harmony with **Abd El-Aal and Salem** (**2018**) on moringa plants, and **Radwan *et al*.,** (**2021**) who showed that the highest increase in enzyme activities (dehydrogenase and nitrogenase) were recorded in the treatment inoculated the mixed free living - nitrogen fixers bacteria (*Azotobacter* spp. and *Azospirillum* spp.) rather than that of individual treatments. It has also been reported that compost, compost tea and potassium humate can be incorporated into the soil as organic matter and as a source of enzymes because they produce extracellular acid and alkaline phosphatases that are active in solution or present in the plasmatic space of the cell.



(T_1) Control, (T_2) Compost + Compost- tea, (T_3) Compost + Potassium humate, (T_4) Compost + Biofertilizer, (T_5) Compost + compost- tea + Potassium humate, (T_6) Compost + Compost- tea + Biofertilizer, (T_7) Compost + Potassium humate + Biofertilizer and (T_8) Compost + Compost- tea + Potassium humate + Biofertilizer

Figure 3. The Soil enzymes activities

2- Effect of organic and biofertilizer treatments on the growth parameters of roselle plants

Data in Table (1) showed that, the plant height and numer of branches/ plant of hibiscus plants as affected by all treatments of organic and biofertilizer compaired to recomended dose of mineral fertilizer (control treatment) during the two seasons.

Table (1). Effect of different organic and biofertilizer treatments on growth parameters of roselle plants

Treatments	Plant height (cm.)		Number of branches/ plant		Number of fruits/ plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
T_1 Mineral fertilizer	223.33	285.67	12.00	13.33	48.00	61.33
T_2 Compost + Compost tea	200.67	241.67	8.33	8.67	55.00	66.00
T_3 Compost + Potassium humate	204.00	245.00	7.33	8.00	56.00	67.67
T_4 Compost + Biofertilizer	205.00	252.67	8.67	9.00	60.67	72.67
T_5 Compost + Compost tea + Potassium humate	207.33	259.33	9.00	9.00	67.33	89.33
T_6 Compost + Compost tea + Biofertilizer	208.33	269.33	9.00	9.67	73.33	102.00
T_7 Compost + Potassium humate + Biofertilizer	212.67	276.00	10.00	10.33	75.00	115.33
T_8 Compost + Compost tea + Potassium humate + Biofertilizer	216.67	281.67	11.33	13.00	79.33	119.00
$LSD_{0.05}$	**3.51**	**1.73**	**1.12**	**0.93**	**2.15**	**2.14**

Results showed that, heighest plants as a growth character were not affected by biofertilizer when compared with the mineral fertilizer. The best results of plant height and number of branches/ plant were obtained by recommended dose of mineral fertilizer during first and second seasons, T_1 (mineral fertilizer) which recorded (223.33 and 285.67 cm.) and (12 and 13.33 branches/ plant) followed by T_8 (compost + compost tea + K- humate + biofertilizer) being (216.67 and 281.67 cm) and (11.33 and 13 branches/ plant) then T_7 (compost + K- humate + biofertilizer) and then all treatments treated with organic and biofertilizers either mixture or single forms during first and second seasons, respectively. In case of the number of fruits $plant^{-1}$, it was found that T_8 (compost + compost tea + K- humate + biofertilizer) gave the highest values (79.33 and 119) in the two growing seasons, respectively, followed by T_7 then all treatments treated with organic and biofertilizer compaired to mineral fertilizer (control treatment) which gave the lowest results being 48 and 61.33 fruits/ plant in the two growing seasons, respectively. These results were agreement with **(Sharma and Chetani, 2017)** said that, mineral fertilizer have many advantages as they do not require direct decomposition, their nutrients are relatively high and they are released quick, so that, mineral fertilizer increase the rate of vegetative growth more rapidly. Also, **Bunu *et al.*** (**2020)** on okra plant, **Abou-El-Hassan and El-Batran (2020)** on sweet corn plant and **(El-shaieny *et al*., 2022)** on onion said that, compost and compost- tea contains many useful components such as soluble bioactive components, macro- and micronutrient. Therefore, they can increase soil fertility and improve plant growth by providing nutrients to plants, improving root growth and overall health of plants and increasing microbial population densities in the soil**.** In addition, (**El-Serafy, 2018)** said that, humic acid acts an indirect role for stimulating plant growth through its interactions with plant membrane transporters responsible for nutrients uptake which regulate plant growth and development.

3- Effect of organic and biofertilizer treatments on the yield production of roselle plants

The results presented in Table (2) showed the positive effect of compost, biofertilizer, compost- tea and k- humate on fruit fresh weight, sepals fresh weight and sepals dry weight of hibiscus plant (*Hibiscus sabdariffa* L.).

Table (2). Effect of different organic and biofertilizer treatments on fruit fresh weight, sepals fresh weight, and sepals dry weight of roselle plant

Treatments	Fruit fresh weight (g./plant)		Sepals fresh weigh (g./plant)		Sepals dry weigh (g./plant)		Sepals dry weigh (kg./feddan)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T_1 Mineral fertilizer	411.83	509.76	202.33	249.31	41.40	45.62	517.50	530.25
T_2 Compost + Compost tea	445.67	549.52	205.33	280.12	42.79	46.98	532.74	584.90
T_3 Compost + Potassium humate	460.07	622.38	208.67	348.83	47.25	49.50	585.30	613.80
T_4 Compost + Biofertilizer	484.33	640.00	222.33	351.09	49.86	50.64	604.30	613.76
T_5 Compost + Compost tea + Potassium humate	538.33	733.80	244.00	409.90	50.65	52.26	622.93	642.80
T_6 Compost + Compost tea + Biofertilizer	580.73	852.38	248.00	440.28	52.91	58.34	661.38	719.25
T_7 Compost + Potassium humate + Biofertilizer	598.67	877.14	264.00	443.24	54.82	58.50	685.15	725.20
T_8 Compost + Compost tea + Potassium humate + Biofertilizer	615.87	888.33	347.33	453.09	60.26	64.68	735.18	776.16
LSD $_{0.05}$	**1.32**	**2.82**	**2.86**	**1.93**	**1.05**	**0.62**	**0.08**	**0.08**

The organic and biofertilizer treatments in single or in combination forms gave the highest significant values of all results compared to mineral fertilizer in the first and second growing seasons. The highest results were obtained from treatment T_8 (compost + compost tea + K- humate + biofertilizer) being (615.87 and 888.33 g/plant), (347.33 and 453.09 g/plant), (60.26 and 64.68 g/plant) and (735.18 and 776.16 kg/fed.) in fruit fresh weigh, sepals fresh weights and sepals dry weights g./ plant and kg/fed. in both first and second seasons, respectively. Followed by other treatments received organic and biofertilizer in combination forms and in single form compared to mineral fertilizer which recorded the lowest values. Several studies were conducted to determine the role of PGPR (Strains like *Azotobacter*, *Azospirillum* and *Bacillus* spp. have the capability to act as Biofertilizers) in increasing nutrient availability and promoting plant growth, the use of microbial inoculants has been stimulating the root and shoot growth, enhanced nutrient uptake and increase the yield of different crops **(Etesami *et al*., 2021; Jiao *et al*., 2021; Patel *et al*., 2021 and Santoyo *et al*., 2021b**). Also, **Khater (2021)** explained that, the positive effect of the compost that leads to the dissolution of nutrients, increase the availability of macro and micro-nutrients as well as stimulation of metabolic and photosynthesis processes, in addition to the compost contains 2- 5% of humic and falvic acids which is positively reflected on the increase the production and quality of plants.

In various studies conducted to inoculation with *Azotobacter chroococcum* increased the contents of total nitrogen (N) and total phosphorus (P) in maize plants compared to uninoculated treatment (**Song *et al*., 2021).** Also **(Aly *et al*., 2015; Singh and Gupta, 2018; Tiwari *et al*., 2018; Vimal *et al*., 2018; Al- sayed *et al*., 2020 and Basu *et al*., 2021)** said that, the most important growth-stimulating bacteria are *Azospirillum* spp., *Azobacter* spp and phosphate dissolving bacteria. they stimulated biological fixation of nitrogen, their positive effect on mineralization and solubility of organic and inorganic phosphorus, increase the concentration of beneficial soil organisms and the plant nutrients availability and reduction in soil pH which increased the solubility of some nutrients such as P, Fe, Zn, Mn and Cu which in turn increased the nutrients uptake by plants which considerably effect on plant growth regulators especially auxin, gibberellins and cytokinin and improved the plant performance overall plant growth and crop yield.

4- Effect of organic and biofertilizer treatments on the plant chemical analysis of roselle plants

4- 1- Total chlorophyll, Total carbohydrates, total soluble solid and total acidity

The results listed in Table (3) showed that the chemical analysis as a quality parameters of total plant chlorophyll (mg/100 g f.w.), total carbohydrates (%), total soluble solid (%) and total acidity (%) in sepals were significantly affected by all organic and biofertilizer treatments during both seasons compared to mineral fertilizer treatment.

Table (3). Effect of different organic and biofertilizer treatments on total chlorophyll, total carbohydrates %, total soluble solid and total acidity of roselle plant

Treatments	Total chlorophyll (mg/100 g f.w.)		Total carbohydrates (% of d.w.)		Total soluble solid (%)		Total acidity (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T_1 Mineral fertilizer	47.44	47.41	25.09	25.35	6.50	6.60	1.23	1.22
T_2 Compost + Compost tea	46.87	47.14	26.01	26.38	6.70	6.88	0.95	1.01
T_3 Compost + Potassium humate	48.24	48.79	26.75	26.93	6.93	7.03	1.05	1.08
T_4 Compost + Biofertilizer	49.58	49.24	26.48	26.82	7.00	7.13	1.11	1.26
T_5 Compost + Compost tea + Potassium humate	53.87	52.44	27.08	27.76	7.27	7.28	1.20	1.37
T_6 Compost + Compost tea + Biofertilizer	56.96	56.27	27.12	28.53	7.30	7.35	1.28	1.38
T_7 Compost + Potassium humate + Biofertilizer	57.73	56.99	28.11	29.03	7.37	7.62	1.34	1.41
T_8 Compost + Compost tea + Potassium humate + Biofertilizer	57.74	58.07	28.51	29.32	7.63	7.72	1.38	1.51
LSD 0.05	**0.89**	**0.56**	**0.51**	**0.33**	**0.16**	**0.09**	**0.02**	**0.04**

The highest mean values were obtained from treatment T_8 (compost + compost- tea + K- humate + biofertilizer) which recorded (57.74 and 58.07 mg/100 g f.w.), (28.51 and 29.32 % of d. w.), (7.63 and 7.72 %) and (1.38 and 1.51) in both seasons, respectively. Followed by T_7 (compost + K-humate + biofertilizer) then T_6 (compost + compost- tea + biofertilizer) then T_5 (compost + composr- tea + K-humate) and then other treatments with the single forms compare to mineral fertilizer which recorded the lowest values being (47.44-47.41), (25.09- 25.35), (6.5- 6.6) and (1.23-1.22) in both seasons, respectively.

These results have been supported by other published research **(Kahil *et al*., 2017 and Al-sayed *et al*., 2020).** In addition, **Umesha *et al*. (2018)** approved that, nitrogen- fixing bacteria have the ability not only to fix nitrogen but also to release certain phytohormons of GA3 and IAA nature that could stimulate plant growth, absorption of nutrients and photosynthesis process. These results were consistent with **Mohamed *et al*. (2021)** found that, the organic and biofertilizer applications recorded significantly higher values than the chemical fertilizer in total carbohydrate, ascorbic acid, TSS content, phenols and flavonoides contents in broccoli. Moreover, **Retab *et al*.** (**2022)** in hibiscus explained that, the application of potassium humate resulted the best significant values of carbohydrates content in sepals in the first and second seasons, respectively. This result may due to increased contents of chlorophylls and carotenoids, which enhanced the efficiency of photosynthesis that is a good explain to the increasing of dry matter production.

4-2- Flavonoids, total phenol content, scavenging activity, ascorbic acid (vitamin C) and anthocyanin

The results in Table (4) showed the positive effect of all organic and biofertilizer treatments on total flavonoids, total phenol content, scavenging activity, ascorbic acid (vitamin C) and anthocyanin of roselle plant (*Hibiscus sabdariffa* L.).

Table (4). Effect of different organic and biofertilizer treatments on flavonoids, total phenol contents, scavenging activity, ascorbic acid and anthocyanin of roselle plant

Treatments	Flavonoids %		Total phenol contents %		scavenging activity %		Ascorbic acid (g./100g d.w.)		Anthocyanin (mg/100g d.w.)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T_1 Mineral fertilizer	1.09	2.17	0.73	1.38	53.11	89.61	1.12	1.35	206.67	358.00
T_2 Compost + Compost tea	1.30	2.29	0.83	1.53	57.15	83.66	1.06	1.38	465.33	381.33
T_3 Compost + Potassium humate	1.38	2.40	0.83	1.59	60.04	82.91	1.23	1.39	484.00	447.67
T_4 Compost + Biofertilizer	1.47	2.37	0.84	1.57	59.30	82.88	1.32	1.39	488.33	420.67
T_5 Compost + Compost tea + Potassium humate	1.58	2.44	0.93	1.66	52.73	87.84	1.33	1.41	489.00	578.33
T_6 Compost + Compost tea + Biofertilizer	1.64	2.47	1.06	1.86	52.60	89.74	1.35	1.44	506.00	609.00
T_7 Compost + Potassium humate + Biofertilizer	1.72	2.51	1.10	1.88	39.78	89.40	1.38	1.54	525.67	711.00
T_8 Compost + Compost tea + Potassium humate + Biofertilizer	1.83	2.62	1.20	1.93	59.21	89.84	1.43	1.57	552.33	727.67
LSD $_{0.05}$	**0.11**	**0.37**	**0.26**	**0.34**	**5.16**	**6.03**	**0.103**	**0.05**	**1.69**	**1.83**

The results showed that, all treatments which received the organic and biofertilizer recorded higher results than the control. The treatment T_8 (compost + compost - tea + K-humate + biofertilizer) recorded the highest significant values, which are (1.83 and 2.62), (1.2 and 1.93), (59.21 and 89.84 %), (1.43 and 1.57 g/100g f. w.) and (552.33 and 727.67mg/100 g) in both seasons, respectively compared to the control one. Then followed by other treatments which received the organic and biofertilizer in mixed forms and then treatments in the single forms. all parameters gave the lowest significant values by the control treatment (mineral fertilizer).

Similar results were obtained by **Al- sayed *et al*. (2019)** in hibiscus, found that the anthocyanin and flavonoide content were significant increase with bio- organic fertilization treatments**.** In addition, **Norhayati *et al*. (2019)** referred that, organic fertilizer increased the availability and uptake of nutrients, which resulted in increased photosynthetic activity and thus increased the amounts of anthocyanin.

4-3- Total macronutrients (total nitrogen, phosphorus and potassium %) and protein content %

The results in Table (5) showed the positive effect of all organic and biofertilizer treatments on total nitrogen, phosphorus, potassium % and protein content %.

The data presented in Table (5) declared that, the highest values of N, P and K as well as protein % of roselle plants were recorded by T_8 (compost + compost - tea + K- humate + biofertilizer) in both seasons. Which recorded (3.28 and 3.31), (0.70 and 0.86), (3.94 and 3.97) and (20.52 and 20.71) in both seasons respectively, followed by T_7 (compost + K-humate + biofertilizer) then other combined treatments and single treatments. Otherwise, these parameters gave the lowest significant values by control treatment. Similar results were reported by **Umesha *et al*. (2018)** who indicated a significant increase in the total contents of macro elements in plants inoculated with *Azotobacter* pp. and *Azospirillum* spp. compared to un-inoculated plants, this may be due to these microorganisms also growth promoting substances that lead to more efficient absorption of nutrients**.** In addition, (**Al-Sayed *et al*., 2020)** said that, non-symbiotic N_2-fixing bacteria produced sufficient amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching which increase the uptake of nutrients from the soil to the plant. K-humate play an important role in the uptake of nutrients. Also, Results obtained reveal that plants grown under the application of K-humate, showed significant increases in the contents of N, P and K. As it acts as a nutrients carrier and improves nutrient uptake, these effects were reflected positively to plant growth and thus increasing the yield (**Mahdi *et al*., 2021).** Using compost- tea as soil drench has a positive effect on plants by providing nutrients in the area around the roots. So it increases the delivery of water and nutrients to the root zone and increase uptake of nutrients and formation of protein and carbohydrates that is reflected in increased growth by increasing all the different physiological processes in the plant (**Khater, 2021).**

Table (5). Effect of different organic and biofertilizer treatments on total nitrogen, total phosphorus, total potassium and total protein % of roselle plant

Treatments	Total nitrogen %		Protein %		Total Phosphorus %		Total Potassium %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T_1 Mineral fertilizer	2.34	2.37	14.55	14.81	0.27	0.28	2.91	3.24
T_2 Compost + Compost tea	2.04	2.08	12.75	13.01	0.33	0.34	2.24	2.66
T_3 Compost + Potassium humate	2.13	2.10	13.33	13.13	0.31	0.31	2.92	3.15
T_4 Compost + Biofertilizer	1.99	2.07	12.46	12.94	0.37	0.38	2.77	3.08
T_5 Compost + Compost tea + Potassium humate	2.34	2.70	14.63	16.88	0.38	0.38	3.19	3.24
T_6 Compost + Compost tea + Biofertilizer	2.67	2.54	16.71	15.86	0.39	0.39	3.80	3.85
T_7 Compost + Potassium humate + Biofertilizer	2.78	2.77	17.38	17.31	0.40	0.42	3.89	3.89
T_8 Compost + Compost tea + Potassium humate + Biofertilizer	3.28	3.31	20.52	20.71	0.70	0.86	3.94	3.97
LSD 0.05	**0.37**	**0.35**	**2.30**	**0.19**	**0.01**	**0.05**	**0.08**	**0.02**

Conclusion

In conclusion, the use of organic and biofertilizers as substitute for mineral fertilizers in order to grow the medicinal and aromatic plants, should be seen as a means of improving environmental conditions and human health. From the results of this study, it can be concluded that organic fertilization treatments with biofertilizers gave the best results in all studied characteristics, and it was the best in all fruit yield and quality characteristics. Therefore, it is possible to use organic fertilizer with biofertilizer as an alternative to mineral fertilizer, and this will certainly preserve the environment and human health without negatively affecting the growth, yield quality and productivity of hibiscus plants.

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RESEARCH ARTICLE

Improving productivity and quality of roselle plant (*Hibiscus sabdariffa* L.) by adopt environmentally friendly organic fertilization practices

Authors' contributions

Author details: Abeer M.O. Shoeip and Samia M. Abd-El Hameed, Agricultural Research Center, Giza, **Egypt**

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