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Medically active ingredient in Tarragon (*Artemisia dracunculus* L.) affected by adding fish emulsion and Vermicompost

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Abstract

The experiment was carried out in the canopy of the Department of Horticulture and Landscape - Faculty of Agriculture - Kerbala University during the spring semester 2023. The study was carried out as a factor experiment by designing complete random block design(R.C.B.D) with three repetitions. The experiment included two factors, the first was a fish emulsion and was added in four concentrations, namely 0, 1, 2, 3%, and the second factor was the addition of Vermicompost with four levels 0, 25, 50, 100 g Pot^{-1}, the averages were compared using the lowest significant difference at the probability level of 0.05. The results showed that the addition of fish emulsion significantly affected each type of flavonoid glucosides (Vitexin, Luteolin-3-galctasides, Rutin, Eriodityol, Quercetin-3-rhamnoside), where the concentration (3%) treatment gave the highest rate, reaching (1119.73 ،914.28 ،1013.6 ،1193.81 ،1308.91) on the relay . The addition of Vermicompost affected the types of glucosides (Vitexin, Luteolin-3-galctasides, routine, Eriodityol), the concentration (g pot^{-1}) gave the highest rate, reaching (1056.22, 962.07, 1008.0, 1055.79) sequentially The interaction between the two study factors showed a significant effect on glucosides (Vitexin, Luteolin-3-galctasides, Rutin, Eriodityol, Quercetin-3-rhamnoside), where the (3%, and 100 g Pot^{-1}) interference treatment gave the highest rate (1448.60 ،1464.72 ،1423.3 ،1463.82،1441.84) on the relay. It can be concluded from this study that fertilizers resulting from organic waste can be used as sources of fertilization for plants to reduce the use of chemical fertilizers to reduce environmental damage and follow the system of sustainable agriculture.

Keywords: organic fertilizers, fish waste, earthworm, vermicompost, Tarragon.

Introduction

The tarragon plant *Artemisia dracunculus* L. from herbs that are not commonly cultivated in Iraq, tarragon is grown in Germany, France, the Netherlands, Bulgaria, Hungary, Belarus, Central Asia, Iran, India and Ukraine[1].

It was found in studies that tarragon extracts can produce antioxidant effects by evaluating the total phenolic level and total flavonoids [2]. The medicinal effect in tarragon is due to the presence of phenolic compounds and flavonoids such as Chlorogenic acid, caffeic, Luteolin or Quercetin [3]. It is used in the cosmetic industry as an ingredient in skin care products, perfumes, face masks and compresses, as it is used in the production of moisturizing creams, shampoos and body cleansers, and these products are used to care for the scalp, body and face, and essential oil can be used as an ingredient for perfumes [4]. The concentration of the main mineral elements and the content of medically active substances in plants is affected as a result of the addition of various fertilizers, including organic fertilizers, as this change reflects the role that these fertilizers play in the readiness of nutrients, increased vegetative growth and vital processes of the plant, which increases the production of chemicals during primary and secondary metabolism, and this has been proven in previous studies and research, it was found that the addition of decomposed fish manure and its comparison with chemical fertilizer (N P K), as the results showed that fish manure resulted in giving a high content of phenols equal to that produced by the treatment of chemical fertilizer for cowpea plant *Vigna unguiculata* L. [5]. When assessing the suitability of fertilizers resulting from the decomposition of fish waste for agricultural use, it was found that the addition of fish waste fertilizer to the soil of agricultural anvils led to an increase in the major elements represented by an increase in nitrogen by 78.6%, phosphorus by 61.8% and potassium by 56.3% in ice lettuce ice lettuce leaves *Lactuca sativa* L. [6].

Vermicompost had a significant effect on all the compounds of the secondary metabolism under study and the volatile oils of the thyme plant *Thymus vulgaris* L. It was found that the use of 75% Vermicompost gave the highest levels of Alpha and Kama turbinates and the proportion of volatile oils [7]. The use of Vermicompost fertilizer has improved the content of the basil plant (*Ocimum basilicum* L.) [8]. apply combinations of organic and chemical fertilizers to find out their effect on the medically effective compounds of the marigold plant *Calendula Officinalis* L. The combinations with Vermicompost fertilizer gave the highest content of total chlorophyll (7.35 mg g-1 soft weight), the highest content of flavonoids (365,000 mg g-1 dry weight) and the highest content of total phenols (0.453 mg g^{-1} dry weight) [9].

Highest content of total phenols and some types of alkaloids of medical and therapeutic importance from leaves and bulbs was found when treating Leucojum acstivum in proportions between 10-50% of Vermicompost fertilizer. [10]. The study aimed to determine the tarragon response to organic fertilizer treatment (fish emulsion and Vermicompost) and its content of medically active compounds**.**

Materials and Methods

The experiment was carried out at the Department of horticulture and landscape - Faculty of Agriculture - Kerbala University for the period from 20/2/2023, where seeds were planted in dishes until 20/6/2023.the study includes two factors, the first is the addition of fish emulsion in four concentrations of 0, 1, 2, 3%, and its symbol

(F1,F2.F3, and F4) In sequence, which was added five times every two weeks . The second factor is four levels of worm fertilizer (Vermicompost), namely (0, 25, 50, 100) g pot^{-1} and its symbol (V1,V2,V3, and V4) In sequence. It was added before transferring the seedlings to pots. The seeds were planted in dishes to prepare the seeds, and then the seeds were transferred to pots with a volume of 5 kg soil. The experiment was carried out according to Randomized Complete Block Design(R.C.B.D) with three replicates . The planting medium was prepared by mixing the soil with ptomose in a ratio (1 soil:1 ptomose) and was placed in the pots and sterilized the pots with Beltanol two weeks before planting, Agricultural Service operations were carried out regularly on all seedlings until the completion of the experiment.The seedlings were transferred to pots with a volume of 5 kg soil at 3-4 true leaves ,Agricultural service operations were carried out for the plants, including adding neutral chemical fertilizer (NPK) (20, 20, 20) at a rate of 1 g L^{-1},. At the end of the experiment on 6/20/2023, samples were taken from the experimental units and some HPLC column; Lichrospher CN (4.6 mm × 100 mm, 3µm)

Mobile phase: 1% acetic in deionized water: acetonitrile, linear gradients from 0%B- 100%B in13 minutes.

detection: Uv set wavelength was 280 nm.

Flow rate; 1.3 m1/min, Temperature: 30 C

The sequences of the eluted material of the standard were as follow, each standard was 25 **µg** /ml.

Seq	Subjects	Retention time minute	Area µ volt
1	Vitexin	2.175	130491
2	Luteolin-3-galctasides	3.417	142920
3	Rutin	4.487	146509
4	Eriodityol	5.427	136509
5	Quercetin -3-rhamnoside	6.323	142619
6	Quercetin-3-galactoside	7.255	149792
7	Kempferol-3-rhamnoside	8.317	131495
8	Kempferol-3-rutinoside	9.422	146588

Calculation

$$\text{Concentration of sample } \mu g \text{ /ml} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. Of standard} \times$$

Instruments

The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV –Vis 10A-SPD spectrophotometer.

Sample Preparation

Briefly, 1 g of the leaves powder were weighed and pulverized, and then soaked with 400 mL water. The mixtures were then boiled for 30 min, leave the mixture to still for 15 min , The decoctions were then concentrated to by vacuum drying at 45^0C and aliquots were stored at -20^0C until used.

Investigation of Hydrolysis Conditions

(1) Hydrolysis

Volume of HCl (1.2N) taken equal the Volume of artemisia leaves extract above Were mixed, and the mixtures were heated in a water bath at 80^0C for 1h,. The mixture was extracted by an equal volume of ethyl acetate and centrifuged at 4,000 xg for 10 min. The supernatant was collected and dried under nitrogen gas. The residue was dissolved in 1 ml methanol and subjected for HPLC analysis.

The liquid sample were filtered on disposable sep-pak cartridge filter (Supelco Co, Ltd), Then 20 **µl** were injected on HPLC column.The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the samples under the same optimum separation condition. [11].

The results were statistically analyzed according to the statistical program Genestat and the averages were compared using the lowest significant difference (L.S.D.) at the probability level of 0.05 [12].

Results and Discussion

Virtexin glycosideglycoside (µgm.ml^{-1})

Results in Table 1 indicate that there is a significant difference between the fish emulsion concentrations and Vermicompost fertilizer levels and the interaction between them in the character of vitexin glycoside in tarragon leaves. The results indicate that the fish emulsion was superior to the F4 concentration and recorded the highest average of 1119.73 µgm.ml-1), while the F1 concentration gave the lowest average of 547.47 µgm.ml-1). The results of the same table indicate the superiority of the Vermicompost fertilizer addition treatment at level V4, which gave the highest average of 1056.22 µgm.ml-1), while the V1 addition treatment recorded a lower average of 530.48 µgm.ml-1) the interaction between the vermicompost fertilizer levels and the fish emulsion addition concentrations was significant, as the interaction treatment was superior in recording the highest rate of 1448.60 µgm.ml^{-1}) in the F4V4 treatment when it gave the lower average F1V1 was 107.27 µgm.ml^{-1}).

Table (1): Effect of fish emulsion and vermicompost on Virtexin glycoside of Tarragon plant

Mean of vermicompost g pot^{-1}	Fish Emulsion%				Mean of vermicompost g pot^{-1}
	F1	F2	F3	F4	
V1	257.26	443.94	541.76	878.96	530.48
V2	456.09	1192.48	820.64	1057.85	881.76
V3	631.17	897.66	767.79	1093.51	847.53
V4	845.38	107.27	859.64	1448.60	1056.22
L.S. D	4.804				2.402
Mean of Fish Emulsion %	547.47	901.34	747.46	1119.73	
L.S. D	2.402				

Luteolin-3- lctasides glycoside (µgm.ml^{-1}**)**

Table 2 shows a significant difference between the concentrations of fish emulsion and vermicompost fertilizer levels and the interaction between them in the character of luteolin-3-galctasides glycoside in tarragon leaves, as the results indicate that the fish emulsion addition treatment was superior in concentration F4 and recorded the highest average of 914.28 µgm.ml^{-1}), while the concentration F1 gave the lowest average of 555.11 µgm.ml^{-1}. The results of the same table indicate the superiority of the Vermicompost fertilizer addition treatment at level V4, which gave the highest average of 962.07 µgm.ml^{-1}, while the V1 addition treatment recorded the lowest average of 561.18 µgm.ml-1. The interaction between the levels of vermicompost concentrates and the addition of fish emulsion fertilizer was significant, as the interference treatment outperformed in recording the highest rate of 1464.72 µgm.ml-1 in the F4V4 treatment, while the F4V2 treatment gave the lowest average of 297.68 µgm.ml^{-1}.

Table (2): Effect of fish emulsion and vermicompost on Luteolin-3- lctasides glyco-side(µgm.ml^{-1}) of Tarragon plant.

Vermicompost g pot^{-1}	Fish Emulsion%				Mean of Vermicompost g pot^{-1}
	F1	F2	F3	F4	
V1	448.39	496.93	599.36	700.05	561.18
V2	447.73	1101.16	766.60	297.68	653.29
V3	565.89	701.12	878.96	1194.65	835.16
V4	758.42	914.32	710.81	1464.72	962.07
L.S. D	9.830				4.915
Mean of Fish Emulsion %	555.11	803.38	738.93	914.28	
L.S. D	4.915				

Rutin glycoside (µgm.ml^{-1})

There was a significant difference between the fish emulsion and Vermicompost fertilizer levels and the interaction between them in the character of glycoside Rutin glycoside in tarragon leaves, as the results indicate that the fish emulsion concentrations were treated with a higher concentration of F4 and recorded an average of 1013.6 µgm.ml^{-1}, while the F1 concentration gave a lower average of 638.5 µgm.ml^{-1}. The results of the same table indicate the superiority of Vermicompost fertilizer addition treatment at the V4 level, which gave the highest average of 1008.0 µgm.ml^{-1}, while the V1 addiction treatment recorded the lowest average of 575.2 µgm.ml^{-1} . The interaction between the addition of fish emulsion concentrations and Vermicompost fertilizer levels was significant, as the interference treatment outperformed in recording the highest rate of 1423.3 µgm.ml^{-1} in the F4V4 treatment, while the F1V1 treatment gave the lowest average of 515.8 µgm.ml^{-1}.

Table(3): Effect of fish emulsion and vermicompost on Rutin glycoside(µgm.ml^{-1})

Vermicompost g pot^{-1}	Fish Emulsion%				Mean of Vermicompost g pot^{-1}
	F1	F2	F3	F4	
V1	515.8	540.0	600.2	644.7	575.2
V2	540.6	1017.7	780.6	1077.7	854.2
V3	718.1	712.1	1105.5	908.8	861.1
V4	779.3	945.5	884.0	1423.3	1008.0
L.S. D	11.97				5.98
Mean of Fish Emulsion %	638.5	803.8	842.6	1013.6	
L.S. D	5.98				

Eriodityol glycoside (µgm.ml^{-1})

The results in Table 4 showed a significant difference between the concentrations of fish emulsion and Vermicompost fertilizer levels and the interaction between them in the content of Iodityol glycoside in tarragon leaves, as the results Table 4, indicates the superiority of the treatment of fish emulsion with concentration F4 and recorded

the highest average of 1193.81 µgm.ml^{-1}, while the concentration F1 gave the lowest average of 495.08 µgm.ml^{-1}, and the results of the same table indicate the superiority of the treatment of adding Vermicompost fertilizer at level V4, which recorded the highest average of 1055.79 µgm.ml^{-1}, while the level V1 addition treatment recorded the lowest average of 666.25 µgm.ml^{-1}, the interaction between the fish emulsion addition concentrations and the levels of Vermicompost fertilizer is significant, as the f4v4 interference treatment outperformed in recording the highest average of 1463.82 µgm.ml^{-1}, while the F1V1 treatment gave the lowest average of 352.04 µgm.ml^{-1}).

Table (4): Effect of fish emulsion and vermicompost on Erioditol glycoside (µgm.ml^{-1}) of Tarragon plant

Vermicompost g pot^{-1}	Fish Emulsion%				Mean of vermicompost g pot^{-1}
	F1	F2	F3	F4	
V1	352.04	716.92	850.78	745.26	666.25
V2	452.03	1098.45	910.00	1419.93	970.10
V3	533.93	920.00	1141.92	1146.23	935.52
V4	642.30	1104.20	1012.82	1463.82	1055.79
L.S. D	9.657				4.828
Mean of Fish Emulsion %	495.08	959.89	978.88	1193.81	
L.S. D	4.828				

Quercetin-3-rhamnoside glycoside (µgm.ml^{-1}).

The results in Table 5, indicate that there is a significant difference between the concentrations of fish emulsion and Vermicompost fertilizer levels and the interaction between them in the character of Quercetin-3-rhamnoside glycoside in tarragon leaves, as the results indicate the superiority of the treatment of fish emulsion with concentration F4 and recorded the highest average of 1308.91 while concentration F1 gave the lowest average of 694.41 µgm.ml^{-1}), and the results of the same table indicate the superiority vermicompost at level V3, which gave the highest average of 1153.98 µgm.ml^{-1}), while the level V1 addition treatment recorded the lowest average of 812.44 µgm.ml^{-1}), and the interaction between vermicompost fertilizer levels and addition concentrations was Significantly, the F4V4 interference treatment outperformed the highest average of 1441.84 µgm.ml-1), while the F1V1 treatment gave the lowest average of 515.82 µgm.ml^{-1}).

Table (5): Effect of fish emulsion and Vermicompost on Quercetin-3-rhamnoside glycoside (µgm.ml^{-1}) of Tarragon plant.

Vermicompost g pot-1	Fish Emulsion%				Mean of Vermicompost g pot^{-1}
	F1	F2	F3	F4	
V1	515.82	757.58	955.17	1021.19	812.44
V2	651.80	1199.26	1007.79	1356.20	1053.76
V3	695.15	1082.28	1422.09	1416.42	1153.98
V4	914.87	1175.30	938.99	1441.84	1117.75
L.S. D	5.276				2.638
Mean of Fish Emulsion %	694.41	1053.60	1081.01	1308.91	
L.S. D	2.638				

The synthesis of secondary metabolic compounds is controlled by genetic factors, but these vital processes are influenced by soil factors, the environment and agricultural processes, including the fertilization process, so it is natural that the production of these substances is stimulated by dietary changes [13]. The accumulation of secondary metabolic compounds, including glucosides in plant tissues, especially in leaves during the plant life cycle, depends mainly on the indicators of vegetative growth, including the rate of carbon metabolism and then the accumulation of its products during the stages of plant growth [14], the results of this study showed as shown in the tables (1،2،3،4،5) ،the use of organic fertilizers under study (fish emulsion and vermicompost), either singly or in combination, had a significant effect in improving vegetative growth indicators related to an increase in carbon metabolism (chlorophyll content), which contributes to an increase in glucosides and their types in tarragon leaves (table 1،2،3،4،5). In addition to the role that organic fertilizers play in increasing the soil nutrients necessary in the construction of secondary compounds in the plant, these fertilizers contribute to the activation and improvement of the soil microbial community, maintain the water content of the soil, resist environmental stresses and thus lead to improved conditions of carbon metabolism, increased carbon fixation, carbohydrate production and protein synthesis [15]. There are many studies that support the results of this study, including those found by. (14), where they found an increase in total glucosides and their types in the stevia plant (Stevia rebaudiana Bertoni) as a result of the use of organic fertilizers and surpassed even chemical fertilizers, and with what was found by replacing part of the mineral fertilizer recommendations with organic fertilizers led to an increase in flavonoid glucosides and their various types in tea leaves [16]. It was also found that organically produced crops are characterized by high concentrations of flavonoids [17]. Moreover, Singh et al. [18] pointed to a lot of studies that illustrate the role of macro-and micro-nutrients that can be obtained by using organic fertilizers in increasing the content of various crops of various secondary metabolic compounds.

Tarragon treated with a combination of fish emulsion and vermicompost had the greatest average of medically active ingredient . It is clear from the results that various plants, like tarragon, may be grown using locally made organic fertilizer mixtures without suffering financial losses or environmental contamination.

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