Response of cauliflower (*Brassica oleracae* var *botrytis* L.) to soil application with olive mill wastewater, vinasse and potassium humate and its reflection on growth, curds yield, dry seed yield and Enzyme activity

ABSTRACT

A field experiment was achieved in two successive winter seasons of 2018/2019 and 2019/2020 at Qaha Research Farm, Qaliobia Governorate, Horticulture Research Institute, Agriculture Research Center (ARC), Egypt. This experiment was suggested to study the beneficial effects of Olive Mill Wastewater (OMW), vinasse and potassium humate at 0, 50 and 100% of each as soil application on plant growth, curds yield parameters, dry seed yield and its components, quality and seeds chemical composition of cauliflower Amshiry cv. So that, the physical and chemical characteristics of the soil in this study included that; enzymatic activity in soil rhizosphere of the oxidoreductase enzymes (catalase and peroxidase). The experiment was arranged in a randomized complete block design (RCBD) with seven treatments and three replicates. The obtained results indicated that adding venasse followed with K-humates at 50% led to improve the vigorous growth plants, increasing the total yield of curds besides enhancing dry seed yield and its components as well as improve the physical and chemical properties of the investigated soil. Addition of 50% olive and vinasse recorded the best mean values of all tested compared to the other treatments or the control and at the same time save and reduce mineral fertilizer recommendation of cauliflower by 50 %. The superior treatment *i.e.* vinasse 50% led to higher values of total yield of curds of cauliflower (ton/ fed.) by 34.7 and 31.6 % and obtained higher values of dry seed yield (kg/fed) by 28.7 and 30.6 % over the control (the untreated plants) in the both experimental seasons respectively.

Key words: Cauliflower, olive mill wastewater, vinasse, potassium humate, soil application, growth, curds yield and dry seed yield as well as oxidoreductase enzyme

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the significant vegetable crops in the Brassicaceae family. It is a vegetable with a wealth of nutrients that can be consumed fresh, boiled, or pickled **(Noor *et al.*, 2014).** It includes vitamins like vitamin A, thiamine, riboflavin, niacin, and vitamin C as well as minerals like calcium and iron that have nutritional significance **(Ahmed and Ali 2013).** Additionally, according to **Eimon *et al.*, (2019)**, cauliflower has a higher concentration of dietary fiber, folic acid, water, and ascorbic acid, which has a high nutrient density. The apical meristem, often known as curds, is the pre-floral white fleshy edible portion of cauliflower.

The majority of Olive Mill Wastewater produced worldwide comes from the Mediterranean nations, with Egypt ranking among the top five producers. It depends on a variety of elements, including the types and maturities of the olives, the climate in the region of origin, the farming practices, and the equipment employed in the extraction process. The majority of nations that produce olive oil have been deeply concerned about the environmental impact of olive mill waste (OMW) and olive waste pomace. According to **FAOSTAT (2007)**, the global and particularly in the Mediterranean Basin olive oil industries have been expanding steadily over the past 15 years, with an average annual rise of roughly 5% of global production. The three most popular methods of disposing of OMW are as follows: storing it in evaporation ponds during the extraction of olives, which has negative environmental effects and pollutes shallow ground water; dumping it in the sewer system and moving it to dumping sites, which is more expensive and may result in future point sources of pollution in the nearby areas. OMW's are complex composition poses serious technical and financial challenges for appropriate wastewater treatment and disposal. Controlled OMW land spreading is, thus, an alternative and logical solution **(Youness *et al.*, 2022).** As an organic fertilizer, evaporation ponds, thermal concentration, physico-chemical and biological treatment methods as well as direct application to agricultural soil have been proposed as different disposal methods **(Mostafa *et al.*, 2022).**

Vinasse is a byproduct of the sugar industry, **Hidalgo (2009)**. Crystalline sugar, pulp, and molasses are produced through the processing of sugarcane. These latter are then fermented to produce ethanol, ascorbic acid or other compounds. Vinasse is the substance left over after the desired product has been removed (such as alcohol, ascorbic acid, etc.). In terms of volume, the conversion of one liter of cane alcohol yields approximately 13 L of vinasse. **Vadivel *et al.*, (2014)** pointed out that appropriate time and rate of vinasse application in agriculture has obtained significant amounts of nutrients, improved the soil quality of degraded land and increase crop yields. Also, **Abd-El-Kaway (2006)** demonstrated that, the addition of vinasse and potassium Sulphate caused a slight decrease in pH and a slight increase in salinity EC of the soil. Vinasse has been mostly used on practices of fertigation, reducing the water input for plant growth, **Walter *et al.*, (2011).** The use of vinasse in fertigation systems has advantages because it can contribute substantial amounts of water and mineral nutrients, support soil quality and crop productivity and finally, but no less importantly can solve the environmental problem of the disposal of this agro-industrial residue (**Abou-Husssien *et al.*, 2020**). Moreover, **Soha *et al.*, (2020)** illustrated that the addition of [50% of the recommended rate of K-fertilizer; 24 kg K_2O as potassium sulphate

+ 50% of the biologically treated vinasse (408.85 liters)/fed can be recommended to get an economical root and sugar yields, which led to saved 50% of required inorganic potassium needed for plant growth. Many research work is suggested to investigate the effect of the long term use of vinasse on soil properties such as soil permeability; salinity and pH.

Potassium humate is the salt of humic acid and water soluble. The physical, chemical, and biological characteristics of the soil are improved as a result of humic acid, which also has an indirect effect on plant growth. According to **Tejada *et al.*, (2006),** it has direct effects on plant growth through altering metabolic activity, therefore, when plants were treated with potassium humate, chlorophyll contents were enhanced. Also, entering in plant cell, the functional group of humic and fulvic acids can serve as supplementary source of respiratory catalysts, **(Ryosuke *et al*., 2006)**. Humic substances may be absorbed by the roots and transported to shoots, thus enhancing the growth of the whole plant **(Shahein *et al*., 2015)**.

Organic molecules known as humic acid (HA) have crucial functions in enhancing soil characteristics, plant development and agronomic aspects. In addition, humic acid (HA) is primarily responsible for influencing the physical, chemical, and biological properties of soil, such as its texture, structure, water-holding capacity, cation exchange capacity, pH, soil carbon content, enzyme activity, nitrogen cycle, and availability of nutrients **(Kwame *et al.*, 2022).** The review emphasizes the significance of HA on crop development, plant hormone production, nutrient intake and assimilation, yield, and protein synthesis. Humic substances may be classified into three categories; humic acid, fulvic acid and humin **(Solange and Rezende, 2008)**. Moreover, **Wafaa *et al.*, (2017)** studied the effects of various nitrogen fertilization rates, there is a substantial positive correlation between total yield (straw and grains) and total nutritional content, the results showed that the mixture of bentonite and potassium humate were beneficial for the chemical soil properties of sandy soil, which has an impact on increasing soil fertility. **Hassan *et al.*, (2021)** mentioned that soil applications of potassium humate and vinasse could effectively be used in sandy soil to enhance soil nutrient status as well as enhance crops growth and productivity. Furthermore, **Ayman *et al.*, (2022)** pointed out that the impacts of humic acid (HA) as a soil amendment improved the soil's hydraulic qualities, HA addition changed the soil's structure by promoting the rapid production of macro aggregates, lowering bulk density and pH while raising porosity and electrical conductivity.

Soil enzymes are an important parameter in carbon sequestration and soil nutrient dynamics (**Lemanowicz *et al.*, 2023).** Soil enzymes activities are indicators for soil quality measuring soil microbial activity related to nutrient transformations, **Ahamadou and Huang (2012)**. Soil dehydrogenases (EC 1.1.1.) represent oxidoreductase enzymes that do not accumulate extracellularly in soil but occur inside microbial cells linked to their oxido-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, **Wolinska** and **Stepniewsk (2012).** Peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) activities were used as indicators to quantify the stress conditions facing lettuce, specifically in their leaves, **Aires *et al.*, (2021); Leitão *et al.*, (2021)**.

Therefore, the purpose of this work was to investigate the impact of soil application with olive mill wastewater, vinasse and potassium humate on cauliflower growth, curds yield and dry seed yield as well as enzyme activity.

MATERIALS AND METHODS

The present investigation was performed out at Qaha Research Farm, Qaliobia, Horticulture Research Institute, Agriculture Research Center during the two consecutive winter seasons of 2018/2019 and 2019/2020 in order to study the effects of Olive Mill Wastewater (OMW), vinasse and potassium humate as soil application on vegetative growth, curds yield parameters, dry seed yield components, chemical components and seed quality of cauliflower Amshiry cv. as well as enzyme activity. The soil type of this experimental field was clay loam. Surface soil samples were taken randomly of each year before planting at the depth of 0 – 30 cm to represent the different physical and chemical properties. Samples were air dried, crushed, sieved to pass through 2.0mm sieve and analyzed for their chemical and physical properties according to the standard methods outlined by **Page *et al.*, *(*1982)** and **Klute (1986).** These chemical and physical properties are recorded in Table1.

Table 1: Mean values of the physical and chemical properties of Qaha fine textured soil before planting during the two winter seasons of 2018/2019 and 2019/2020.

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Textural class	O.M mg kg-1	$CaCO_3$ mg kg-1
14.2	8.31	26.5	51	Clay	1.51	38.3

pH (1:2:5)	EC (dS/m)	Cations (mmolcL-1)				Anions (mmolcL-1)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{--}_4
7.3	2.54	**10.61**	4.98	9.38	0.24	2,36	9.32	13.52

Macronutrients (mg/kg)			Micronutrients (mg/kg)			
N	P	K	Fe	Mn	Zn	Cu
53.3	4.2	60.3	3	2.17	2.18	6.8

The experiment was contained seven treatments as follows:

T1-The control (NPK 100%).

T2- Soil application with olive mill wastewater 100%.

T3- Soil application with olive mill wastewater 50%.

T**4-** Soil application with vinasse 100%.

T5- Soil application with vinasse 50%.

T6- Soil application with potassium humate 100%.

T7- Soil application with potassium humate 50%.

Organic fertilizer:

Potassium humate was obtained from the Agricultural Research Center (ARC) at Giza governorate, Egypt. Chemical composition of this compound is illustrated in Table 2.

Table 2: The chemical properties of K-humate

Parameter	Value	Parameter (ppm)	Value
pH	8.10	P	9.6
OC %	0.63	Ca	400
OM %	1.08	Mg	336
C/N	1.21	Fe	10.9
N %	0.52	Mn	1.7
K %	4.00	Zn	0.3
Na %	0.83	Cu	0.5

Waste originated conditioner:

Olive Mill Wastewater was obtained from Olive Oil Production unit (OOP) at the Horticultural Research Institute, Agricultural Research Center (ARC), Giza governorate, Egypt.

Table 3. Physic-chemical characterization of Olive Mill Wastewater

pH	E.C d.s/m	Cations (meq/l)			Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	HCO_3^-	Cl^-	SO_4^{--}
4.76	15.8	38.7	35.5	68.9	4.2	115.5	11.3

Macro nutrients			Micro Elements (ppm)			Organic materials (g/L)			
N%	P%	K%	Zn	Fe	Cu	COD	Carbohydrates	TSS	Phenolic compound
1.63	0.13	2.45	0.660	0.577	0.052	121.8	14.57	34.36	6.95

Vinasse was obtained from Hawamdia Sugar and Distillation Company. Egypt.

Table 4. Physic-chemical characterization of concentrated vinasse:

pH	E.C ds/m	Color conc.	Density	Total phenol (ppm)	COD g/L	BOD g/L	OM %	TSS g/L	HMF g/L	N %	P %	K %
4.31	21.70	74061	1.028	0.41	48.5	27	25.9	4.6	12	3.05	0.44	6.4

The experimental design was complete randomized block design with three replicates. Seeds of cauliflower Amshiry cv. were firstly sown in 209-cell styrophom trays in the nursery to produce the transplants (45 days old) then, the seedlings were transplanted on the 1^{st} week of November in the two experimental seasons. Seedlings were transplanted on one side of each ridge in 70 cm width and 50 cm apart. Each plot consists of five ridges, each one is 3.0 m long, the plot area was 10.5 m^2. NPK fertilizers were added to the soil in the forms of ammonium sulphate (20.5%N), super phosphate (15.5% P_2O_5) and potassium sulphate. (48% K_2O), respectively. The recommendation of 100% NPK was (200kg of ammonium sulphate +200kg calcium super phosphate +100kg potassium sulphate), 50% of NPK was (100 kg of ammonium sulphate +100kg calcium super phosphate +50kg potassium sulphate). Whereas, the three types of organic fertilizers were applied in a liquid form individually in irrigation water (fertigation) either as 100% or 50% of recommended dose ($5m^3fed^{-1}$ each of olive mill wastewater, vinasse and k-humate), while 50% of NPK recommended dose was combined with that 50% of organic fertilizers (**Hossain and Ryu, 2017;Rokia, *et al*., 2023).** However, the control were 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 5.

Table 5: 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

Data Measurements:

1- Vegetative growth characteristics:

A random sample of three plants from each experimental plot was collected to measure each of the plant height (cm), number of leaves/ plant, leaf area (cm^2), leaves fresh weight (g), leaves dry matter (%). The foliage of plants was dried at 70 C^o until they reached to a constant weight then, the dry weight per plant was calculated.

2- Curds yield and its physical attributes:

At 115 day after planting, a sample of three curds plot was taken to determine fresh weight (g), curd diameter (cm), curd dry matter (%), number of days to maturity and total curd yield (ton/fed).

3- Dry seed yield, its components and seed germination ratio:

Random samples of ten dry pods of cauliflower at the end of the harvesting date (after the physiological maturity) from each plot were taken to determine the following data *i.e*. No. of dry seeds/dry pod, 1000 seeds weight (g), seed yield (g/plant) and total dry seeds yield (kg/fed.). Dry seeds of cauliflower were treated with Tobsen fungicide then put it in filter paper inside germination incubator at 25 C^o and the germination ratio(%) was calculated.

4- Chemical constituents in curds:

N, P and K were measured according to **AOAC (1990)**. Carbohydrates content (%) was determined according to **Mazumdar and Majumder (2003)**.

5- Statistical analysis procedure:

All obtained data of the present study were subjected to the analysis of variance techniques according to the design used by the MSTATC computer software program variance and the mean of treatments were compared according to the Least Significant Differences (L S D) test at the 0.05 probability level, the method described by **(Bricker, 1991)**.

6- Chemical and physical Characteristics of soil

Samples of the soils under this study were air dried, crushed, sieved to pass through a 2.0 mm sieve and analyzed for their chemical and physical properties according to the standard methods outlined by **Page *et al*, (1982)** and **Klute (1986)** as follows:

*** Electrical conductivity (EC)** was determined in the soil paste extract by electrical conductivity meter soil electric conductivity (EC, dSm^{-1}) was determined according to **Rhoades (2018).**

*** Soil pH** was measured in 1:2.5 soil: water suspension using pH meter. The soil pH values were measured according to **Thomas (2018)**.

*** Soluble carbonates and bicarbonates** were determined in a soil paste extract by titration against 0.01M sulphuric acid in presence of phenolphthalein and methyl orange indicators, respectively.

*** Calcium and magnesium** were determined in a soil paste extract using the titration methods by versinate (0.01M) in presence of ammonium purpurate (murexide) and Eriochrome black T (EBT) indicators, respectively.

*** Chloride concentration** was determined in a soil paste extract using the silver nitrate (0.01M) in presence of potassium chromate as an indicator.

*** Sulphate** was calculated by subtracting total summation of total determined soluble anions from summation of total soluble cations.

*** Sodium and potassium** were determined in a soil paste extract by using flame photometer according to **(Page *et al.*, 1982).**

*** The Organic matter** was determinate by **Walkely and Black (1934).**

Cations and anions were determined to have a complete knowledge about the experimental conditions (**Jackson, 1973**).

The data in three treated olive mill wastewater, vinasse and potassium humates samples were recorded for the experimental soil after 30 days from the last application of treatments then.

*** Total phenols** were estimated using spectrophotometrically according to **Swain and Hillis (1959).**

*** Hydroxymethylfurfural** (HMF) was determined as mentioned by **Zappala *et al*., (2005)**.

*** Potassium (K) and phosphorus (P) contents** were estimated using Atomic Absorption Spectrophotometer according to (**Cottenie *et al.*,1982).**

*** Total nitrogen (T.N)** was determined by the Kjeldahl method (**Cottenie *et al*., 1982**). Organic carbon was calculated according to **(Page *et al.*, 1982).**

7- Enzyme activities:

Soil and plant biological activities determination for enzymes activities in soil rhizosphere and fresh plant. Some biological activities were determined in collected soil samples from cauliflower rhizospheres at periods of 105 days from planting.

Fresh sample material of 0.5g was homogenized in 10 ml cold phosphate buffer (50 mM, pH 7). The homogenates were centrifuged at 4000 rpm at 20°C for 20 min. The supernatant was used as a raw extract for the enzymatic assay. Reagents used in the following assays were prepared in the same previously indicated buffer.

*** Peroxidase activity** (EC 1.11.1.7) was determined using methylene blue assay according to **Magalhaes *et al.*,(1996)**. One unit of peroxidase was defined as the amount of enzyme required to oxidize 1.0 µmol of methylene blue per min.

*** Catalase activity** (EC 1.11.1.6) was assayed based on the breakdown of H_2O_2 detected in UV at 240nm, as mentioned by **Beers** and **Sizer (1952)**. The method steps were done according to **Pine *et al.*, (1984)**.

*** Units of catalase** were calculated by using a molar absorbance index for H_20_2 of 43.6. One unit of catalase was defined as the amount of enzyme required to degrade 1.0 µmol of H_2O_2 per min.

*** Dehydrogenase activity** (DeH-ase) (EC 1.1.1.) was assayed using tri-phenyl tetra-zolium chloride (TTC) method according to **Casida *et al.*, (1964).** One unit of dehydrogenase was defined as the amount of enzyme required to hydrolyze of TTC to form 1.0 µmol of tri-phenyl formazan (TPF) per hour (µmol $g^{-1}h^{-1}$).

*** Phosphatase activity** (P-ase) **(E.C 3.1.3)** was measured using para nitro phenyl phosphate according to **Tabatabai and 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** (E.C.1.18.6.1) was measured by acetylene reduction assay as described by (**Johnsen and Apsley, 1990**).

RESULTS AND DISCUSSION

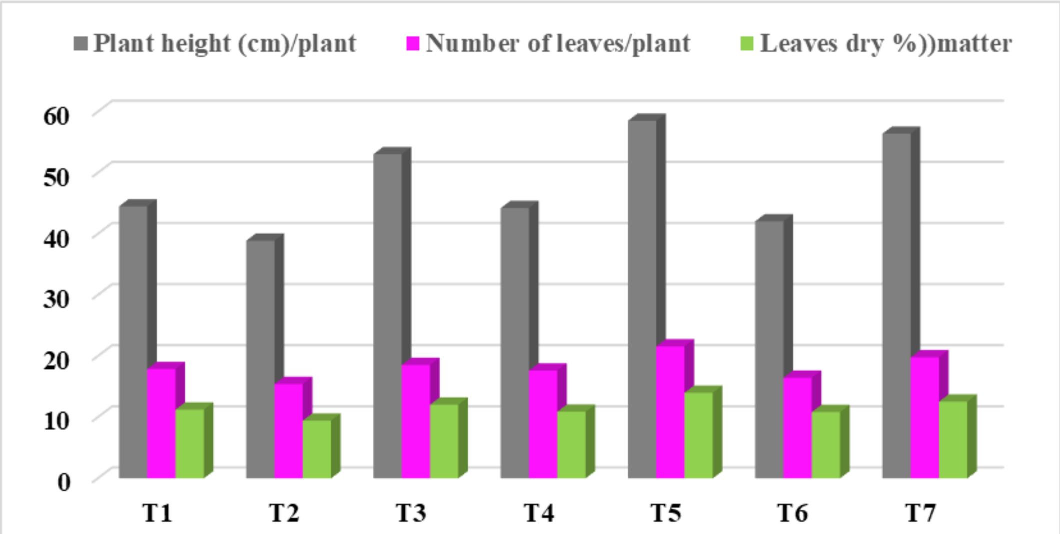
1- Vegetative growth characteristics:

Data in Table (6 and Fig. 1) revealed that the treatments used had an impact on the vegetative growth parameters of the cauliflower plants. This was in relation to the effect of adding olive mill wastewater, vinasse, and humate as soil application and its reflection on cauliflower vegetative growth characteristics as compared to the control, all treatments considerably improved all growth parameters, including plant height (cm), number of leaves per plant, leaf area (cm2), fresh weight per plant, and leaf dry matter (%). The most successful therapy, however, for boosting all prior growth indices in the two growing seasons was vinasse 50% followed by humate 50%.

Table (6): Vegetative growth characteristics of cauliflower plants as affected by soil application with olive mill wastewater, vinasse and potassium humate during the two winter seasons of 2018/2019 and 2019/2020.

Treatments	Plant height (cm)/plant		Number of leaves/plant		Leaf area (cm^2)		Fresh weight /plant		Leaves dry %)(matter	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control(NPK100%)	44.47	44.54	17.66	18.12	9412.3	10254.2	1384.1	1436.7	10.8	11.7
Olive mill waste 100%	38.34	39.44	15.12	15.76	7452.3	8541.3	1235.1	1257.4	9.2	9.7
Olive mill waste 50%	52.73	53.36	18.42	18.68	11125.1	12574.3	1532.4	1577.3	11.8	12.3
Vinasse 100%	43.80	44.64	17.53	17.77	9654.1	10234.2	1442.4	1524.3	10.7	11.1
Vinasse 50%	57.71	59.32	21.48	21.72	16574.5	17231.1	1678.4	1784.3	13.8	14.2

Humate 100%	41.61	42.53	16.14	16.78	8741.1	9651.3	1325.6	1374.1	10.5	11.2
Humate 50%	55.11	57.69	19.52	20.11	13584.2	14758.3	1651.7	1723.1	12.3	12.8
L.S.D. at 0.05	**2.05**	**3.14**	**1.27**	**0.39**	**2543.1**	**2457.3**	**101.2**	**112.3**	**1.14**	**1.11**



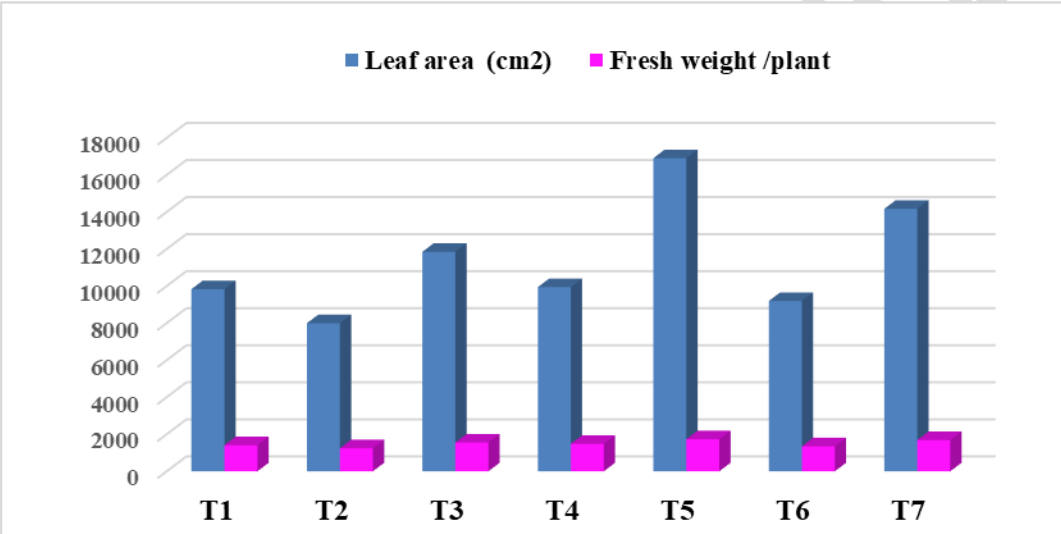


Fig 1. Vegetative growth characteristics of cauliflower plants affected by Treatments

The enhancement effect of vinasse on cauliflower plants growth may be attributed to that vinasse which is rich in organic matter and potassium which can be used as alternative fertilization sources and offers great possibilities for improving growth (**Oliveira *et al.*, 2014**). In this perspective, the increment in growth parameters may be due to that humic acid HA are extremely important component led to constitute stable fraction of carbon, thus regulating the carbon cycle and release of nutrients, including nitrogen, phosphorus, and sulfur which decreasing the need for mineral fertilizer for plant growth **(Ulukan, 2008)**. These obtained results agreed with those reported by **Ahmed *et al.*, (2020)** revealed that leaves number, stem, plant length, plant weights, and even chlorophyll of cauliflower significantly enhanced by application with humic acid. **Abbas *et al.*, (2020)** found that humic acid had a significant increase in chlorophyll, dry matter % in cauliflower leaves. **Moreover, Ismail (2016)** illustrated that fertilizing common bean plants with (HA) at 12kg/fed led to significant increases in the all growth parameters as compared with the control (100% NPK recommendation without HA) in both seasons. **Hassan *et al.*, (2021)** pointed out that all vinasse and potassium humate combinations resulted in significantly increased shoot length, leaf number, and leaf area in pomegranate. Also, **Ayman (2022)** reported that wheat plants treated with humic acid (HA) 0.4 gradually increased wheat morpho physiological parameters, namely, shoot length, root length, shoot dry weight and root dry weight. **Abd El-Rhman (2017)** recorded that potassium humate at 25 and 50g/tree was superiority and more effective in increasing the growth in pomegranate.

2 - Curds yield and its physical attributes:

The data in (Table 7 and Fig 2.) regarding the impact of adding olive mill wastewater, vinasse, and K-humate as soil applications on cauliflower curds showed that these treatments significantly increased curd yield at the edible stage compared to the control. The data also showed that adding vinasse 50% followed by potassium humate 50% as soil applications to cauliflower plants were significantly sufficient to produce the superiority values of curds compared to the other treatments or the control. The superior treatment, vinasse 50%, resulted in higher values for the total yield of cauliflower curds (ton/fed.) in the first and second places, respectively, by 34.7 and 31.6% over the control (the untreated plants).

Table (7): Curds yield parameters and its physical quality of cauliflower plants as affected by soil application with olive mill wastewater, vinasse and potassium humate during the two winter seasons of 2018/2019 and 2019/2020.

Treatments	Curd weight (g)		Curd diameter (cm)		Curd dry matter %		Days to curd maturity		Curds Yield (ton/fed)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Control(NPK100%)	1493	1524	17.4	18.7	7.30	7.40	93.47	93.56	21.20	21.81
Olive mill waste 100%	1314	1354	16.8	17.3	6.24	6.54	94.71	94.66	18.23	19.18
Olive mill waste 50%	1557	1576	18.1	18.6	7.73	7.81	92.42	92.67	23.48	23.75
Vinasse 100%	1475	1521	17.8	18.7	7.52	7.65	93.47	93.33	22.38	22.64
Vinasse 50%	1798	1823	21.1	22.6	8.06	8.16	91.11	91.23	27.86	27.91
Humate 100%	1368	1385	17.5	18.9	7.22	7.36	94.44	94.32	20.18	2054

Humate 50%	1607	1657	19.4	20.0	7.80	7.88	91.87	91.91	24.98	25.11
L.S.D. at 0.05	**115**	**136**	**0.68**	**0.41**	**0.20**	**0.24**	**N.S**	**N.S**	**3.09**	**3.14**

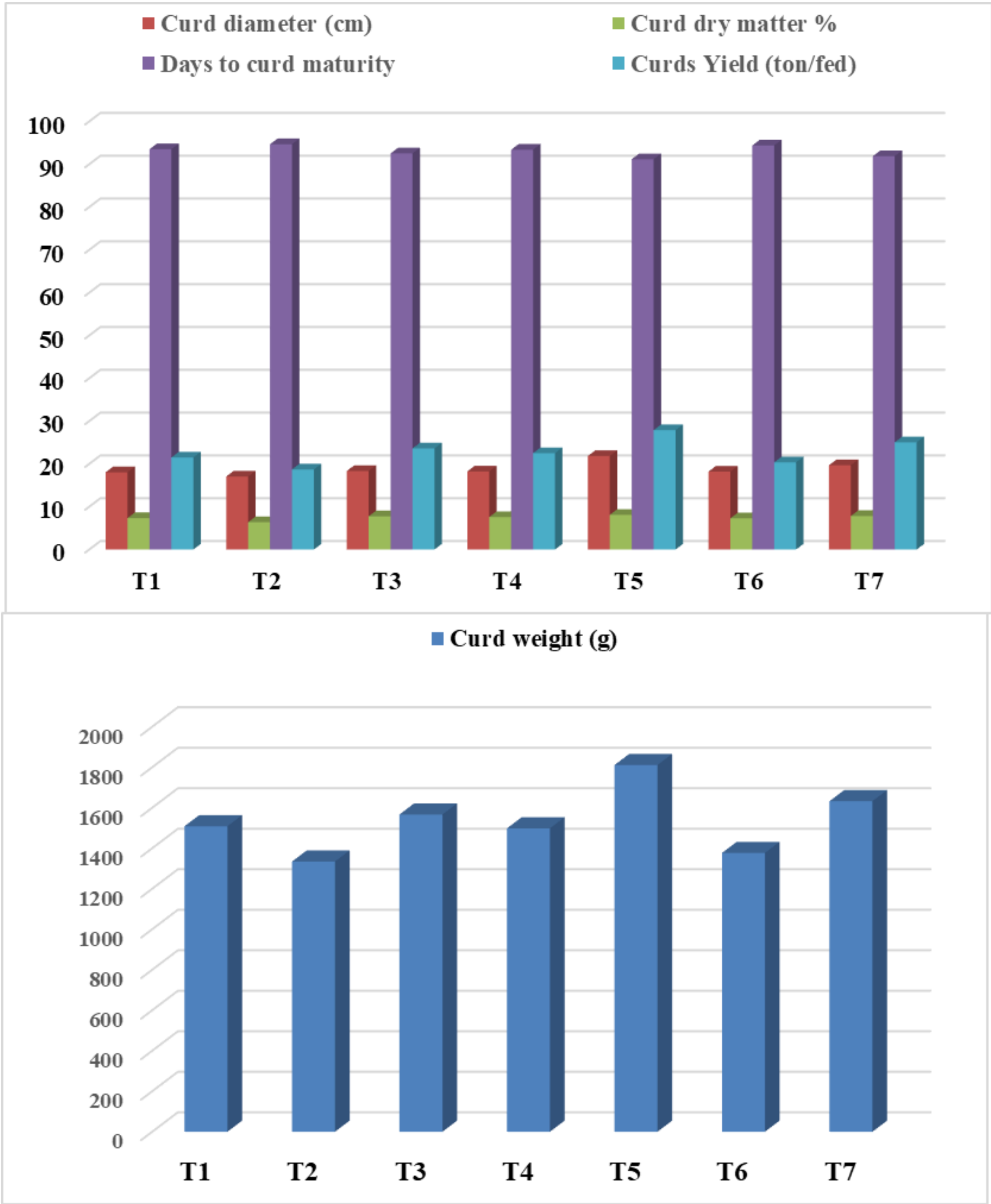


Fig2. Curds yield parameters of cauliflower plants affected by Treatments

These results are in harmony with those of **Ahmed *et al.*, (2020)** they decided that percentage of dry fruit weight of cauliflower was enhanced by the application with HA. Also, **Abbas *et al.*, (2020)** reported that humic acid significantly increased curd weight. **Hassan *et al.*, (2021)** on pomegranate illustrated that soil applications of 20 g and 40 g potassium humate with 500 mL or 1000 mL vinasse applications recorded the highest values of perfect flower %, fruit set %, yield, fruit weight, aril/ fruit%. Also, **Badawy *et***

***al*., (2019)** found that application of potassium humate plus yeast extract increased the potato tubers weight with 34.9%, 21.3% and 35.6% compared to control (without application). **Abd El-Rhman (2017)** demonstrated that there were statistically significant increases in fruit yield and fruit quality by soil application with potassium humate treatments in pomegranate.

3 - Dry seed yield, its components and seed germination ratio:

As for the effect of soil application of some organic compounds; olive mill wastewater, vinasse and potassium humate on cauliflower seed yield and its components *i.e.* number of seeds/pod, 1000 seeds weight (g), seed yield (g/plant) and total dry seed yield (kg/ fed), as well as seed germination ratio (%), data in (Table 8 and Fig 3.) illustrated that dry seed yield and its components were significantly increased by adding all organic compounds. Whereas, adding vinasse 50% followed with potassium humate 50% to cauliflower plants were significantly sufficient to produce the superiority values of dry seed yield components compared to the other treatments or the control. In other meaning, it can be concluded that, the favorable treatments produced the superior dry seed yield either per plant or / fed. and seed germination ratio were obtained by adding vinasse 50% followed with potassium humate 50% as soil application to cauliflower plants. This increment was clear during the two both seasons. The total dry seed yield of cauliflower (kg/ fed.) value under vinasse 50% treatment occurred higher value by 28.7 and 30.6 % over the control (the untreated plants) in the both experimental seasons respectively.

Table (8): Total dry seed yield, its components and seed germination ratio (%) of cauliflower plants as affected by soil application with olive mill wastewater, vinasse and potassium humate during the two winter seasons of 2018/2019 and 2019/2020.

Treatments	No. of dry seeds /dry pod		1000 seeds weight (g)		Seed yield (g/plant)		Total dry seeds yield (kg/fed.)		Germination ratio (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control(NPK100%)	9.9	10.1	2.7	2.8	19.6	20.3	174.6	177.1	80.2	80.6
Olive mill waste 100%	9.2	9.4	2.1	2.2	16.4	16.7	150.1	152.5	72.3	72.8
Olive mill waste 50%	10.1	10.4	2.9	3.1	20.7	22.4	183.2	189.7	82.3	83.8
Vinasse 100%	9.8	10.6	2.6	2.7	20.4	21.6	179.4	184.3	82.4	83.3
Vinasse 50%	11.4	11.7	4.1	4.2	26.7	27.6	211.1	219.4	90.1	90.8
Humate 100%	9.7	9.9	2.3	2.4	18.3	18.6	157.8	160.2	77.6	76.5

Humate 50%	10.7	10.9	3.4	3.6	22.4	23.8	196.2	200.7	86.4	87.8
L.S.D. at 0.05	**0.41**	**0.34**	**0.10**	**0.13**	**1.5**	**1.4**	**4.48**	**6.54**	**2.2**	**2.4**

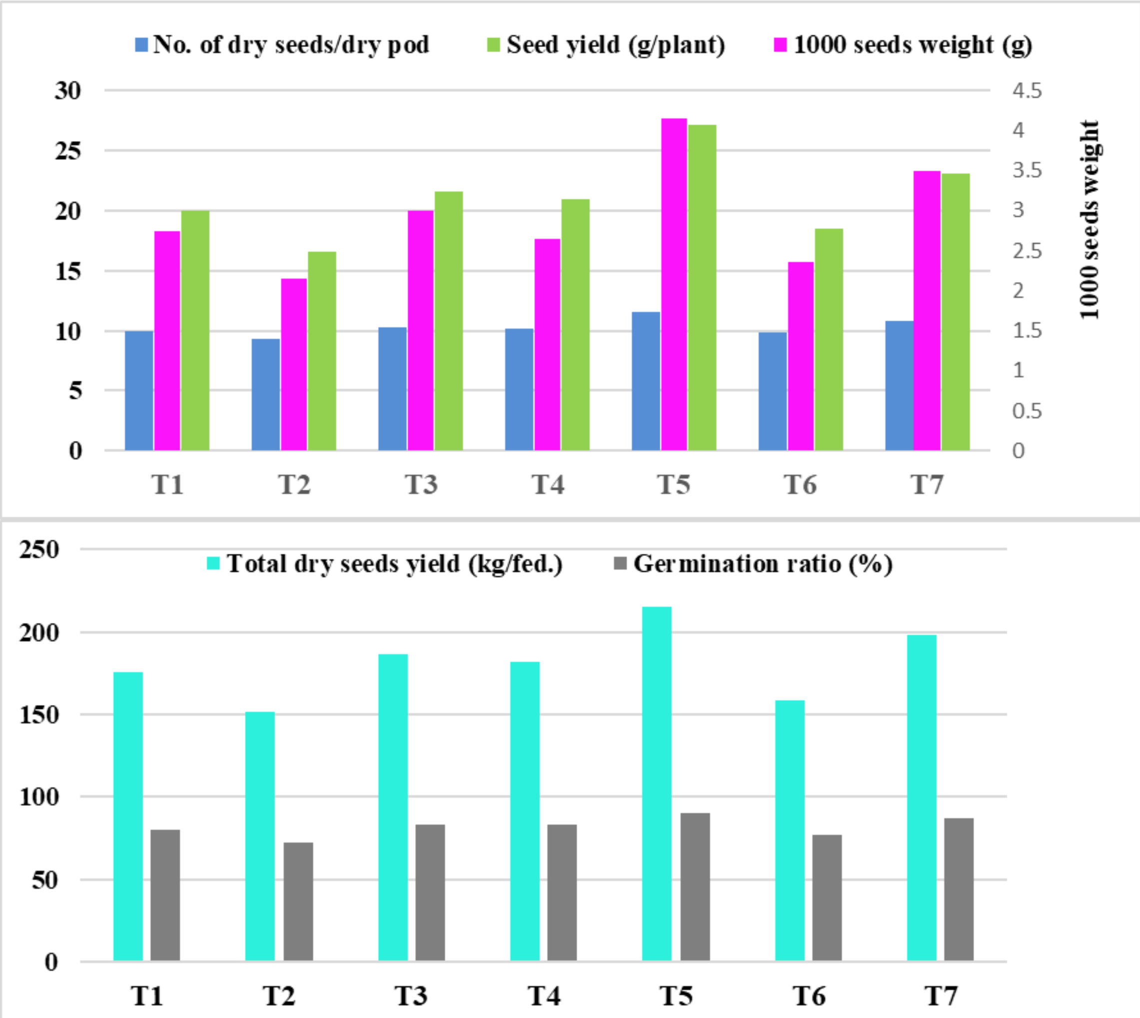


Fig3. Total dry seed yield and its components of cauliflower plants affected by Treatments

The favorable effect of using this treatment in dry seed yield production could be referred to that vinasse is used as an organic fertilizer because it contains macronutrients, as well as the ability to chelate organic material with micronutrients. In addition, Humic substances may possibly enhance the uptake of minerals through the stimulation of microbiological activities **(Mayhew, 2004)**. When adequate humic substances are present within the soil, the requirement for nitrogen, phosphorus and potassium fertilizer applications may be reduced (**Pettit, 2004**). Also, Humic substances will maximize the efficient use of residual plant nutrients, reduce fertilizer costs, and help release those plant nutrients presently bound in minerals and salts. This favorable role of humic acid application on dry seed yield and its components are in agreement with those results obtained by **Ismail (2016)** on common bean indicated that dry seed yield and its components expressed as, seed index, dry seed yield/plant and per fed as well as seed germination ratio (%) were significantly increased by adding HA as soil application in both seasons. **Ayman (2022)** mentioned that wheat grain yield and components significantly differed in response to the main effect of soil applied HA.

4- Chemical constituents in curds:

According to chemical properties in curds of cauliflower which harvested in edible stage, the data in Table (9) revealed that the quality of curds was increased after adding all tested treatments of humate, vinasse and olive mill wastewater (OMW) as soil application to cauliflower plants. It was cleared that N, P, K and carbohydrates (%) in curds were significantly increased. It is noticed that, adding vinasse 50% followed with potassium humate 50% to cauliflower plants were the superior treatments to obtain the maximum of N, P, K and carbohydrates (%) in curds compared with the other treatments or the control (100 % NPK). This obtained result was true during the both seasons. The stimulatory effects of vinasse is used as a soil adjustment by the production of beneficial microorganisms in the soil because of the high concentration of vitamins and amino acids **(Fito *et al.*, 2019)**. Added to that, the effects of humic substances have been directly correlated with enhancing uptake of macronutrients, such as nitrogen, phosphorus and sulfur **(Chen and Aviad, 1990)** and micronutrients as, Fe, Zn, Cu and Mn **(Chen *et al.*, 1999)**.

Table (9): Nitrogen, phosphorus, potassium and carbohydrates (%) on curds of cauliflower plants as affected by soil application with olive mill wastewater, vinasse and potassium humate during the two winter seasons of 2018/2019 and 2019/2020.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Carbohydrates (%)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Control(NPK100%)	1.81	1.94	0.366	0.377	1.88	2.02	24.86	25.11
Olive mill waste 100%	1.44	1.51	0.237	0.256	1.64	1.78	22.35	22.74
Olive mill waste 50%	1.86	1.93	0.361	0.378	1.97	2.22	26.47	27.23
Vinasse 100%	1.74	1.85	0.348	0.357	1.83	1.93	24.11	24.66
Vinasse 50%	2.47	2.68	0.517	0.533	2.67	2.84	31.45	32.73
Humate 100%	1.57	1.68	0.246	0.263	1.72	1.85	23.47	23.87
Humate 50%	1.91	2.14	0.411	0.452	2.33	2.65	28.67	29.34
L.S.D. at 0.05	**0.05**	**0.09**	**0.33**	**0.54**	**0.05**	**0.06**	**0.87**	**0.63**

The obtained results in this respect are confirmed with many investigators, **Ismail (2016)** illustrated that fertilizing common bean plants with HA gave the highest values of N, P, K and crude protein content in common bean dry seeds compared with the other tested treatments during both seasons. **Hassan *et al.*, (2021)** reported that soil applications of 20 g and 40 g potassium humate with 500 mL or 1000 mL vinasse applications recorded the highest values of concentrations in pomegranate. Also, **Ayman (2022)** showed that soil application with HA 0.2 resulted in significant increases in grain nutrients on wheat *i.e.* N, P, K, Zn and total protein.

5- Additive fertilizer effects on enzyme activities for plants:

More than 75% of Earth's land surface is affected by poor farming practices and changing climatic scenarios, and enzymes were crucial in maintaining soil degradation that resulted in (salinized, eroded, low organic matter), **Talukder *et al*., (2021)**. In dry land areas, a variety of soil additives have been investigated to improve deteriorated soil. The effectiveness of applying soil amendments including olive mill wastewater, vinasse, and potassium humate varies widely depending on the land use, soil type, and ecosystems globally.

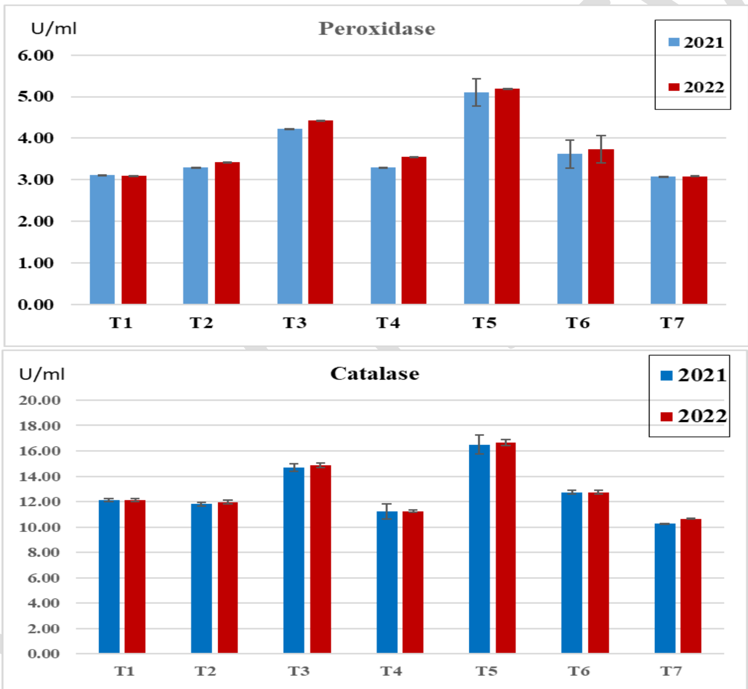


Fig 4.Effect of additive olive mill wastewater, vinasse and k-humate on catalase and peroxidase activity for cauliflower plant.

In the first and second seasons, the lowest levels of vinasse and olive mill wastewater in the soil led to an increment in catalase and peroxidase activity. The treatment with vinasse in particular, significantly increased peroxidase activity in the first and second seasons up to (5.10 and 5.18 U/ml, respectively), as well as the maximum catalase activity in the two seasons up to (16.5 and 16.7 U/ml, respectively). This is followed by adding olive mill wastewater to the soil 50% (4.22 and 4.42 U/ml peroxidase activity and 14.67 and 14.87 catalase activity). Catalase and peroxidase are an oxidoreductase associated with aerobic microbial activity, **Rodrı´guez and Truelove (1982)** and its activity was significantly and positively correlated with dehydrogenase soil showed no significant differences between treatments. This result may be explained by the improved soil aeration in the byproduct amended soils as a consequence of an increasing in soil total porosity (**Bin *et al.*, 2022).** Catalase activity in leaves of cauliflower was found to share in scavenging the reactive oxygen species resulting from oxidative stress. In plants, catalase scavenges H_2O_2 generated mainly during mitochondrial photo respiratory oxidation and electron transport, beside β-oxidation of the fatty acids, **Leitão *et al.*, (2021).**

Soil enzymes activities

Ecosystem functioning depends on soil enzyme activities that affect nutrient transformation, carbon (C) sequestration, and the biogeochemical cycling of carbon, nitrogen (N), phosphorous (P) and sulfur (S). In humid and semi-arid soils, the activities of enzymes activities have been utilized as sensitive indicators of changes in soil quality associated with management and land uses**, Acosta (2014)**. Dehydrogenases are oxidoreductase enzymes that play a vital role in the respiration of microorganisms, catalyzing a variety of reactions in the process. As such, they have the potential to serve as a marker for microbial activity in soils. Dehydrogenases activity (De-Hase) monitoring was employed in this investigation to determine the impact of various organic fertilizer applications on soil microbial activity.

The presented data in Table 10 showed that De-Hase activity in the 50% vinasse and 50% olive applications was either higher than 100% of all other treatments and significantly different from the control. This may indicate that 100% application did not benefit soil microbial community and thus wasted. The control treatment had the lowest De-Hase levels, while 50% vinasse appliaction had the highest level (55.81and 56.32 g TPF/g soil in the 1st and the 2nd season, respectively) which was significantly higher than the same treatment supplemented with 50% olive mill wastewater (43.18 and 43.93 g TPF/g soil in the 1st and the 2nd season, respectively). These results may be explained by the presence of more organic matter and fewer phenolic compounds than in the 100% treatments, which would be beneficial to the soil microbial community possessing De-Hase. According to data in Table (10) 50% vinasse application had the highest Phosphatase (p-ase) activity (28.38 and

25.90mg PNP/g soil in the first and second seasons, respectively), followed by 50% olive mill wastewater application (23.73 and 23.43 mg PNP/g soil in the first and second seasons, respectively). These applications were either higher than 100% of all other treatments or significantly different from the control. Both De-Hase and Phosphatase (p-ase) are main sources in soil combinations of microbial origin, reflecting the whole residual microbial activities at harvest time. Microbial DeH-ase and P-ase had their impact on cauliflower growth extended during cultivation period that were translated into cauliflower yield and analysis at harvest time.

Table10. Effect of additive olive mill wastewater, vinasse and k-humateon soil characterization and soil enzymes activity for cauliflower plant rhizosphere

Treatments	pH		EC dS/m		OM %		De H-ase µg TPF/g/day		P-ase mg PNP/g/day		N- ase NmolesC_2H_4/g/day	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	7.5	7.5	1.35	1.34	1.30c	1.13a	28.62e	28.54e	12.53b	13.20b	60.17e	61.33e
T2	7.3	7.3	1.41	1.57	1.82a	1.85ab	32.65d	32.49d	20.83a	22.87a	77.83b	78.83b
T3	7.5	7.4	1.39	1.43	1.81b	1.90ab	43.18d	43.93a	23.73ab	23.43ab	80.73c	80.97c
T4	7.3	7.1	1.67	1.75	2.58a	2.83b	37.19c	39.32a	24.73a	23.23a	53.83f	53.95f
T5	7.4	7.3	1.49	1.53	2.89a	2.85c	55.81a	56.32c	28.38a	25.90a	102.67a	103.33a
T6	7.55	7.57	1.45	1.32	2.34d	2.46c	43.66b	44.53b	22.93ab	22.57a	66.33d	66.57d
T7	7.65	7.8	1.33	1.22	2.03d	2.48c	44.99b	45.12d	22.90a	23.13a	96.17b	98.10b
LSD=0.05					2.3	2.35	2.8143	3.1276	7.440	8.35	1.159	1.078

De H-ase (Dehydrogenase), P-ase (Phosphatase), N2- ase (Nitrogenase), OM (Organic Matter)

Enzyme activities varied widely among the treatments studied. The treatment in which the highest activity occurred was found to vary depending on the enzyme, Fig. 5. Basically, enzymes involved in Intracellular microbial metabolism, such as dehydrogenase and phosphatase increased with the addition of vinasse and olive mill wastewater. In addition, adding 50% of vinasse had the highest nitrogenase activity levels (102.67and 103.33 N moles C2H4/g soil in 1^{st} and 2^{nd} season, respectively) followed by the treatments using 50% olive mill wastewater treatment, 50% and 100% k-humate in the soil. Cauliflower quality traits such as nitrate contents were also improved with application of several organic fertilizers such as bio digested vinasse, digested livestock manure, rice bran and molasses, **Santos *et al*., (2010) and Liu *et al*., (2014**). Additional studies demonstrated that soil application of organic materials like food pomace compost, garlic stalk by-product enhanced soil enzyme activity in cauliflower cultivation, **Lee (2004) and Wojewódzki *et al.*, (2022)**. The use of untreated olive wastewater in the fertigation of tomato growth led to an increment in the amount of total soil organic carbon, extractable N and C, accessible P, and extractable Mn and Fe. In addition, soils treated with olive wastewater displayed greater levels of soil respiration, dehydrogenase, urease activity, and microbial biomass (**Piotrowska *et al.*, 2006).**

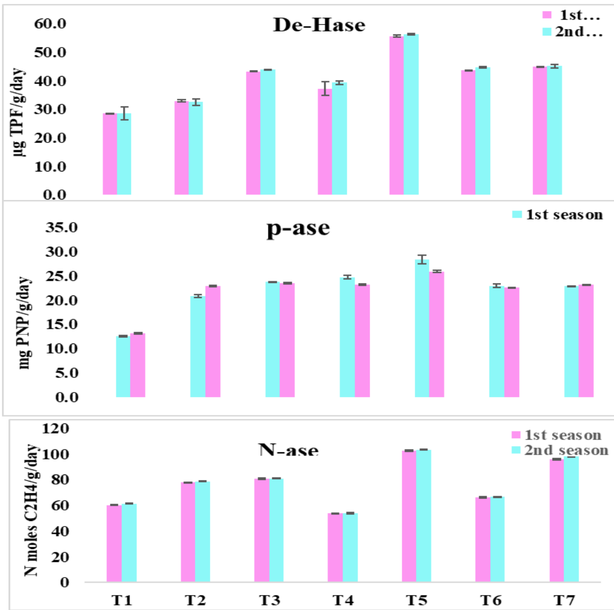


Fig 5. Dehydrogenase, phosphatase and nitrogenase activities in cauliflower plant rhizosphere irrigated with vinasse, olive mill wastewater and k-humate

The results in Table (10) demonstrated that applying the various treatments of vinasse, olive mill wastewater, and K-humate either alone or in combination with NPK over the two growing seasons had a substantial impact on the soil characteristics as compared to that provided 100% NPK alone (control). The results also showed that the treatment of 50% vinasse and 50% olive mill wastewater mixed with 50% NPK led to a slight decrease in pH, possibly due to vinasse and olive mill wastewater is acidic nature, as well as a slight increase in salinity (EC), as compared to the soil pH and EC before their application (Table, 1). These findings concur with those made by **Abd-El-Kaway (2006).** Because vinasse and olive mill wastewater are an acidic with pH between 4 and 5, these results may be explained by the fact that an increasing in vinasse and olive mill wastewater rate resulted in a slight increment in the insoluble acid fraction **(Arafat and Yassen, 2002; Christofoletti *et al.*, 2013).** In a study on cauliflower plants, 100% olive mill wastewater and 100% vinasse irrigation resulted in increased soil salinity and decreased plant weights, whereas 50% olive mill wastewater and

50% vinasse irrigation improved plant development and a lowering of soil pH, this can be attributed to the elimination of phenols and other phytotoxic substances from raw olive mill wastewater and vinasse **(Rusan *et al.,* 2016).** According to the review analysis, plants irrigated with treated OMWW grew more quickly and produced more than those irrigated with tap water **(Mekki *et al*., 2013).**

EC values in Table 10 showed that vinasse 100% treatment had a higher EC value than olive mill wastewater and K-Humate due to relatively high concentration of dissolved salts in the vinasse. In a comparison of all treatments, two treatments of K-humates at 100% and 50% have the lowest EC values. This attributed to the high concentration of monovalent cations, particularly sodium **(Paz *et al.,* 2009 and Wafaa *et al.,* 2016)**. As compared to all treatments, pH values in Table 10 generally decreased when vinasse was applied 100% at the two examined seasons. According to **Jiang *et al*., (2012)** this may be caused by the oxidation of organic matter (H+ acts as an electron acceptor) and the high quantity of free hydrogen ions. **Wafaa *et al*., (2016)** found that the application of vinasse and feldspar mineral at both study seasons often resulted in pH values decreasing due to the acidic influence of vinasse. When using 100% olive mill wastewater for irrigation, pH value also slightly decreases. Whereas, when K-humate was utilized, there was no obvious distinction from the control, which may be due to K-humate acts as a buffer to stabilize soil against substantial changes in pH brought on by fertilizer application. This aligns with the final result of **Campitelli *et al*., (2008).**

On the other hand, pH and EC with other properties were more suitable in 50% olive mill wastewater and 50% vinasse resulting higher fresh weight of cauliflower. It can improve the total marketable fresh weight of cauliflower. Additionally, **Andriolo *et al.* (2005)** revealed that fresh yield and plant growth were decreased by EC values above 2.0 and 2.6 dS m^{-1}. Vinasse and olive mill wastewater applied to the soil in varying concentrations greatly increased cauliflower growth and yield. As a result, they might be crucial to maintaining the rizosphere environment. Additionally, applying the olive to soils in organic farming systems is an intriguing possibility for completing the cycle of resources-residues compared to other treatments, 50% vinasse seems to have the strongest impact on growth and yield **(Roig *et al*., 2006)**.

Organic matter (OM) is a fundamental component of the agro-ecosystem, serving as a vital link between the many chemical, physical and biological soil qualities. Results in Table 10 indicated that there were a significant positive responses observed with OM values obtained with different fertilizers treatments compared to control treatments. The superior treatment was observed at treatment in a combination with (50% vinasse+ 50% NPK) and 100% vinasse. Of course, this may be due to vinasse relatively high content of organic matter. Furthermore, results showed that 100% K- humate has high values of organic matter content (OM) because potassium humate works as an organic matter which promote microorganisms in soil **(Khaled and Fawy 2011).** The positive effect of vinasse may be due to its beneficial contents of some chelate organic micro and macro nutrients and increasing the bio-availability of NPK, which is an important function in the formation of photosynthetic pigments as mentioned by **Parnaudeau *et al*., (2007).**

CONCLUSION

Regarding to the results of this study, it could be concluded that fertilizing cauliflower plants Amshiry cv. with vinasse 50% followed with potassium humate 50 % mixed with 50% NPK in order to increase plant growth, curds yield, dry seed yield/fed and improve yield components. These treatments also led to save mineral fertilizer for cauliflower requirements by 50% of NPK and play a fundamental role in the maintenance of rhizosphere ecosystem. Also, for organic farming systems application to soils of these olive represents an interesting option, closing the cycle of residues-resources. Further, studies should be conducted in the future to know the effect of the combined addition of vinasse, olive mill wastewater and K-humate on the physical, biological and chemical properties of soils, as well as their effect on improving the efficiency of water and nutrient use.

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