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ROLE OF FOLIAR APPLICATION WITH PLANT EXTRACTS, AND ADDING HUMIC ACID IN GROWTH OF *Calendula officinalis* AND ITS CONTENT FROM ZEAXANTHIN AND LYCOPENE

Saja N. Al-Badri *[1] Hussein A. Al-Amrani [2],
[1] Department of Horti. and Land. Gard College of Agricultural Eng. Science , University of Baghdad.
[2] Unit of Medi. and Arom. Plants Res. College of Agricultural Eng. Science , University of Baghdad

ABSTRACT

The research was conducted at Research Station, College of Agricultural Engineering Sciences - University of Baghdad during fall seasons of 2021-2022 and 2022-2023 with the aim of studying the effect of foliar application with Moringa extract, yeast suspention, and adding humic acid on the vegetative and flowering growth of marigold plant and its content of plant pigments. A factorial experiment including three factors in (RCBD) with three replicates . First factor was foliar application with Moringa leaf extract at concentrations 4,6,8 % (M1,M2,M3), as well as yeast suspension at a concentration 5 and 10 g .L^{-1} (Y1,Y2), in addition to the control treatment (Y0), and Adding humic acid to the soil at concentrations 0 and 5 ml l^{-1} (H0,H1), respectively. The results showed a significant superiority for the interaction treatment M3Y2H1 in leaf area, plant and flower dry weight, and the content of Lycopene and Zeaxanthin for both seasons. It reached 75.92 dm^2, 23.81 g $plant^{-1}$, 0.520 g flower $plant^{-1}$, 22.95 µg g^{-1}, and 47.27 µg g^{-1}, respectively, for the first season, and 68.27 dm^2, 22.03 g $plant^{-1}$, 0.477 g flower $plant^{-1}$, 17.63 µg g^{-1}, and 44.26 µg g^{-1}, respectively, for the second season compared to the control treatment.

Keywords: antioxidants, Organic farming, plant extracts, plant pigments.

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INTRODUCTION

Marigolds (*Calendula officinalis* L.) belonging to the composite family Asteraceae, is considered one of the medicinal plants. It is a perennial herbaceous plant, and there are dwarf varieties suitable for borders and as pot plants. Marigolds are grown in containers, and the compact varieties are desirable and suitable for harvesting (Al-Qaisi, and Altai, 2023). Marigolds have numerous medical uses due to the presence of many active compounds in their flowers, such as carotenoids, minerals, flavonoids, and beta-carotene, which are used in the treatment of uterine and breast cancer, throat inflammations, wound healing, and bruises and varicose veins. It used as a diluted wash for bruises and varicose veins, as well as for dermatological diseases, joint treatment, antiseptic, detoxifier, rabies, fever reducer, blood pressure reducer, headache and insomnia relief, cooling agent, blood purifier (Al-Bayati, et al . 2019 , Kadhim, 2019) . Bio-based extracts play a role in improving the quantitative and qualitative properties of medicinal plants, including marigold (Salehi, et al. 2022). In recent years, there has been an increasing use of plant extracts to supply plants with part of their nutritional needs and growth-promoting substances, contributing to reducing useing of chemical fertilizers (Alwan,

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et al, 2023, Rafiee, et al. 2016). *Moringa oleifera leaf* extract is readily available, environmentally friendly, and beneficial in integrated pest management (Al-Taweel, and Al-Anbari, 2019). It is also a major source of calcium, potassium, sulfur, zinc, magnesium, iron, copper,vitamins proteins, carotenoids, amino acids, and various phenolics (Salman, and Abdul Rasool, 2022). Abdel-Rahman and Abdel-Kader (Abdel-Rahman, et al. 2020) reported the effect of aqueous and alcoholic extracts of *Moringa oleifera* leaves on *Foeniculum vulgare*, Mill compared to Benzyladenine, was investigated. 5% aqueous Moringa extract was found to be more effective than benzyladenine, showing better results in increasing the concentration of minerals such as potassium, phosphorus, and nitrogen in the leaves, as well as chlorophyll a and b. It also increased plant height, number of branches, plant dry weight, individual plant yield, and total yield. Sardar et al. (2021) concluded the possibility of using foliar application with Moringa leaf extract to enhance growth and mineral content in *Stevia rebaudiana* Bertoni plants using different concentrations (0,10,20,30%). The treatment with 20% concentration showed the highest plant height , number leaves plant, branches number, leaf area, total chlorophyll, total phenolics, and carotenoids. It also led to an increase in leaf mineral content of nitrogen, phosphorus, potassium, calcium, zinc, iron, and magnesium compared to the control treatment. Yeast is considered a safe biological extract for plants as it is rich in auxins, gibberellins, and cytokinins (Alwan, and Sadiq. 2019, De Miccolis, et al. 2019). In addition to its content of sugars, proteins, and amino acids, as well as many vitamins including Vitamin B (Paul, 2009). It also contains numerous essential nutrients and compounds important for plant growth such as magnesium, iron, and manganese (Al-Dulaimi,and Jumaa, 2020). Hammad, and Ali (2014) found that foliar application marigold with active yeast suspension at a concentration of 6 gL^{-1} ‘;significantly improved plant height, number of lateral branches, number of leaves, both fresh and dry weights of vegetative biomass, leaf nutrient content, as well as flower diameter, number, dry weight, flowering period, total flower yield, and carotenoid content. According to Malik and Almrani (2023), when spraying peppermint (*Mentha piperita* L.) with a yeast suspension foliarly, there was a significant increase in the concentration of 7.5 gm-1, as it gave the highest rates of Volatile oil yield and some active substances, and Taih *et al.*, (2023) observed significant improvement in morphological, physiological, and floral properties, as well as plant content of active compounds, when marigold was treated with 10 g L^{-1} yeast and 1 g L^{-1} humic acid. Humic acid is one of the organic nutrients used in plant fertilization due to its content of many essential nutrients necessary for growth. Humic compounds are of great importance in plant nutrition as they are environmentally friendly, improve soil properties, and play an active role in obtaining high-quality flowers (Al-Asheebi, et al. 2020). Nassif *et al.* (2023) found that treating black cumin (*Nigella Sativa* L.) with humic acid resulted in a significant superiority of the treatment at the level of 6 g m^{-2} over humic acid treatment in terms of both single plant yield and total yield compared to the control treatment. Additionally, Salehi et al, (2022) mentioned in a study conducted on marigold plants that fertilization with humic acid had a significant effect on improving vegetative and floral growth indicators and increasing the active compounds in the plant. In the context of promoting sustainable agriculture, this research investigated the potential of foliar application with moringa extract, yeast suspension, and organic humic

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acid fertilization to enhance marigold plant growth, yield, and plant pigments contents .

MATERIALS AND METHODS

The experiment was conducted in the open field during the fall seasons of 2021-2022 and 2022-2023 at Research Station A, College of Agricultural Engineering Sciences, University of Baghdad, on marigold plants (W-Blak variety). Seeds were sown in the nursery to obtain homogeneous seedlings, which were then transplanted in the open field on 29 December, 2021, for the first season, and 29 December, 2022, for the second season. The plants were planted in rows spaced 30 cm apart and 22 cm between plants, with a density of 20 plants per experimental unit. The experiment was factorial with three factors within a (RCBD) with three replicates, including moringa extract at concentrations of 4%, 6%, and 8% (denoted as M1, M2, and M3, respectively), (Abdalla, 2013). foliar application yeast suspension at concentrations of 5 and 10 g L^{-1} (denoted as Y1 and Y2, respectes) (Taih *et al.*, (2023), and humic acid is added to soil at a concentration of 5 ml/L (denoted as H1) **(**Al-Zubaidi, and Al-Hasnawi, 2021), in addition to the control treatment for each of the three study factors. The plants were sprayed three times during the growth season, with a ten-day interval between each spraying. Additionally, all plants were fertilized with N-P-K compound fertilizer (20-20-20) at a concentration of 0.5 g L^{-1}, applied in two doses with a one-month interval. Random samples consisting 5 plants from each experimental unit were selected to measure plant height (cm), main branches number (branches per plant), leaf area (dm^2) using Digmaezer software, dry weight of plants and flowers (g), and the content of plant pigments including Lycopene µg g^{-1} (Cucu, et al. 2012)، and Zeaxanthin µg g^{-1} (Xu, et al. 2023).

RESULTS AND DISCUSSION

Vegetative growth traits

The results of **Table 1**. indicate that vegetative growth traits were significantly affected by the study factors for both seasons. The results showed a significant superiority for the treatment M3Y2H1 in leaf area (75.92 dm^2) and plant dry weight (23.81 g $plant^{-1}$) for the first season, and 68.27 dm^2 and 22.03 g $plant^{-1}$ for the second season, respectively, compared to the control treatment which recorded 35.08 dm^2 and 13.93 g $plant^{-1}$ for the first season, and 26.46 dm^2 and 12.48 g $plant^{-1}$ for the second season, respectively.

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Table 1. Effect of three-way interaction foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid on some vegetative traits of the plant for two seasons.

M* Y* H	First season 2021-2022				Second season 2022-2023			
	Plat height cm	Branch number (plant^{-1})	Leaf area(cm^2)	Plant dry weight (g plant^{-1})	Plat height cm	Branch number (plant^{-1})	Leaf area (cm^2)	Plant dry weight (g plant $^{-1}$)
M0Y0H0	35.53	5.96	35.08	13.93	32.80	4.91	26.46	12.48
M0Y0H1	42.80	6.93	44.89	18.14	40.47	6.18	38.90	15.60
M0Y1H0	37.22	5.87	38.74	14.15	35.73	5.39	29.28	12.97
M0Y1H1	48.40	7.37	46.21	17.70	44.43	6.46	38.21	15.78
M0Y2H0	45.58	7.80	41.78	16.82	42.57	6.93	33.64	15.10
M0Y2H1	52.98	9.60	61.57	20.56	51.00	8.00	53.10	19.86
M1Y0H0	43.89	6,66	40.58	15.70	42.20	5.97	32.40	14.15
M1Y0H1	46.57	7.04	52.04	18.12	42.73	6.32	43.26	17.41
M1Y1H0	40.70	7.20	40.51	15.81	39.30	6.73	33.49	14.64
M1Y1H1	43.34	7.65	59.91	19.29	42.13	6.80	51.57	18.24
M1Y2H0	40.39	8.64	52.70	17.87	39.20	7.52	44.42	16.52
M1Y2H1	45.14	10.48	67.03	21.64	42.50	8.69	57.74	20.52
M2Y0H0	50.71	7.09	43.57	17.00	46.67	6.34	37.26	15.80
M2Y0H1	52.25	8.40	64.52	20.55	49.03	7.33	57.93	20.02
M2Y1H0	50.89	8.74	56.28	19.08	47.40	7.86	47.53	17.45
M2Y1H1	52.80	10.20	71.16	21.99	49.87	8.88	62.15	20.59
M2Y2H0	49.46	9.93	63.83	20.07	46.27	8.56	55.88	18.56
M2Y2H1	56.25	11.20	73.35	23.01	51.80	9.44	64.35	21.03
M3Y0H0	48.66	7.74	52.88	18.78	45.30	7.12	49.44	17.37
M3Y0H1	53.53	9.49	69.14	22.44	51.43	8.58	60.67	21.27
M3Y1H0	49.06	9.64	64.55	20.12	46.00	8.21	56.75	18.66
M3Y1H1	52.73	11.86	74.88	23.48	49.50	11.14	67.01	21.89
M3Y2H0	53.68	11.40	69.01	21.21	51.67	9.78	61.10	19.66
M3Y2H1	56.32	12.93	75.92	23.81	52.17	11.85	68.27	22.03
LSD	N.S	N.S	2.18	0.76	N.S	N.S	1.60	0.68

The results revealed a significant influence of the study factors on the vegetative growth traits in both seasons. Results showed a significant superiority for the M3Y2 interaction treatment table 2. in plant height, number of main branches, leaf area, and plant dry weight. It recorded 55.00 cm and 12.16 branches per plant, 72.47 dm², and 22.51 g/plant^{-1} for the first season, and 51.92 cm and 10.82 branches per plant, 64.69 dm², and 20.92 g/plant^{-1} for the second season, respectively, compared to the control treatment which recorded 39.17 cm and 6.45 branches per plant, 39.98 dm², and 13.03 g/plant^{-1} for the first season, and 36.63 cm and 5.55 branches per plant, 32.68 dm², and 14.04 g/plant^{-1} for the second season, respectively. Moreover, the M3H1 interaction treatment table 2. also exhibited superiority in plant height, main branches number, and leaf area, (54.19 cm and 11.42 branches per plant, 73.31 dm²) for the first season respectively, and 51.03 cm and 10.52 branches per plant, 65.32 dm² for the second season, respectively, compared to the control treatment which recorded 39.44 cm and 6.54 branches per plant, 38.53 dm² for the first season, and 37.03 cm and 5.74 branches per plant, 29.79 dm² for the second season, respectively. Additionally, the Y2H1 interaction treatment **table 2**. outperformed in plant leaf area, at 69.47 dm² and 60.87dm² for both seasons, respectively, compared to the control treatment which recorded 43.03 dm² and 36.39 dm² for both seasons, respectively.

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Table 2. Two-ways interaction effect of foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid on some vegetative traits of the plant for two seasons

M*Y	First season 2021-2022				Second season 2022-2023			
	Plat height (Cm)	Branch number (plant^{-1})	Leaf area (cm^2)	Plant dry weight (g plant^{-1})	Plat height (cm)	Branch number(plant^{-1})	Leaf area (cm^2)	Plant dry weight (g plant^{-1})
M0Y0	39.17	6.45	39.98	16.03	36.63	5.55	32.68	14.04
M0Y1	42.81	6.62	42.47	15.93	40.08	5.92	33.75	14.38
M0Y2	49.28	8.70	51.67	18.69	46.78	7.46	43.37	17.48
M1Y0	45.23	6.85	46.31	16.91	42.47	6.14	37.83	15.78
M1Y1	42.02	7.42	50.21	17.55	40.72	6.76	42.53	16.44
M1Y2	42.76	9.56	59.87	19.75	40.85	8.10	51.08	18.52
M2Y0	51.48	7.74	54.05	18.77	47.85	6.84	47.59	17.91
M2Y1	51.85	9.47	63.72	20.53	48.63	8.37	54.84	19.02
M2Y2	52.86	10.56	68.59	21.54	49.03	9.00	60.12	19.79
M3Y0	51.10	8.61	61.01	20.61	48.37	7.85	55.05	19.32
M3Y1	50.89	10.75	69.72	21.80	47.75	9.67	61.88	20.28
M3Y2	55.00	12.16	72.47	22.51	51.92	10.82	64.69	20.92
LSD M	3.94	0.55	1.54	0.53	3.02	0.49	1.13	0.48
M* H								
M0H0	39.44	6.54	38.53	14.97	37.03	5.74	29.79	13.52
M0H1	48.06	7.96	50.89	18.80	45.30	6.88	43.41	17.08
M1H0	41.66	7.50	44.60	16.46	40.23	6.74	36.77	15.10
M1H1	45.01	8.39	59.66	19.68	42.46	7.27	50.86	18.73
M2H0	50.36	8.59	54.56	18.71	46.78	7.59	46.89	17.27
M2H1	53.77	9.93	69.68	21.85	50.23	8.55	61.48	20.55
M3H0	50.47	9.59	62.15	20.04	47.66	8.37	55.76	18.61
M3H1	54.19	11.42	73.31	23.24	51.03	10.52	65.32	21.73
LSD	3.22	0.45	1.26	N.S	2.46	0.40	0.92	N.S
Y * H								
Y0H0	44.70	6.86	43.03	16.35	41.74	6.08	36.39	14.95
Y0H1	48.79	7.96	57.65	19.81	45.92	7.10	50.19	18.58
Y1H0	44.47	7.86	50.02	17.29	42.11	7.05	41.76	15.93
Y1H1	49.32	9.27	63.04	20.61	46.48	8.32	54.74	19.13
Y2H0	47.28	9.44	56.83	18.99	44.92	8.20	48.76	17.50
Y2H1	52.67	11.05	69.47	22.25	49.37	9.49	60.87	20.86
LSD	N.S	NS	1.09	N.S	N.S	N.S	0.80	N.S

The results also indicate a significant influence of the study factors on the vegetative growth traits in both seasons **table 3**. The treatment M3 exhibited superiority in plant height, number of main branches, leaf area, and plant dry weight, recording 52.33 cm and 10.51 branches per plant, 67.73 dm², and 21.64 g plant^{-1} for the first season, and 49.34 cm and 9.45 branches per plant, 60.54 dm², and 20.17 g plant^{-1} for the second season, respectively, compared to the control treatment which recorded 43.75 cm and 7.25 branches per plant, 44.71 dm², and 16.88 g plant^{-1} for the first season, and 41.17 cm and 6.31 branches per plant, 36.60 dm², and 15.30 g plant^{-1} for the second season, respectively. Similarly, the treatment Y2 table 3. outperformed in the same traits, recording 49.97 cm and 10.24 branches per plant, 63.15 dm², and 20.62 g for the first season, and 47.15 cm and 8.84 branches per plant, 54.81 dm², and 19.18 g plant^{-1} for the second season, respectively, compared to the control treatment which recorded 46.74 cm and 7.41 branches per plant, 50.34 dm², and 18.08 g plant^{-1} for the first season, and 43.83 cm and 6.59 branches per plant, 43.29 dm², and 16.76 g plant^{-1} for the second season, respectively. Furthermore, the treatment H1 table 3. also demonstrated superiority, recording 50.26 cm and 9.43 branches per plant, 63.39 dm², and 20.89 g plant^{-1} for the first season, and 47.26 cm and

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8.30 branches per plant, 55.27 dm², and 19.52 gplant^{-1} for the second season, respectively, compared to the control treatment which recorded 45.48 cm , 8.05 branches per plant, 49.96 dm², and 17.54 g for the first season, and 42.92 cm , 7.11 branches per plant, 42.30 dm², and 16.12 g plant^{-1} for the second season, respectively.

Table 3 . Main effect of foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid on some vegetative traits of the plant for two seasons

	First season 2021-2022				Second season 2022-2023			
M	Plat height (Cm)	Branch number (plant^{-1})	Leaf area (cm^2)	Plant dry weight (g plant^{-1})	Plat height (cm)	Branch number(plant^{-1})	Leaf area (cm^2)	Plant dry weight (g plant^{-1})
M0	43.75	7.25	44.71	16.88	41.17	6.31	36.60	15.30
M1	43.34	7.94	52.13	18.07	41.34	7.00	43.81	16.91
M2	52.06	9.26	62.12	20.28	48.51	8.07	54.18	18.91
M3	52.33	10.51	67.73	21.64	49.34	9.45	60.54	20.17
LSD $_M$	2.28	0.31	0.89	0.31	1.74	0.28	0.65	0.28
Y								
Y0	46.74	7.41	50.34	18.08	43.83	6.59	43.29	16.76
Y1	46.89	8.56	56.53	18.95	44.30	7.68	48.25	17.53
Y2	49.97	10.24	63.15	20.62	47.15	8.84	54.81	19.18
LSD $_Y$	1.97	0.27	0.77	0.27	1.51	0.24	0.57	0.24
H								
H0	45.48	8.05	49.96	17.54	42.92	7.11	42.30	16.12
H1	50.26	9.43	63.39	20.89	47.26	8.30	55.27	19.52
LSD $_H$	1.61	0.22	0.63	0.22	1.23	0.20	0.46	0.20

Flower growth indicators and antioxidants

The results from **Table 4.** indicate a significant impact of the study factors on the floral growth traits and their antioxidant content in both seasons. The results showed a significant superiority for the three-ways interaction treatment M3Y2H1 in the dry weight of flowers and their content of Lycopene and zeaxanthin. It recorded 0.520 g plant^{-1}, 22.95 µg g^{-1}, and 47.27 µg g^{-1} for the first season, and 0.477 g, 17.63 µg g^{-1}, and 44.26 µg g^{-1} for the second season, respectively, compared to the control treatment which recorded 0.302 g plant^{-1}, 18.05 µg g^{-1}, and 41.17 µg g^{-1} for the first season, and 0.286 g plant^{-1}, 12.32 µg g^{-1}, and 37.09 µg g^{-1} for the second season, respectively.

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Table 4. Effect of three-ways interaction foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid on the dry weight of chrysanthemum flowers and some antioxidants for the two seasons.

M* Y* H	First season 2021-2022			Second season 2022-2023		
	Inflorescences dry weight (g plant^{-1})	Lycopene (µg g^{-1})	Zeaxanthin (µg g^{-1})	Inflorescences dry weight (g plant^{-1})	Lycopene (µg g^{-1})	Zeaxanthin (µg g^{-1})
M0Y0H0	0.302	18.05	41.17	0.286	12.32	37.09
M0Y0H1	0.356	18.80	42.35	0.300	13.02	37.87
M0Y1H0	0.310	18.34	41.99	0.241	12.62	37.58
M0Y1H1	0.362	19.17	42.95	0.327	13.34	38.43
M0Y2H0	0.369	18.52	42.64	0.340	12.84	38.14
M0Y2H1	0.435	19.91	43.59	0.413	13.98	39.33
M1Y0H0	0.327	18.93	43.21	0.261	13.23	38.80
M1Y0H1	0.358	20.16	43.92	0.318	14.24	40.02
M1Y1H0	0.339	19.85	43.89	0.285	13.78	39.78
M1Y1H1	0.396	20.95	44.29	0.372	15.23	40.64
M1Y2H0	0.385	20.77	44.40	0.358	15.03	40.87
M1Y2H1	0.464	21.08	44.86	0.416	15.43	41.65
M2Y0H0	0.378	19.48	43.50	0.308	13.56	39.03
M2Y0H1	0.453	21.31	45.02	0.380	15.66	41.86
M2Y1H0	0.416	20.39	44.17	0.339	14.58	40.25
M2Y1H1	0.489	22.03	45.73	0.454	16.45	42.92
M2Y2H0	0.445	21.71	45.36	0.372	16.03	42.37
M2Y2H1	0.512	22.26	46.27	0.468	16.94	43.69
M3Y0H0	0.407	20.56	44.64	0.334	14.83	41.15
M3Y0H1	0.479	21.54	45.18	0.442	15.88	41.96
M3Y1H0	0.427	21.90	46.04	0.349	16.21	43.64
M3Y1H1	0.501	22.70	46.57	0.462	17.27	43.93
M3Y2H0	0.471	22.16	45.62	0.431	16.50	42.67
M3Y2H1	0.520	22.95	47.27	0.477	17.63	44.26
LSD $_{M*Y*H}$	0.008	0.35	0.43	0.007	0.10	0.38

Furthermore, the results from **Table 5.** also demonstrate a significant influence of the study factors on the floral growth traits and their pigment content in both seasons. The findings revealed a significant superiority for the M3Y2 interaction treatment in the dry weight of flowers and their content of Lycopene and zeaxanthin. It recorded 0.495 g plant^{-1}, 22.55 µg g^{-1}, and 46.45 µg g^{-1} for the first season, and 0.454 g plant^{-1} and 17.07 µg g^{-1} for the second season, respectively, compared to the control treatment which recorded 0.329 g plant^{-1}, 18.43 µg g^{-1}, and 41.76 µg g^{-1} for the first season, and 0.293 g plant^{-1}, 12.67 µg g^{-1}, and 37.48 µg g^{-1} for the second season, respectively. Additionally, the treatment M3Y1 outperformed in the zeaxanthin content of flowers, recording 43.78 µg g^{-1} for the second season compared to the control treatment which recorded 41.76 µg g^{-1} for the first season, and 37.48 µg g^{-1} for the second season, respectively. Additionally, M3H1 interaction treatment table 5. outperformed in flowers dry weight and their content of Lycopene and zeaxanthin, (0.500 g plant^{-1}, 22.40 µg g^{-1} , and 46.34 µg g^{-1}) for the first season, and 0.460 g plant^{-1}, 16.93 µg g^{-1} , and 43.38 µg g^{-1} for the second season, respectively, compared to control treatment which recorded 0.327 g, 18.30 µg g^{-1} , and 41.93 µg g^{-1} for the first season, and 0.289 g plant^{-1}, 12.59 µg g^{-1} , and 37.60 µg g^{-1} for the second season, respectively. Furthermore, Y2H1 interaction treatment table 5. also showed superiority in the dry weight of flowers and their content of Lycopene,

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recording 0.483 g plant^{-1} and 21.55 µg g^{-1} for the first season, and 0.444 g^{-1} plant and 16.00 µg g^{-1} for the second season, respectively, compared to the control treatment which recorded 0.353 g plant^{-1} and 19.26 µg g^{-1} for the first season, and 0.297 g plant^{-1} and 13.49 µg g^{-1} for the second season, respectively.

Table 5. Effect of Two-ways interaction foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid and the interaction between them on the dry weight of chrysanthemum flowers and some antioxidants for the two seasons.

	First season 2021-2022			Second season 2022-2023		
M*Y	**Inflorescences dry weight (g plant^{-1})**	**Lycopene (µg g^{-1})**	**Zeaxanthin (µg g^{-1})**	**Inflorescences dry weight (g plant^{-1})**	**Lycopene (µg g^{-1})**	**Zeaxanthin (µg g^{-1})**
M0Y0	0.329	18.43	41.76	0.293	12.67	37.48
M0Y1	0.336	18.76	42.47	0.284	12.98	38.01
M0Y2	0.402	19.22	43.11	0.377	13.41	38.73
M1Y0	0.342	19.55	43.57	0.290	13.74	39.41
M1Y1	0.368	20.40	44.09	0.329	14.51	40.21
M1Y2	0.425	20.92	44.63	0.387	15.23	41.26
M2Y0	0.415	20.40	44.26	0.344	14.61	40.44
M2Y1	0.452	21.21	44.95	0.396	15.51	41.59
M2Y2	0.478	21.99	45.82	0.420	16.49	43.03
M3Y0	0.443	21.05	44.91	0.388	15.36	41.56
M3Y1	0.464	22.30	46.30	0.406	16.74	43.78
M3Y2	0.495	22.55	46.45	0.454	17.07	43.46
LSD $_{M*Y}$	0.006	0.25	0.30	0.005	0.07	0.27
M* H						
M0H0	0.327	18.30	41.93	0.289	12.59	37.60
M0H1	3.84	19.30	42.96	0.347	13.44	38.54
M1H0	0.350	19.85	43.83	0.301	14.01	39.82
M1H1	0.406	20.73	44.36	0.369	14.97	40.77
M2H0	0.413	20.53	44.34	0.340	14.72	40.55
M2H1	0.485	21.87	45.68	0.443	16.35	42.82
M3H0	4.35	21.54	45.43	0.371	15.85	42.49
M3H1	0.500	22.40	46.34	0.460	16.93	43.38
LSD $_{M*H}$	0.005	0.20	0.25	0.004	0.06	0.22
Y * H						
Y0H0	0.353	19.26	43.13	0.297	13.49	39.02
Y0H1	0.412	20.46	44.12	0.360	14.70	40.43
Y1H0	0.373	20.12	44.02	0.304	14.30	40.31
Y1H1	0.437	21.21	44.89	0.404	15.57	41.48
Y2H0	0.418	20 79	44.51	0.375	15.10	41.01
Y2H1	0.483	21.55	45.50	0.444	16.00	42.23
LSD $_{Y*H}$		0.17	0.004	0.003	0.05	N.S

Moreover, the results from **Table 6**. also indicate a significant influence of the study factors on the floral traits and their antioxidant content in both seasons. The treatment M3 demonstrated superiority in the dry weight of flowers and their content of Lycopene and zeaxanthin, recording 0.468 g plant-1, 21.97 µg g^{-1} , and 45.89 µg g^{-1} for the first season, and 0.416 g plant^{-1}, 16.39 µg g^{-1} , and 42.93 µg g^{-1} for the second season, respectively, compared to the control treatment which recorded 0.356 g plant^{-1}, 18.80 µg g^{-1} , and 42.45 µg g^{-1} for the first season, and 0.318 g plant^{-1}, 13.02 µg g^{-1} , and 38.07 µg g^{-1} for the second season, respectively. Additionally, the Y2 treatment Table 6. showed superiority in the same traits, recording 0.450 g, 21.17 µg g-1 , and 45.00 µg g^{-1} for the first season, and 0.410 g plant^{-1}, 15.55 µg g^{-1} , and 41.62 µg g^{-1} for the second season, respectively, compared

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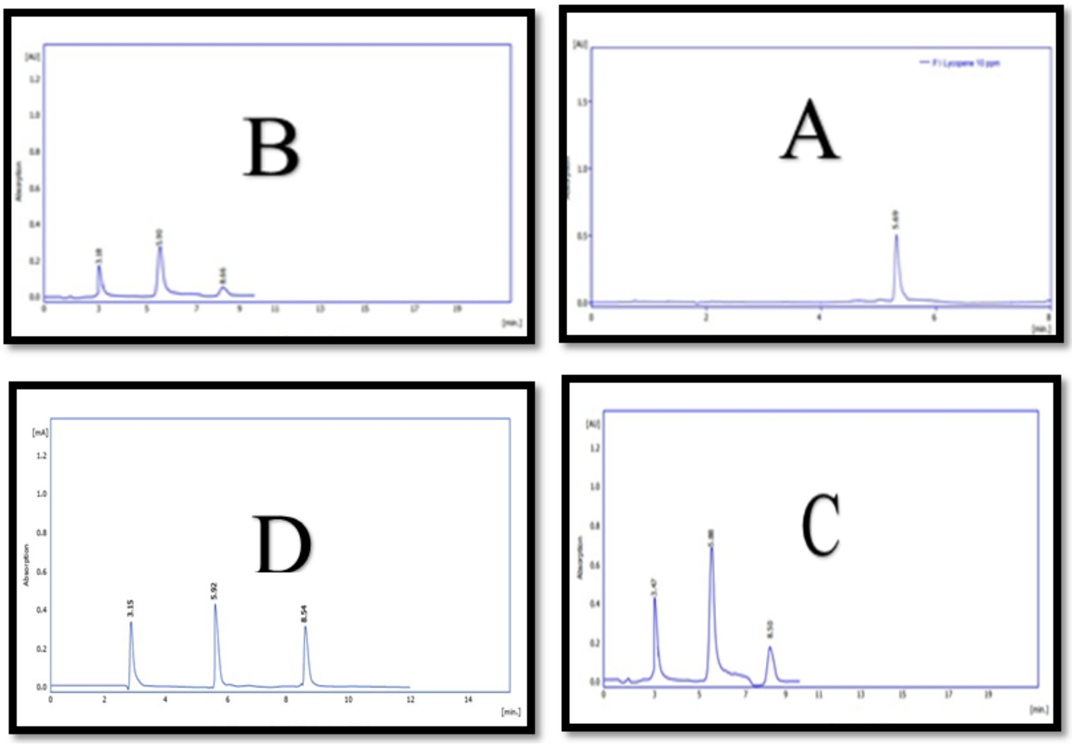
to the control treatment which recorded 0.382 g plant^{-1}, 19.86 µg g^{-1} , and 43.62 µg g^{-1} for the first season, and 0.329 g plant^{-1}, 14.09 µg g^{-1} , and 39.72 µg g^{-1} for the second season, respectively. Moreover, the H1 treatment Table 6. also outperformed in the same mentioned traits, recording 0.444 g plant^{-1}, 21.07 µg g^{-1} , and 44.83 µg g^{-1} for the first season, and 0.403 g plant^{-1}, 15.42 µg g^{-1} , and 41.38 µg g^{-1} for the second season, respectively, compared to the control treatment which recorded 0.381 g plant^{-1}, 20.05 µg g^{-1} , and 43.89 µg g^{-1} for the first season, and 0.325 g plant^{-1}, 14.29 µg g^{-1} , and 40.11 µg g^{-1} for the second season, respectively.

Table 6. Main effect of foliar application with moringa leaf extract, dry yeast suspension, and the addition of humic acid on the dry weight of chrysanthemum flowers and antioxidants for the two seasons. Content

M	First season 2021-2022			Second season 2022-2023		
	Inflorescences dry weight (g plant^{-1})	Lycopene (µg g^{-1})	Zeaxanthin (µg g^{-1})	Inflorescences dry weight (g plant^{-1})	Lycopene (µg g^{-1})	Zeaxanthin (µg g^{-1})
M0	0.356	18.80	42.45	0.318	13.02	38.07
M1	0.378	20.29	44.10	0.335	14.49	40.29
M2	0.449	21.20	45.01	0.387	15.54	41.68
M3	0.468	21.97	45.89	0.416	16.39	42.23
LSD $_M$	0.003	0.14	0.17	0.003	0.04	0.15
Y						
Y0	0.382	19.86	43.62	0.329	14.09	39.72
Y1	0.405	20.67	44.45	0.354	14.93	40.90
Y2	0.450	21.17	45.00	0.410	15.55	41.62
LSD $_Y$	0.003	0.12	0.15	0.002	0.03	0.13
H						
H0	3.810	20.05	43.89	0.325	14.29	40.11
H1	0.444	21.07	44.83	0.403	15.42	41.38
LSD $_H$	0.002	0.10	0.12	0.002	0.03	0.11



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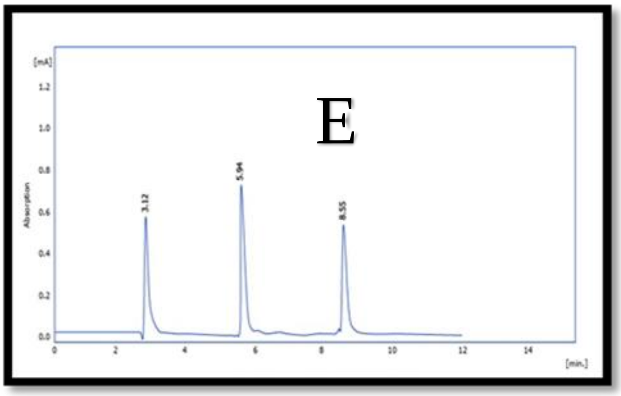
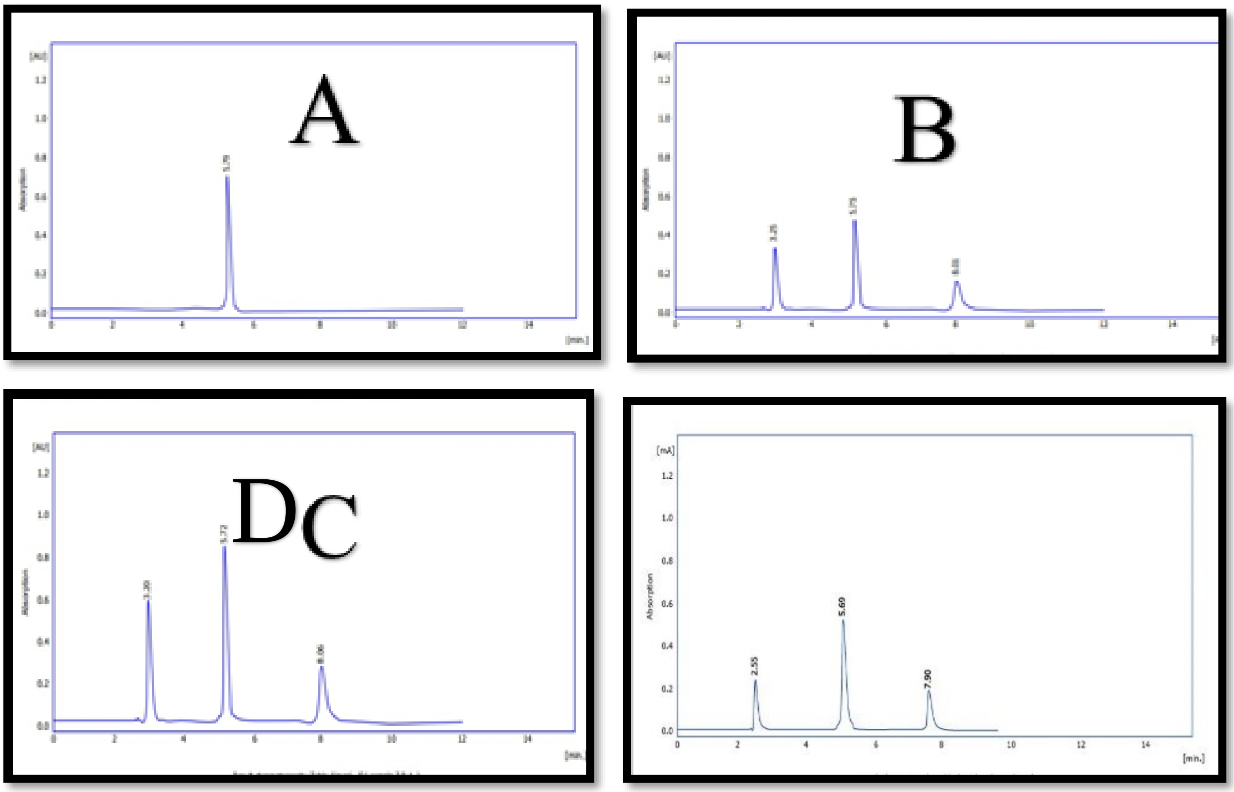


Figure 1 . HPLC chromatograms of Lycopene content in flowers of (*Calendula officinalis* L.) A-Standard(B-Control and C-Highest treatment) for the first season (D-Control and E-Highest treatment) for the second season



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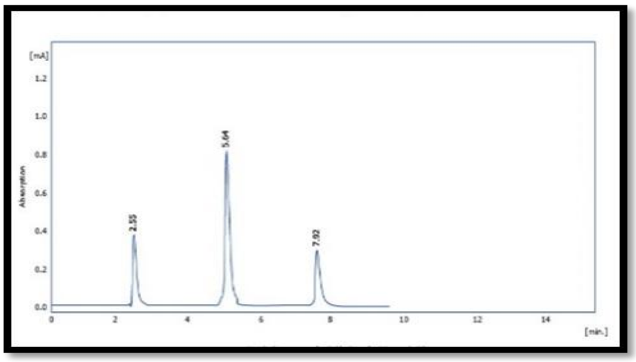
E

Figure 2 . HPLC chromatograms of Zeaxanthin content in flowers of (*Calendula officinalis* L.) A-Standard (B-Control and C-Highest treatment)) for the first season (D-Control and E-Highest treatment) for the second season

The increase in plant height when foliar application with moringa extract and yeast suspension may be attributed to their content of growth regulators. These plant hormones play a crucial role in cell division and cell expansion, leading to a balance in physiological and biological processes, enhancing carbon metabolism, and improving growth characteristics (Al-Asadi, etal. 2019). Additionally, their richness in cytokinins helps reduce apical dominance and redirect auxin flow to lateral buds, promoting their growth and increasing the number of main branches in the plant (Abdul-Hussein,et al. 2010). This may directly contribute to promoting plant growth and development, increasing leaf area, positively impacting growth traits and dry weight of the vegetative mass. This allowed for better flower growth and development, increasing their size and enabling the accumulation of abundant amounts of manufactured compounds in the flowers. Consequently, this led to an increase in their fresh weight and subsequently their dry weight. This result is consistent with Sardar *et al*,. (2021) when foliar application moringa extract on *Stevia rebaudiana* Berton plants, and with Hammadi (Hammad, and Ali. 2014) when foliar application yeast suspension on Marigolds plants. The increase in plant pigments in Marigolds flower petals may be attributed to the improvement in vegetative growth indicators such as plant height, number of main branches, and leaf area. e mass, number of flowers, and flower set percentage, while reducing damage and loss in weight. This reflects on carbon metabolism, where the breakdown of these sugars produces Acetyl CoA, the main substance in lycopene synthesis (Preedy, and Patel, 2021., Saloom, et al. 2019) Lycopene is considered one of the biological pathways for the formation of Zeaxanthin (Zafar, et al. 2021). The significant effect of ground fertilization with humic acid in improving the studied traits may be attributed to its similar role to that of plant hormones such as auxins and cytokinins, leading to increased plant height and the main branches number. Additionally, its addition has an effect on increasing leaf area (Table 1), which may be attributed to its role in enhancing the availability of elements, including potassium, important in physiological processes by enhancing enzyme



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activity, facilitating the transfer of carbon metabolism products, and promoting cell division and elongation (Oosterhuis, 2014). Moreover, it increases the levels of available nitrogen in the soil, improving the physical and chemical properties of the soil, positively affecting nutrient absorption, thus enhancing vegetative growth indicators, increasing carbon metabolism, and the accumulation of carbohydrates and proteins, thereby increasing the dry weight of the vegetative mass (Table 1). Furthermore, it improves floral growth traits and increases the dry weight of flowers (Table 2). Additionally, increasing carbon metabolism efficiency increases the production of compounds involved in the formation of antioxidants such as Lycopene and Zeaxanthin by providing the basic material for their biosynthesis (Nassif, et al. 2023). Furthermore, humic acid in the soil helped in the formation of a good vegetative and root mass, aiding in the absorption and accumulation of this nutrients in the tissues. Alternatively, the increase in photosynthetic efficiency due to the increase in leaf area positively reflected in the increase in photosynthetic products. High levels of these products increase NH_4^+ absorption due to the increased ammonia metabolism within the plant (Gad, et al. 2012). The increase in plant size and the number of branches led to an increase in the number of floral buds, as a positive correlation was found between buds number and branches number (Hasan, 2019) , The reason for the superiority of the interaction treatments may be attributed to improving the nutritional status of the plant and increasing carbon metabolism, leading to an increase in manufactured substances, which is reflected in vegetative growth indicators and then transferred to the flowers, enhancing their plant pigments.

CONCLUSION

It could be concludes from the foregoing, conclude that foliar application marigolds plants with 8% concentration of moringa extract, a yeast suspension at a concentration of 10 g L^{-1}, and adding humic acid at a concentration of 5 ml L^{-1} has a clear role in improving vegetative and floral growth indicators and floral antioxidants content. This has led to an increase in the yield of economically important flowers along with an increase in qualitative yield. Furthermore, the study treatments are environmentally friendly, which is important in reducing chemical use and environmental pollution. We recommend using the same concentrations on other medicinal plants and higher concentrations on marigolds plants.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript. Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds:

AUTHOR'S CONTRIBUTION STATEMENT: All authors contributed to the conception and design of the experiment, data collection, statistical analysis, interpretation of results, and writing of the manuscript. All

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authors reviewed and approved the final version of the manuscript.

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دور الرش بالمستخلصات النباتية واضافة حامض الهيومك في نمو نبات الاقحوان Zeaxanthin و Lycopene ومحتواه من *Calendula officinalis* L.

سجى نجيب عبد الرزاق البدري[1] حسين عنيد هميم العمراني[2]

المستخلص

أُجري البحث في المحطة البحثية A التابعة الى كلية علوم الهندسة الزراعية – جامعة بغداد في الموسمين الخريفي 2021-2022 و 2022-2023 بهدف دراسة تاثير الرش بمستخلص المورنجا والخميرة واضافة حامض الهيومك في النموالخضري والزهري لنبات الاقحوان ومحتواه من الصبغات النباتية ، نفذ البحث كتجربة عاملية ضمن تصميم R.C.B.D. بثلاثة مكررات ضمت ثلاثة عوامل للدراسة هي الرش الورقي بمستخلص أوراق المورينجا بالتراكيز4،8،6 % (M_1،M_3،M_2) فضلا عن معاملة القياس M0 على الترتيب ومعلق الخميرة بالتركيز 5،10 غم. لتر$^{-1}$ (Y_1،Y_2) فضلا عن المعاملة بدون رش Y0 وأضافة حامض الهيومك الى التربة وبتركيزين 5،0 مل. لتر$^{-1}$ (H0،H1) على الترتيب، اظهرت النتائج تفوقا معنويا لمعاملة التداخل M3Y2H1 في المساحة الورقية والوزن الجاف للنبات والازهار ومحتوى الازهار من صبغة Lycopene و Zeaxanthin لكلا الموسمين إذ بلغت 75.92 دسم^2 و 23.81 غم و 0.520 غم نورة نبات$^{-1}$ و 22.95 µg/g و 47.27 µg/g على التتابع للموسم الأول و 68.27 دسم^2 و 22.03 غم و 0.477 غم نورة نبات$^{-1}$ و 17.63 µg/g و 44.26 µg/g على التتابع للموسم الثاني مقارنة بمعاملة القياس .

كلمات مفتاحية: مضادات اكسدة، الزراعة العضوية، مستخلصات نباتية، الصبغات النباتية

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