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EFFECT OF HUMIC AND SALICYLIC ACIDS ON OIL YIELD AND FLAVONOID GLYCOSIDE OF SAFFLOWERS (*CARTHAMUS TINCTORIUS* L.) AS MEDICINAL PLANTS GROWN IN IRAQ

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ABSTRACT: This study was conducted to investigation the effect of humic acid and salicylic on oil yield and flavonoids glycoside of safflower plant (*Carthamus tinctorius* L.) grown in Iraq. After soil preparation the plant seeds were cultivated during winter growing seeds at 2015-2017 at Karbala region, South Baghdad in Iraq. The humic acid was applied at 1000, 2000 and 4000 mg/lit conception as organic flower fertilizes while the application of salicylic acid was used 50, 100 and 200 mg/lit. Twister application of bath humic and salicylic acids were done at vegetable growth stage all treatment were arranged by according randomized complete block design (RCBD) flower leaves or petals ethylic extract was done by used Soxhlet apparatus, the use HPLC his prefect liquid climter to determine the type and concentration of Pt flavonoids glycoside. Also the seed oil was obtained by expression mthai. The redaction were infeed to the S2 F3 treatment (100 mgl^{-1} of salicylic acid with 400 mgL^{-1} of humic acid) was gave height value of oil yield and reached to 20 - 20 kg ha^{-1}, the results are chromatoryted by HPLC were referred to petals safflowers were contain different flavonoids glycoside such as quercetin, salicylic yellow apigenin and kaempferol.

INTRODUCTION: Safflower is a genus of species of flowering plants in the Compositae family they are native in southern Europe, Northern Africa and South West Asia [1]. *Carthumus tinctorius* L. is one of the most important medicinal plants in the world **Fig. 1** [2]. In Iraq *C. tinctorius* usually found in fields of industrial crops which used for different industrial property such as oil production [3]. Safflower seeds are mainly used in preparation of edible oil, conserve, margarine, soap and the like.

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Safflower, it has the highest percentage of oil among industrial crops [4]. Now, the petals leaf and seed oil of this plant a real used in herbal or traditional medicine for treatment different diseases such as dysmenorrhea, amenorrhea, postpartum abdominal pain and mass, trauma and pain of joints [5]. In general the active compound of herbs or medicinal plants are affected by different factors such as field practices and environmental factors, fertilizers and other additives are very important to increasing the biosynthesis and accumulation [6]. This study was conducted to investigation the effect of humic and salicylic acids application on active compounds biosynthesis and concentration of this plant.

MATERIALS AND METHODS: The field experiment was conducted at Karbala region during

2015 - 2016 growing season, the soil was a sandy loam of 50% sand, 25% silt and 25% clay with 1.3% organic matter content and pH of 7.5. Two ploughing operations were done, one by chisl to a depth of 20 to 25 cm then followed by rotary cultivator. The seeds were sown at 70 cm among rows and 25 cm among plants, the date of sowing was 25/11/2015. After emergence of the seeds completed, one plant was selected. The trail was designed in randomized complete block design (RCBD), by using three replicates. The treatments used in this study were includes four levels of humic acid were (0, 1000, 2000 and 4000 ton ha^{-1}) while other level were (0, 50, 100 and 200) mgL^{-1} of salicylic acid. Analysis of variance of the data was performed using MSTAT-C statistical program and differences between means were determined by LSD test [7]. During flowering stage the flower of plants were collected randomly and preparation to extraction.

Extraction of Flower Petal: Air dried powder of the flower petals (100 g) were defatting with hexane (500 ml) by Soxhlet apparatus till exhaustion then the mare was extracted by Soxhlet with methanol (90% , 1000 ml) till exhaustion. The extract was filtered to get rid of plant ashes, then the filtrate was concentrated to 400 ml and divided into two fractions:

The first part was evaporated by rotary evaporator dryness and taken as the total methanolic extract (TME). The other fraction was taken and 100 ml, of distilled water is ethyl acetate (3 × 100 ml). The combined ethyl acetate layers (EAE) were dried with anhydrous sodium sulphate, filtered, evaporated under vacuum and weighted. HPLC technique mainly utilizes a column that holds filling material (the stationary phase), a pump that transfers the mobile phase through the column and the detector that show the retention times of the molecules. The retention time differs depending on the interactions between the stationary phase, the molecules that analyzed, and the solvent used [8].

Sample: Ethyl acetate fraction.

Column: 3 micrometer particle size (50 × 4.6 mm ID) Shim pack C – 18.

Mobile Phase: 0.1% phosphoric acid: acetonitrile (52:42 v/v).

Detection UV set at 285 nm.

Flow rate 1.5 ml / min.

Tem: 35 ºC.

The concentration for each compound were quantitatively determine by comparison the peak area of the standard with that of the sample. The determination of fixed oil of seeds was done by expression methanol in Al-Raaed factory for plant oils production the calculated the percentage of oil of each treatment.

RESULTS: The results of this study were referred to significant effect of salicylic acid on percentage of oil with oil yield and gave height values compared with control treatment and reached to 24.20% - 22.49% and 21.14 at 100, 200 and 400 mil respectively **Table 1**.

TABLE 1: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON OIL PERCENTAGE OF SAFFLOWER PLANTS

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
19.85	21.82	21.53	21.48	14.58	S_0
21.14	23.14	23.10	22.21	16.11	S_1
24.20	28.49	24.61	23.72	19.97	S_2
22.49	23.52	23.19	23.16	20.09	S_3
	24.24	23.11	22.64	17.69	Organic Fertilizer Rate
	F × S = 1.90	F = 0.95	S = 0.95		LSD 5%

The results were accrued in **Table 2** were referred to same effect of salicylic acid at all levels 100, 200 and 50 mil and were gave 159.48 kg ha^{-1}, 133.50 kg ha^{-1} and 113.62 kg ha^{-1} at 100, 200 and 50 mil respectively. The increasing of oil percentage and oil yield may be belonging to effect of salicylic acid on biosynthesis of active compounds by smaller effect natural pressures [9]. Also the effect of salicylic acid have growth hormone effect and increasing the tolerant of plant to stress conditions such as semi-acid conditions in middle region of Iraq, these results were agreement with other

results in wheat crops was cultivated under dry environmental conditions [10], and agreement these results with other results of safflower plants were treated with salicylic acid [11]. The effect of humic acid as organic fertilizer on oil percentage and oil yield is shown in **Table l** and **2**. And the height values of both oil percentage and oil yield were obtained with 4000 mg was gave 24.24% and 154.35 Kg ha^{-1} respectively, followed by 2000 and 1000 mg levels were gave 23.11 kg ha^{-1} and 22.64 kg ha^{-1} respectively, while the lowest value of both percentage and yield of oil were obtained with control treatment without humic acid application and reached to 17.69 and 65.70 kg ha^{-1} respectively.

The effect of humic acid may be due to its effect on vegetative growth and flowers development that effect on metabolism and accumulative of carbohydrates with oil percentage and oil yield [12, 13].

TABLE 2: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON OIL YIELD OF SAFFLOWER PLANTS

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
92.06	117.68	109.03	101.82	39.72	S_0
113.62	140.73	135.70	124.15	53.92	S_1
159.48	204.20	179.14	175.24	79.34	S_2
133.50	154.80	145.23	144.19	89.80	S_3
	154.35	142.27	136.35	65.70	Organic Fertilizer Rate
	F × S = 13.31	F = 6.66	S = 6.66		LSD 5%

The results in Table **3, 4, 5** and **6** also in chronatyrphy diagram 1, 2, 3 and 4 were referred to presented four major flavonoid compounds of flowers petals extract included apigenin, kaempferol, quercetin and safflower yellow A. The results of application of all levels of salicylic acid was gave significant effect on quantity of compared with control. The height values of apigenin, kampherol, quercetin and safflower yellow A were obtain with 100 ml application of salicylic acid and reached to 16.69%, 51.21%, 15.01% and 14.11% for apigenin, kampherol, quercetin and safflower yellow A respectively, while lowest values of these compounds were obtained with control treatment (without application) and reached to 42.64%, 13.38%, 10.93% and 7.51% for apigenin, kampherol, quercetin and safflower yellow A respectively. Although the increasing the salicylic acid level to 200 $milL^{-1}$ but not a height value of flavonoids compound and reached to 16.69%, 46.96%, 13.64% and 12.26% for apigenin, kampherol, quercetin and safflower yellow A. the effect of application of salicylic acid on flavonoids compounds may be due to role of this acid in activation of biosynthesis path ways of their compounds [14], there results were in agreement with the results of using salicylic acid as flower application in vincorosa plant which gave signifycant effect on active compounds biosynthesis [15, 16].

TABLE 3: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON SAFFLOR YELLOW COMPOUND

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
7.51	8.33	6.82	9.82	5.09	S_0
9.81	18.67	8.16	9.78	2.65	S_1
14.11	12.44	26.56	12.29	5.13	S_2
12.26	13.89	8.40	11.17	15.6	S_3
	13.33	12.46	10.76	7.12	Organic Fertilizer Rate
	F × S = 0.76	F = 0.38	S = 0.38		LSD 5%

TABLE 4: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON QUERCETIN COMPOUND

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
10.93	14.56	12.2	12.54	4.41	S_0
12.43	9.86	13.69	12.47	13.72	S_1
15.01	12.10	16.59	12.43	18.91	S_2
13.64	20.24	11.88	12.88	9.65	S_3
	14.19	13.59	12.58	11.67	Organic Fertilizer Rate
	F × S = 1.44	F = 0.72	S = 0.72		LSD 5%

TABLE 5: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON APIGENIN COMPOUND

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
13.38	15.11	12.60.	12.91	13.00	S_0
14.91	19.54	13.17	17.80	9.15	S_1
16.69	19.88	13.56	16.37	16.96	S_2
15.71	8.67	23.12	15.05	15.99	S_3
	15.80	15.59	15.53	13.80	Organic Fertilizer Rate
	F × S = 1.62	F = 0.81	S = 0.81		LSD 5%

Also the effect of salicylic acid on increasing the flavonoids compounds may be belong to that acid is consider as elicitor in flavonoids biosynthesis [17]. These results were in agreement with results of application salicylic acid in eruca plant which increased the leaves active compounds [18].

The effect of salicylic acid may be belong its effect in increasing the activity of protein kinase and nitrate re-educates with reducing of proteinase that lead to increasing the growth biosynthesis and active compound accumulation [19].

TABLE 6: EFFECT OF SALICYLIC ACID, HUMIC ACID AND THEIR INTERACTION ON KAEMPFEROL

Average salicylic acid	F_3	F_2	F_1	F_0	Organic fertilizers salicylic acid
42.64	56.67	32.50	47.17	34.23	S_0
50.16	57.61	46.53	44.87	51.64	S_1
51.21	45.05	58.62	53.80	47.36	S_2
46.96	51.24	50.79	41.50	44.29	S_3
	52.64	47.11	46.84	44.38	Organic Fertilizer Rate
	F × S = 0.42	F = 0.20	S = 0.20		LSD 5%

The results of this study were referred to increasing the flavonoids compound with increasing the humic acid application compared with control without any fertilized. The 4000 mg level was gave a heights value of apigenin, kampherol, quercetin and safflower yellow A and reached to 15.80, 52.64. 14.19 and 13.33 respectively. The interaction treatment (100 salicylic acid with 4000 humic acid) was gave the height value of apigenin, interaction treatment (100 salicylic acid with 2000 humic acid) was gave height values of kaempferol, interaction treatments (200 salicylic acid with 4000 humic acid) was gave height value of quercetin while the height value of safflower A was obtained with (100 salicylic acid with 2000 humic acid) as interaction treatment. Above value were reached to 19.88, 58.62, 20.24 and 13.89 for apigenin, kampherol, quercetin and safflower yellow A respectively. The increasing of flavonoids compound by using humic acid may be due to the effect of this acid in increasing the resistance plant for stress conditions beside its effect in activation the vegetative growth, photosynthesis and biosynthesis of active compounds such as flavonoids compounds [20, 21].

The increasing of quantity of flavonoids compounds by using humic acid may be due increasing the activity of enzymes and plant hormones which lead to activation of path ways of secondary metabolites biosynthesis [22]. Also these result were in agreement with results of using humic acid in pinella plant that let to increasing the active compound [23, 24].

CONCLUSION: According the results of this study this plant need more phytochemical studies. With isolation the flavonoids compound and used in production herbal drugs especially antioxidant agent.

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