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 over two consecutive winter seasons of 2018/2019 and 2019/2020 at Qaha Research Farm, Qaliobia Governorate, Horticulture Research Institute, Agriculture Research Center (ARC), Egypt. The study aimed to assess the positive impacts of using of Olive Mill Wastewater (OMW), vinasse and potassium humate at varying application rates (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. The physical and chemical characteristics of the soil were evaluated, including enzymatic activity in the soil rhizosphere, specifically oxidoreductase enzymes such as catalase and peroxidase. The experiment was arranged in a randomized complete block design (RCBD) with seven treatments and three replicates. The obtained results demonstrated that applying 50% 0f vinasse followed by 50% of potassium humate led to enhanced plant growth, increased total curd yield, improved dry seed yield and its components, and positively impacted the physical and chemical properties of the investigated soil. The addition of 50% vinasse or 50% K- humate yielded the most favorable outcomes compared to other treatments or the control and at the same time save and reduce mineral fertilizer recommendation of cauliflower by 50 %. The most successful treatment, involving 50% vinasse application, resulted in a 34.7% and 31.6% increase in total curd yield (ton/ fed.) and a 28.7% and 30.6% increase in dry seed yield (kg/fed) over the control for the two 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.) holds a significant position among vegetable crops within the Brassicaceae family. It is valued not only for its culinary versatility – being suitable for consumption fresh, boiled, or pickled **(Noor *et al.*, 2014)** – but also for its robust nutritional content. This cruciferous vegetable is a rich source of essential vitamins such as vitamin A, thiamine, riboflavin, niacin, and vitamin C, in addition to vital minerals including calcium and iron, which contribute to its nutritional significance **(Ahmed and Ali, 2013)**. Remarkably, **Eimon *et al.*, (2019)** highlight cauliflower's exceptional nutrient density, particularly with respect to dietary fiber, folic acid, water content, and ascorbic acid.

Shifting attention to the environmental realm, the production of Olive Mill Wastewater (OMW) is concentrated in Mediterranean countries, with Egypt ranking among the prominent producers. The factors influencing OMW production encompass olive types, maturation stages, regional climates, farming methodologies, and extraction equipment, as revealed by **FAOSTAT (2007).** 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. The global olive oil industry, especially within the Mediterranean Basin, has steadily expanded over the past decade, with a notable average annual growth of approximately 5% in global production. Consequently, the disposal of OMW poses considerable challenges, leading to diverse strategies like evaporation ponds, direct soil application, and various physico-chemical and biological treatments (**Mostafa *et al.*, 2022; Youness *et al.*, 2022).**

In a different context, vinasse, a byproduct of the sugar industry, emerges as a residue subsequent to sugarcane processing, yielding products like crystalline sugar, pulp, and molasses **(Hidalgo, 2009).** This residual substance remains after extracting desired products such as alcohol and ascorbic acid. Vinasse production is voluminous – around 13 liters of vinasse are generated per liter of cane alcohol. Vinasse, when appropriately applied in agriculture, has demonstrated substantial nutrient provision, enhanced soil quality on degraded land, and elevated crop yields **(Vadivel *et al.*, 2014)**. Insights from **Abd-El-Kaway (2006)** revealed the influence of vinasse and potassium sulfate on soil pH and salinity. Furthermore, vinasse has found utility in fertigation practices, effectively reducing water input for plant growth. **Walter *et al.*, (2011**) underline its merits, as it contributes significant water and mineral nutrients, enhances soil quality, and boosts crop productivity. Importantly, vinasse's incorporation into fertigation systems not only offers agronomic benefits but also addresses the environmental challenge of disposing of this agro-industrial residue **(Abou-Husssien *et al.*, 2020)**. **Soha *et al.*, (2020)** further advocate for a balanced approach involving 50% recommended K-fertilizer and 50% biologically treated vinasse, leading to economical root and sugar yields while conserving inorganic potassium resources. Nevertheless, further investigations are required to assess the prolonged impact of vinasse on soil properties such as permeability, salinity, and PH.

Delving into the sphere of potassium humate, a water-soluble salt of humic acid, the manifold enhancements it imparts to soil's physical, chemical, and biological attributes become apparent. Its influence extends to plant growth, with **Tejada *et al.*, (2006)** showcasing its role in metabolic activity alteration. Potassium humate's introduction into plant cells offers a supplementary source of respiratory catalysts, as underscored by **Ryosuke *et al.*, (2006).** The transportation of humic substances – humic and fulvic acids – from roots to shoots further enhances overall plant growth **(Shahein *et al.*, 2015).**

Undoubtedly, humic acid (HA) emerges as an organic molecule with pivotal functions in enriching soil characteristics, fostering plant development, and enhancing agronomic aspects. Its influence extends to the physical, chemical, and biological attributes of soil, encompassing aspects like texture, structure, water-holding capacity, cation exchange capacity, pH, soil carbon content, enzyme activity, nitrogen cycle, and nutrient availability **(Kwame *et al.*, 2022).** This review underscores the multi-faceted impact of HA, spanning crop development, plant hormone production, nutrient assimilation, yield enhancement, and protein synthesis. The classification of humic substances into humic acid, fulvic acid and humic elucidates their varying roles (**Solange and Rezende, 2008**). Notably, the research by **Wafaa *et al.*, (2017)** emphasizes the positive correlation between nitrogen fertilization rates and total yield and nutritional content, reflecting the benefits of bentonite and potassium humate mixture in enhancing soil properties and fertility. Moreover, **Hassan *et al.*, (2021)** advocate for the synergistic use of potassium humate and vinasse in sandy soils to amplify nutrient status and enhance crop growth and productivity. The hydraulic improvement in soil's qualities due to humic acid addition, leading to changes in structure, density, porosity, and electrical conductivity, is highlighted by **Ayman *et al.*, (2022).**

In considering soil enzymes, their pivotal role in carbon sequestration and soil nutrient dynamics is underscored **(Lemanowicz *et al.*, 2023**). These enzymes serve as indicators of soil quality, microbial activity, and nutrient transformation, as established by **Ahamadou and Huang (2012).** Notably, soil dehydrogenases, categorized as oxidoreductase enzymes, are integral to soil's microbial oxidation of organic matter and correlate with overall microbial biomass (**Wolinska and Stepniewsk, 2012**). The activities of peroxidase and catalase, vital enzymes in quantifying stress conditions in lettuce leaves, further emphasize their role in plant health assessment (**Aires *et al.*, 2021; Leitão *et al.*, 2021**).

Consequently, this study sought to discern the impact of olive mill wastewater, vinasse, and potassium humate applied to soil on cauliflower growth, curd yield, dry seed yield, and enzyme activity. By investigating the intricate interplay between these agricultural inputs and the cultivation parameters of cauliflower, the research aimed to contribute valuable insights into optimizing crop productivity and soil health.

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 experimental field's soil type was identified as clay loam. Before the commencement of each planting season, surface soil samples were collected randomly from the experimental field at a depth of 0 – 30 cm. These samples were then subjected to air-drying, followed by crushing and sieving through a 2.0mm sieve. Subsequently, chemical and physical analyses were performed on the soil samples, in line with the standardized methods outlined by **Page *et al.*, (1982)** and **Klute (1986).** These analyses aimed to determine the various chemical and physical properties of the soil, and the recorded data are provided in Table 1.

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 utilized a completely randomized block design with three replications. For the cultivation of cauliflower Amshiry cv., the process began with sowing the seeds in 209-cell Styrofoam trays within a nursery environment. This stage aimed to produce transplants, which were grown for 45 days until reaching the appropriate age.

Subsequently, the matured seedlings were transplanted during the first week of November in both experimental seasons. During the transplanting phase, seedlings were positioned on one side of each ridge, maintaining a width of 70 cm between rows and 50 cm gap between individual seedlings. Each experimental plot comprised five ridges, each measuring 3.0 meters in length, thus yielding a total plot area of 10.5 m^2.

The nutritional requirements, NPK fertilizers were introduced to the soil in the form of specific compounds: ammonium Sulphate (20.5% N), super phosphate (15.5% P2O5), and potassium Sulphate (48% K2O). The recommended application of 100% NPK comprised 200 kg of ammonium Sulphate, 200 kg of calcium super phosphate, and 100 kg of potassium Sulphate. Meanwhile, 50% of NPK was represented by 100 kg of ammonium Sulphate, 100 kg of calcium super phosphate, and 50 kg of potassium Sulphate. The organic fertilizers – olive mill wastewater, vinasse, and potassium humate – were administered in a liquid form, individually mixed with irrigation water through fertigation.

The application rates for organic fertilizers were either 100% or 50% of the recommended dose (5 m^3per feddan for each type). Moreover, a combination of 50% of the NPK recommended dose with 50% of the organic fertilizers was also employed **(Hossain and Ryu, 2017; Rokia *et al.*, 2023).** Conversely, the control received 100% of the recommended NPK dose, aligning with the guidelines set by the Egyptian Ministry of Agriculture. The specific fertilizer application details are provided in Table 5, as outlined in the experiment plan.

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:

Randomly selected sets of three plants from each experimental plot were gathered for measuring several parameters: plant height (cm), number of leaves per plant, leaf area (cm^2), fresh weight of leaves (g), and leaves' dry matter content (%). The plant foliage was subsequently subjected to drying at 70°C until reaching a constant weight, from which the dry weight per plant was determined.

1- Curds yield and its physical attributes:

At 115 days post-planting, a sample consisting of three curds from each plot was collected to determine attributes such as fresh weight (g), curd diameter (cm), curd dry matter content (%), number of days to maturity, and the overall curd yield (ton/fed).

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

At the culmination of the harvesting period (physiological maturity), ten random dry pods were sampled from each plot to ascertain the following data: number of dry seeds per dry pod, 1000 seeds weight (g), seed yield (g per plant), and total dry seeds yield (kg per feddan). Additionally, the dry cauliflower seeds underwent treatment with Tobsen fungicide before being placed in filter paper within a germination incubator set at 25°C to calculate the seed germination ratio (%).

4- Chemical constituents in curds:

The determination of nitrogen (N), phosphorus (P) and potassium (K) content was performed according to **AOAC (1990).** Carbohydrate content (%) was assessed in line with the method outlined by **Mazumdar and Majumder (2003)**.

5- Chemical and physical Characteristics of soil:

Soil samples from the study were air-dried, crushed, and sieved through a 2.0 mm sieve for subsequent analysis of their chemical and physical properties. This analysis followed the standardized methods detailed by **Page *et al.*, (1982)** and **Klute (1986)** for various aspects, including electrical conductivity, soil pH, soluble carbonates and bicarbonates, calcium, magnesium, chloride concentration, sulphate calculation, sodium, potassium, organic matter, cations, and anions. The Organic matter was determinate by **Walkely and Black (1934).**

6- Enzyme activities:

Soil and plant biological activities were assessed through enzyme activity measurements in both soil rhizosphere and fresh plant material. Soil samples from cauliflower rhizosphere were collected after 105 days from planting.

A fresh sample weighing 0.5g was homogenized in 10 ml of cold phosphate buffer (50 mM, pH 7). The resulting homogenates were then centrifuged at 4000 rpm at 20°C for 20 min, with the supernatant serving as the raw extract for enzymatic assays. Enzyme activity assays were conducted for:

- Peroxidase activity (EC 1.11.1.7) using the methylene blue assay according to **Magalhaes *et al.*, (1996).**

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

- Dehydrogenase activity (DeH-ase) (EC 1.1.1.) using the tri-phenyl tetra-zolium chloride (TTC) method according to **Casida *et al.*, (1964**).
- Phosphatase activity (P-ase) (EC 3.1.3) measured using para nitro phenyl phosphateaccording to **Tabatabai and Bremner (1969**).
- Nitrogenase activity (EC 1.18.6.1) determined by acetylene reduction assayas described by (**Johnsen and Apsley, 1990**).

7- Statistical analysis procedure:

All data acquired from the study underwent analysis of variance (ANOVA) using the MSTATC computer software program. Subsequently, the means of various treatments were compared utilizing the Least Significant Differences (LSD) test at a significance level of 0.05, as described by **Bricker (1991).**

RESULTS AND DISCUSSION

1-Vegetative growth characteristics:

The collected data, presented in Table 6 and Figure 1, illustrated the substantial impact of the employed treatments on the vegetative growth characteristics of cauliflower plants. The effects of incorporating olive mill wastewater, vinasse, and K-humate as soil applications were evident, leading to notable improvements in various growth parameters when compared to the control. Across all treatments, there was a significant enhancement observed in key growth indicators, namely plant height (cm), number of leaves per plant, leaf area (cm^2), fresh weight per plant, and leaf dry matter content (%). Notably, the treatments of vinasse 50% followed by K- humate 50% emerged as the most effective interventions, consistently showcasing the greatest promotion of these growth parameters over both growing seasons.

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**

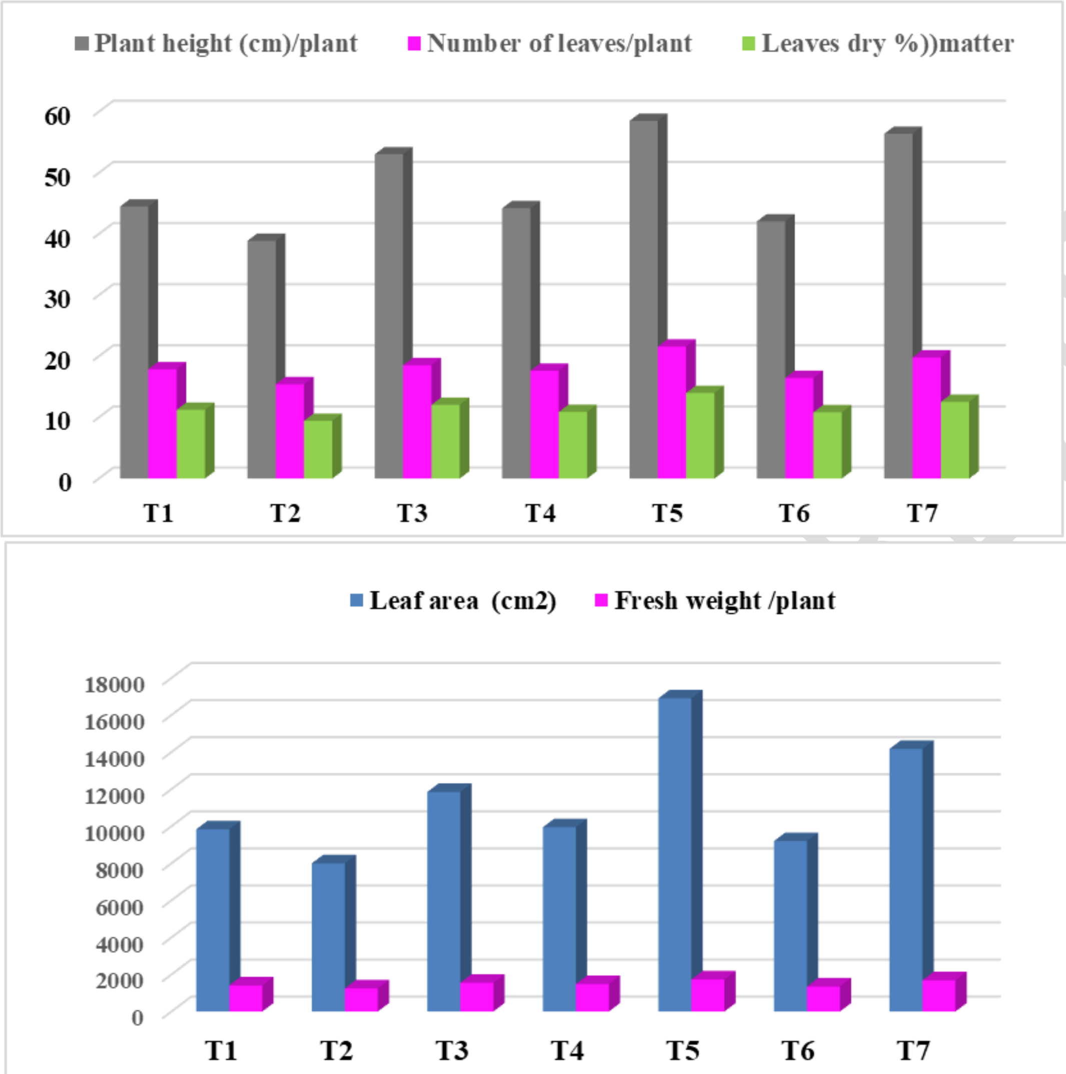

Fig 1. Vegetative growth characteristics of cauliflower plants affected by Treatments

The growth enhancement observed in cauliflower plants due to vinasse can be attributed to its richness in organic matter and potassium, which serve as alternative sources of fertilization, presenting significant potential for growth improvement (**Oliveira *et al*., 2014 [46]**). From this perspective, the increase in growth parameters can be attributed to the presence of humic acid (HA), a vital component that contributes to the formation of a stable carbon fraction. This process regulates the carbon cycle and facilitates the release of essential nutrients such as nitrogen, phosphorus, and sulfur. As a result, there is a reduced dependency on mineral fertilizers for promoting plant growth (**Ulukan, 2008 [20]).**

Consistent findings were reported by **Ahmed *et al*., (2020 [7]**), who observed that the application of humic acid significantly enhanced several growth parameters of cauliflower, including leaf number, stem length, plant length, plant weight, and chlorophyll content.

Similarly, **Abbas *et al.*, (2020 [1])** noted a significant increase in chlorophyll content and dry matter percentage in cauliflower leaves following the application of humic acid. **Ismail (2016 [30])** demonstrated that the addition of HA at a rate of 12 kg/fed led to significant improvements in growth parameters for common bean plants compared to the control (100% NPK recommendation without HA) during both seasons. **Hassan *et al.*, (2021 [27])** emphasized that various combinations of vinasse and potassium humate led to substantial increases in shoot length, leaf number, and leaf area in pomegranate trees. Additionally, **Ayman (2022 [11])** reported that the treatment of wheat plants with humic acid (HA) at a concentration of 0.4 gradually resulted in improved morpho-physiological parameters, including shoot length, root length, shoot dry weight, and root dry weight. **Abd El-Rhman (2017 [21])** documented the superiority and enhanced effectiveness of potassium humate at rates of 25 and 50 g/tree in stimulating growth in pomegranate trees.

2 - Curds yield and its physical attributes:

The data presented in Table 7 and Figure 2 illustrated the significant impact of adding olive mill wastewater, vinasse, and potassium humate as soil applications on cauliflower curd yield. Across the edible stage, these treatments exhibited a notable increase in curd yield when compared to the control. Notably, adding of 50% vinasse followed by 50% potassium humate as soil applications demonstrated remarkable efficacy in enhancing curd yield for cauliflower plants. This treatment, with 50% vinasse, stood out by producing substantially higher curd yields in both the first and second places, surpassing the control (non-treated plants) by 34.7% and 31.6%, respectively, in terms of total curd yield (tons/fed).

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**

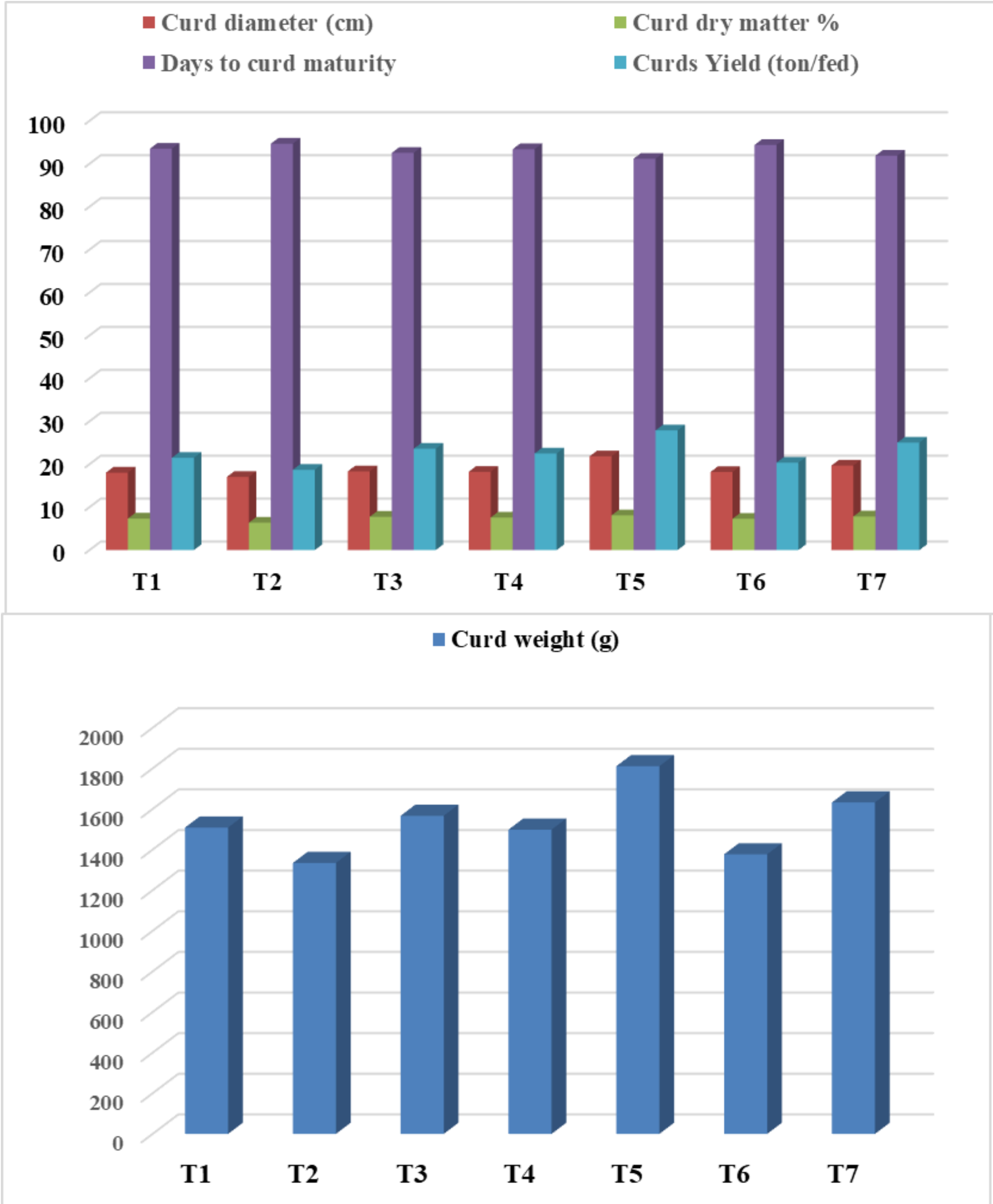

Fig2. Curds yield parameters of cauliflower plants affected by Treatments

These findings are consistent with those of **Ahmed *et al.*, (2020**), who observed an improvement in the percentage of dry fruit weight of cauliflower due to the application of HA. Similarly, **Abbas *et al.*, (2020)** reported a significant increase in curd weight as a result of humic acid application. **Hassan *et al.*, (2021**) conducted research on pomegranate and found that soil applications of 20 g and 40 g of potassium humate along with 500 mL or 1000 mL of vinasse led to the highest values in terms of perfect flower percentage, fruit set percentage, yield, fruit weight, and aril-to-fruit percentage. Furthermore, **Badawy *et al.*, (2019)** observed that the application of potassium humate combined with yeast extract increased potato tuber weight by 34.9%, 21.3%, and 35.6% compared to the control without application. **Abd El-Rhman (2017)** also reported significant increases in fruit yield and fruit quality through soil application of potassium humate treatments in pomegranate.

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

The impact of soil application of various organic compounds, including olive mill wastewater, vinasse, and potassium humate, on cauliflower's dry seed yield and its components, such as the number of seeds per pod, 1000 seeds weight (g), seed yield (g/plant), and total dry seed yield (kg/fed), as well as seed germination ratio (%), is presented in Table 8 and Figure 3. The data revealed significant increases in dry seed yield and its components following the application of all organic compounds. Notably, adding vinasse at 50% followed by potassium humate at 50% as a soil application to cauliflower plants produced notably superior dry seed yield component values compared to other treatments and the control. In other words, the treatments involving vinasse and potassium humate yielded the highest dry seed yield per plant or per feddan. Furthermore, the application of vinasse at 50% followed by potassium humate at 50% as soil treatment resulted in the highest seed germination ratio (%), and this positive effect was consistent across both seasons. Remarkably, the total dry seed yield of cauliflower (kg/fed.) under the vinasse 50% treatment recorded values that were 28.7% and 30.6% higher than the control (100% of NPK) in 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**

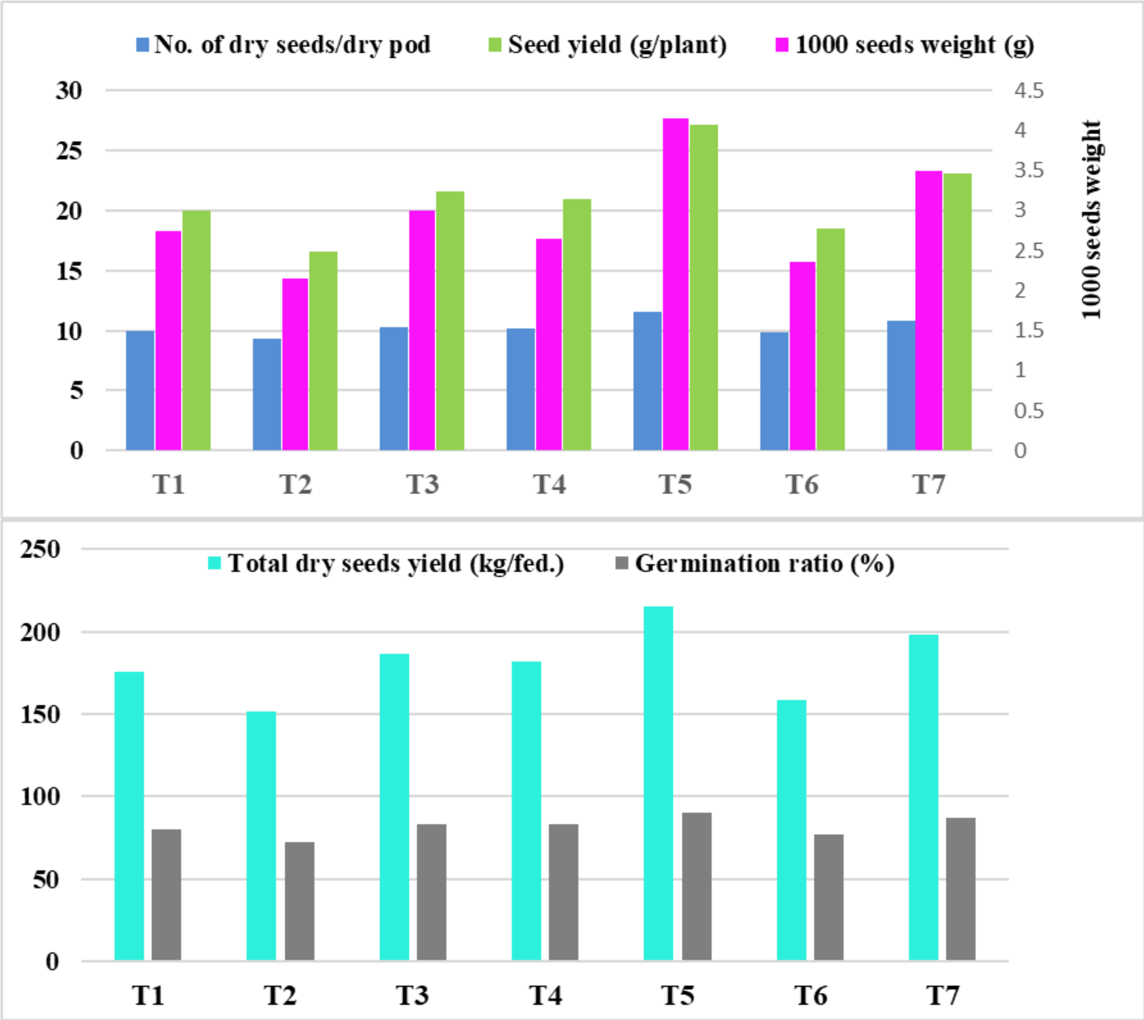

Fig3. Total dry seed yield and its components of cauliflower plants affected by Treatments

The positive impact of using this treatment on dry seed yield production could be attributed to the fact that vinasse serves as an organic fertilizer, containing essential macronutrients and the ability to chelate organic material with micronutrients. Moreover, humic substances have the potential to enhance mineral uptake through the stimulation of microbiological activities (**Mayhew, 2004**). When sufficient humic substances are present in the soil, there might be a reduction in the need for nitrogen, phosphorus, and potassium fertilizer applications (Pettit, 2004). Furthermore, humic substances can optimize the efficient utilization of residual plant nutrients, leading to reduced fertilizer expenses and aiding in the release of plant nutrients that are currently bound within minerals and salts.

These favorable effects of applying humic acid to enhance dry seed yield and its components are consistent with findings by **Ismail (2016)** in common beans, which indicated that dry seed yield and its components such as seed index, dry seed yield per plant and per fed, as well as seed germination ratio, were significantly increased by incorporating humic acid as a soil application in both seasons. **Ayman (2022)** also noted significant differences in wheat grain yield and its components in response to the main effect of soil-applied humic acid.

4- Chemical constituents in curds:

The chemical properties of cauliflower curds harvested at the edible stage were evaluated to assess the impact of different treatments, including olive mill wastewater (OMW), vinasse and K-humate as soil applications. The data presented in Table (9) demonstrated a consistent enhancement in the quality of curds after the addition of all tested treatments to cauliflower plants. Notably, the levels of nitrogen (N), phosphorus (P), potassium (K), and carbohydrates (%) in curds showed significant increases. Particularly striking was the effectiveness of the vinasse 50% treatment, followed by the potassium humate 50% treatment, in producing curds with the highest concentrations of N, P, K, and carbohydrates (%), outperforming the other treatments and even the control (100% NPK recommendation). This trend was consistent across both experimental seasons.

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 stimulating impact of vinasse as a soil amendment on curd quality can be attributed to its ability to promote the growth of beneficial microorganisms in the soil due to its high content of vitamins and amino acids **(Fito *et al.*, 2019).** Additionally, the positive effects of humic substances have been associated with their capacity to enhance the uptake of essential macronutrients like nitrogen, phosphorus, and sulfur (**Chen and Aviad, 1990**) as well as micronutrients such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) (**Chen *et al.*, 1999).**

The findings of the present study align well with the results obtained by various researchers. For instance, **Ismail (2016)** conducted a study on common bean plants and found that the application of humic acid (HA) led to the highest levels of nitrogen (N), phosphorus (P), potassium (K), and crude protein content in dry common bean seeds, surpassing the other treatments tested in both seasons. Similarly, **Hassan *et al.*, (2021)** reported that in pomegranate, soil applications of potassium humate at rates of 20 g and 40 g, combined with vinasse applications of 500 mL or 1000 mL, resulted in the highest concentrations of certain compounds. Furthermore, **Ayman (2022)** demonstrated that the soil application of HA at a rate of 0.2 significantly elevated the concentrations of grain nutrients in wheat, including nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), and total protein. These consistent findings across different plant species and studies reinforce the beneficial impact of organic amendments such as humic acid and vinasse on enhancing nutrient concentrations in plant tissues.

5- Additive fertilizer effects on enzyme activities for plants:

In the context of addressing soil degradation, which affects over 75% of the Earth's land surface due to factors such as poor agricultural practices and changing climate conditions, enzymes play a pivotal role. These enzymes are instrumental in mitigating soil degradation issues, such as salinization, erosion, and low organic matter content, as highlighted by **Talukder *et al.*, (2021)**. In regions characterized by dry land conditions, various soil additives have been extensively studied with the aim of rejuvenating degraded soils. The efficacy of these soil amendments, which encompass substances like olive mill wastewater, vinasse, and potassium humate, exhibits significant variability based on factors including land utilization, soil composition, and global ecosystems.

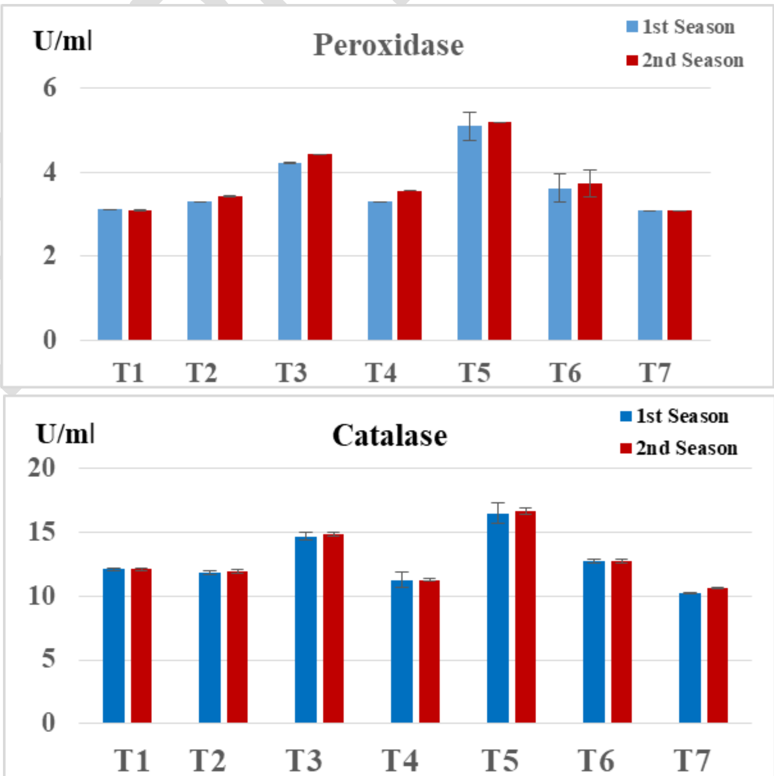

Fig 4.Effect of additive olive mill wastewater, vinasse and k-humate on catalase and peroxidase activity for cauliflower plant.

In the first and second seasons, the impact of different levels of vinasse and olive mill wastewater on soil catalase and peroxidase activity was investigated. Notably, the lowest levels of vinasse and olive mill wastewater in the soil led to an increase in both catalase and peroxidase activity. Specifically, the treatment involving vinasse exhibited a significant rise in peroxidase activity during both seasons, reaching values of 5.10 U/ml and 5.18 U/ml, respectively. Moreover, this treatment also recorded the highest levels of catalase activity, measuring 16.5 U/ml and 16.7 U/ml for the two seasons, respectively.

Following closely was the treatment involving the addition of olive mill wastewater to the soil at a 50% concentration, which resulted in peroxidase activity levels of 4.22 U/ml and 4.42 U/ml, and catalase activity levels of 14.67 U/ml and 14.87 U/ml for the two respective seasons. Catalase and peroxidase enzymes, which are oxidoreductases associated with aerobic microbial activities, have shown significant and positive correlation with soil dehydrogenase activity. Interestingly, the soil treated with the byproducts exhibited improved aeration due to increased soil total porosity, as suggested by **Bin *et al.,* (2022)**. Catalase activity in cauliflower leaves plays a role in scavenging reactive oxygen species resulting from oxidative stress. This is particularly relevant in plants, where catalase helps eliminate H_2O_2 produced primarily during processes such as mitochondrial photo respiratory oxidation, electron transport, and β-oxidation of fatty acids, as noted by **Leitão *et al.,* (2021).**

6- Soil enzymes activities:

The activities of soil enzymes play a crucial role in ecosystem functioning as they influence various processes, including nutrient transformation, carbon sequestration, and biogeochemical cycling of elements like carbon, nitrogen, phosphorous, and sulfur. These enzyme activities are considered sensitive indicators of changes in soil quality due to land management and use practices, as highlighted by **Acosta (2014**). In this context, dehydrogenases, which are oxidoreductase enzymes, are particularly important as they are involved in microorganism respiration and catalyze various reactions.

To assess the impact of different organic fertilizer applications on soil microbial activity, dehydrogenase activity (De-Hase) monitoring was employed in this study. The results presented in Table 10 demonstrate that the application of 50% vinasse and 50% olive mill wastewater led to higher De-Hase activity compared to all other treatments, including the 100% application rates, and these differences were statistically significant. This suggests that the 100% application rates might not have been as effective in benefiting the soil microbial community and could be considered wasteful. The control treatment displayed the lowest De-Hase activity, while the 50% vinasse application exhibited the highest levels (55.81 g TPF/g soil and 56.32 g TPF/g soil in the 1st and 2nd seasons, respectively), significantly surpassing the activity observed in the 50% olive mill wastewater treatment (43.18 g TPF/g soil and 43.93 g TPF/g soil in the 1st and 2nd seasons, respectively).

These findings can be explained by the presence of more organic matter and fewer phenolic compounds in the 50% treatments compared to the 100% treatments. This composition is likely beneficial for the soil microbial community involved in De-Hase activity. Additionally, the data in Table 10 indicate that the 50% vinasse application led to the highest phosphatase (P-ase) activity (28.38 mg PNP/g soil and 25.90 mg PNP/g soil in the first and second seasons, respectively), followed by the 50% olive mill wastewater application (23.73 mg PNP/g soil and 23.43 mg PNP/g soil in the first and second seasons, respectively). Similar to De-Hase, both De-Hase and P-ase activities contribute to soil microbial activities, reflecting the overall microbial activity at the time of harvest. The influence of microbial De-Hase and P-ase activities on cauliflower growth extended throughout the cultivation period and translated into cauliflower yield and quality at harvest time **Acosta (2014)**.

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)

The enzyme activities exhibited significant variations across the different treatments under study, as depicted in Fig. 5. Notably, the treatment yielding the highest activity levels varied depending on the specific enzyme. Enzymes that are integral to intracellular microbial metabolic processes, including dehydrogenase and phosphatase, displayed increased activity with the application of vinasse and olive mill wastewater. Among the treatments, the addition of 50% vinasse resulted in the highest nitrogenase activity levels (measured as 102.67 and 103.33 N moles C2H4/g soil in the 1st and 2nd seasons, respectively). This was followed by treatments involving 50% olive mill wastewater, 50% and 100% k-humate in the soil.

The application of various organic fertilizers, such as bio-digested vinasse, digested livestock manure, rice bran, and molasses, led to improvements in cauliflower quality attributes, including nitrate content, as reported by **Santos *et al.*, (2010) and Liu *et al.*, (2014)**. Other investigations also indicated that the soil application of organic materials, such as food pomace compost and garlic stalk by-products, enhanced soil enzyme activity in cauliflower cultivation, as observed in studies by **Lee (2004)** and **Wojewódzki *et al.*, (2022)**. Furthermore, the utilization of untreated olive wastewater in tomato fertigation contributed to an increase in total soil organic carbon, extractable nitrogen and carbon, accessible phosphorus, and extractable manganese and iron. Additionally, soils treated with olive wastewater exhibited heightened levels of soil respiration, dehydrogenase and urease activity, as well as microbial biomass, as highlighted in the work of **Piotrowska *et al.*, (2006)**.

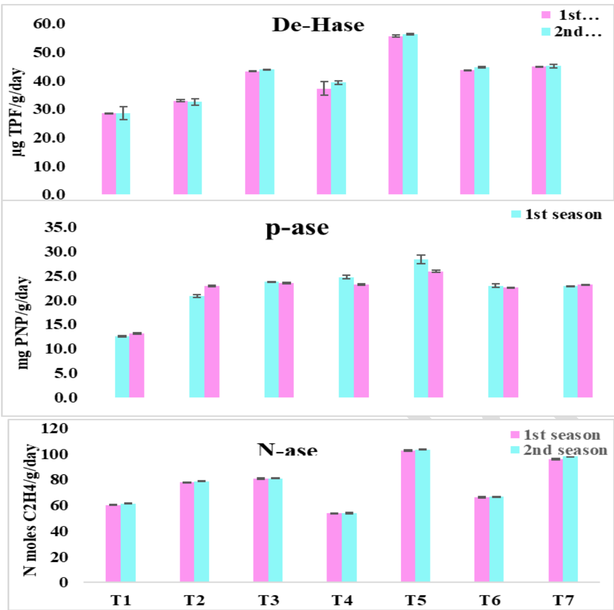

Fig 5. Dehydrogenase, phosphatase and nitrogenase activities in cauliflower plant rhizosphere irrigated with vinasse, olive mill wastewater and k-humate

The outcomes presented in Table (10) underscore the substantial influence of applying diverse treatments of vinasse, olive mill wastewater, and K-humate either independently or in combination with NPK across the two growing seasons, causing significant alterations in soil characteristics compared to the control, which was provided with 100% NPK alone. Notably, the treatment involving 50% vinasse and 50% olive mill wastewater mixed with 50% NPK exhibited a slight decrease in pH, potentially attributed to the acidic nature of vinasse and olive mill wastewater. Additionally, there was a minor increase in salinity (EC) in comparison to the initial soil pH and EC values prior to treatment application (as presented in Table 1). This observation aligns with findings by **Abd-El-Kaway (2006)**. Since vinasse and olive mill wastewater have a pH range of 4 to 5, this result may be attributed to an increase in the insoluble acid fraction due to higher rates of vinasse and olive mill wastewater application (**Arafat and Yassen, 2002; Christofoletti *et al.*, 2013**).

In a study involving cauliflower plants, irrigation with 100% olive mill wastewater and 100% vinasse led to heightened soil salinity and decreased plant weights. In contrast, the use of 50% olive mill wastewater and 50% vinasse irrigation enhanced plant growth, accompanied by a reduction in soil pH. This effect might be attributed to the removal of phenols and other phytotoxic substances from raw olive mill wastewater and vinasse (**Rusan *et al.*, 2016**). A review analysis indicated that plants irrigated with treated olive mill wastewater experienced accelerated growth and higher yields than those irrigated with tap water (**Mekki *et al.*, 2013**).

The EC values in Table 10 highlight that the 100% vinasse treatment displayed a higher EC value compared to olive mill wastewater and K-humate treatments, primarily due to the relatively high concentration of dissolved salts in vinasse. Among all treatments, the two K-humate treatments, at both 100% and 50%, exhibited the lowest EC values. This phenomenon can be attributed to the elevated concentration of monovalent cations, particularly sodium (**Paz *et al.*, 2009 and Wafaa *et al.*, 2016**). Comparing all treatments, pH values generally decreased when 100% vinasse was applied during both seasons. This trend might be attributed to the oxidation of organic matter, where hydrogen ions (H+) act as electron acceptors, resulting in a high quantity of free hydrogen ions. **Wafaa *et al.*, (2016**) also observed that vinasse application often led to a decrease in pH due to its acidic influence. Moreover, when 100% olive mill wastewater was employed for irrigation, pH values slightly decreased. Conversely, when K-humate was used, no significant deviation from the control was observed, potentially due to the buffering effect of K-humate, which helps stabilize soil pH against substantial fluctuations resulting from fertilizer application. This aligns with the findings of **Campitelli *et al.*, (2008**).

Furthermore, a combination of 50% olive mill wastewater and 50% vinasse, along with the resulting pH and EC values, appeared to be more conducive for higher cauliflower fresh weight. This combination contributed to an improvement in the overall marketable fresh weight of cauliflower. Moreover, **Andriolo *et al.*, (2005**) indicated that fresh yield and plant growth tended to decrease when EC values exceeded 2.0 and 2.6 dS m-1. The application of varying concentrations of vinasse and olive mill wastewater to the soil markedly enhanced cauliflower growth and yield, potentially playing a pivotal role in maintaining a favorable rhizosphere environment. Additionally, the application of olive mill wastewater to organic farming systems presents an intriguing opportunity for closing the resource-residue cycle. In comparison to other treatments, 50% vinasse treatment seems to exert the most pronounced impact on growth and yield (**Roig *et al.*, 2006**).

7- Organic Matter and Fertilizer Effects:

Organic matter (OM) holds a pivotal role within agro-ecosystems, acting as a critical bridge that connects various chemical, physical, and biological aspects of soil quality. As evident from the data in Table 10, noteworthy positive responses in OM values were observed across different fertilizer treatments compared to the control group. The most promising outcomes were achieved with treatments combining 50% vinasse and 50% NPK, as well as with 100% vinasse. This enhanced performance could be attributed to the relatively high organic matter content present in vinasse.

Furthermore, the results indicated that 100% K-humate treatment exhibited elevated organic matter content (OM), likely due to the role of potassium humate in promoting soil microorganisms (**Khaled and Fawy 2011**).The favorable impact of vinasse can be attributed to its advantageous composition, which includes chelated organic micro and macro nutrients that enhance the bioavailability of NPK. This enhancement plays a pivotal role in the development of photosynthetic pigments, a function highlighted 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.

Key takeaways from this study include:

1. **Enhanced Growth and Yield:** The recommended treatment resulted in improved vegetative growth, increased curd yield, and enhanced dry seed yield per unit of land (fed).

2. **Nutrient Savings:** The utilization of vinasse and potassium humate along with reduced NPK fertilizer by 50% showcased an effective approach to achieving desired growth while minimizing reliance on mineral fertilizers.

3. **Rhizosphere Ecosystem:** The selected treatments played a crucial role in fostering a favorable rhizosphere ecosystem, where soil enzyme activities and microbial functions were optimized, potentially leading to improved nutrient cycling and availability.

4. **Organic Farming Benefits:** The application of olive mill wastewater, vinasse, and K-humate to soil offers a promising avenue for enhancing organic farming systems, contributing to the recycling of resources and residues within agricultural ecosystems.

Looking forward, future studies could delve deeper into the combined effects of vinasse, olive mill wastewater, and K-humate on various soil properties, including physical, chemical, and biological aspects. Additionally, investigating their potential to enhance water and nutrient use efficiency would contribute to a comprehensive understanding of their agricultural implications.

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