EFFECT OF SPRAYING WITH FOLIC ACID AND YEAST EXTRACT ON THE GROWTH, YIELD AND CALCIUM OXALATE CONCENTRATION OF SPINACH (*SPINACIA OLERACEA* L.)

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Abstract. An experiment was conducted according to randomized complete block design (RCBD) with three replications during the winter growing season of 2019-2020 at the Department of Horticulture and Landscape Gardening Design, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, to investigate the response of spinach plants to different levels of exogenous application of Folic acid and yeast and their interactions in terms of growth, yield, and quality traits. The experiment included two factors the first was Folic acid (0,100 and150 mg.l^{-1}) symbolized as F0, F1 and F2, respectively. The second factor was foliar spraying with three levels of yeast extract (0,5 and10 g/l) symbolized as Y0, Y1 and Y2, respectively. According to the results the outcome of the F2 treatment (150 mg. l^{-1}) was a highly significant effect on all growth and yield traits. Also, Y1 (5 mg.l^{-1}) showed better results for all of the same characteristics except dry matter %, where Y2 treatment (10 g.l^{-1}) showed the highest value. Based on the results the F2Y1 treatment showed the best performance with a plant height of 46.47 cm, leaf width of 13.45 cm, number of leaves at 11.54 leaf.$plant^{-1}$, leaf area of 147.35 cm^2.$plant^{-1}$, oxalate at 61.36 mg.100^{-1} fresh weight, yield per plot at 8.29 kg and total yield of 27.63 ton.ha^{-1}, while F2Y2 treatment gave the highest percentage of dry matter 8.43% and the lowest percentage of nitrate 0.17%.
Keywords: *biostimulators, foliar application, vitamins, Saccharomyces cerevisiae, Chenopodiaceae*

Introduction

Leafy crops are of high nutritional value and stimulate human physiological processes (AL-Mharib et al., 2019). One of the most important crops of the Chenopodiaceae family is spinach (Spinacia oleracea L.). The cultivated area in Iraq is about 538 hectares with a total production of 3234 tons, with an average of 6.0112 ton.ha^{-1} annually (FAO, 2020). Spinach with a high content of beta-Carotene and lutein, has significant role in preventing heart diseases and cancer as it is considered to be an active antioxidant and also has a role in the health of the eyes, skin and hair, it increases immunity, and has significant roles in cell metabolism (Antunes et al., 2005). There are risks when eating spinach in large quantities due to the high accumulation of nitrates and oxalates, which may cause health problems when the permissible limit is exceeded. Al-Tayeb (2012) stated that spinach has high contents of oxalic acid, which causes its taste, its quantity was estimated at 100-400 mg.100 g^{-1} wet weight, while in some European varieties, it may reach more than 930 mg.100 gm^{-1}. There are many nutrients such as amino acids and acids of mineral elements that play an important role in the vegetative and yield traits of plants (Mariush and AL-Mharib, 2020). One of the most important sources of organic nutrients is the yeast extract (Saccharomyces cerevisiae) which had significant effects when it was applied on snap bean, summer squash and pea (Al-Amery and Mohammed, 2017; Salman and Alewi, 2017; Mohammed and Al-

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Ubaidy, 2020). Also, it showed an important role in plant alleviation under abiotic stress due to its contents of proteins, vitamins, and its ability to produce hormones such as (IAA, GA3) which significantly affect plants and symbiotic organisms (Shalaby and El-Nady, 2008; Mady, 2009). Both of Al-Khafaji (1990) and Tawfiq (2010) pointed to the importance of some chemical characters of yeast extract analysis (*Table 1*). Folic acid plays a function in delivering amino acids to their proper positions in protein chains, where it is involved in the methylation of amino and nucleic acids (Kelly, 1998; Lucock, 2004). There is a focus on using natural substances to improve the growth of plants where Folic acid has a synergistic effect on vegetative development and components of yield in many plants where it was so effective in reducing the free radicals (AL-maliky et al., 2019; Heo et al., 2019; Paucean et al., 2018; Sakr, 2009). The objective of this study was to investigate the effects of folic acid, yeast, and their interactions on growth, yield, and quality characteristics in spinach plants.

Table 1. *Chemical analysis of bread yeast extract*

Compounds*	**mg. g^{-1}**
Carb	82
Total nitrogen	90
Amino acids’ nitrogen	40
Chlorides	1-13
Phosphate	38
Sodium	56
Potassium	30
Calcium	0.1
Iron	0.05
Magnesium	2
Copper	0.05
Zinc	0.05
Manganese	0.005
Cobalt	0.005
Produced hormones**	**mcg.ml^{-1}**
IAA	29.86 in 222 nm wavelength
IAA	198 in 280 nm wavelength
GA3	799 in 254 nm wavelength

*Source: Al-Khafaji (1990)
**Source: Tawfiq (2010)

Materials and methods

An experiment was conducted according to randomized complete blocks design (RCBD) with three replications at the Department of Horticulture and Landscape Gardening Design, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, to investigate the response of spinach plants to different levels of exogenous application of Folic acid and yeast and their interactions on growth, yield, and quality traits. The experiment included two factors the first was Folic acid (0,100 and 150 mg.l^{-1}) symbolized as F0, F1 and F2, respectively. The second factor was foliar spraying with three levels of yeast extract (0,5 and10 g.l^{-1}) symbolized as Y0, Y1 and

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Y2, respectively. The field was divided into 2 m long and 1.5 m wide plots, and half a meter was left between each plot and another for the service and one meter at each 9 plots (block), the number of experimental units (plots) reached 27 plots for three replications. The spinach seeds were sown in the field directly in lines on the 21st October for the winter growing season of 2019 where the distance was of 25 cm between one line and another and each experimental unit contained 6 lines. A planted three seeds with an of average 30 kg.ha^{-1}, plant density reached 550000 plant.ha^{-1}, where the area of each experimental unit was 3 m^2, and all other cultural practices, such as irrigation and thinning out and fertilizer were uniform for all the experimental units according to (AL-Ansari, 2014). The estimation of nitrate as percentage was according to (Silveira et al., 2001) and calcium oxalate was measured according to (Mahadevan and Sridhar, 1982) and the other characters of vegetative growth and yield were estimated according to (Al-Mohammed, 2010). The collected data were analyzed statistically by Genstat statistical package software with two-way ANOVA (in Randomized Blocks) and L.S.D was used at 0.05 of probability for comparing the differences between various treatment means.

Results and discussion

The results in *Table 2* referred that spraying folic acid at F2 treatment (150 mg.l^{-1}) showed a highly significant increasing effect on plant height, leaf width, number of leaves, leaf area, dry matter, yield per plot and the total yield reached (42.98 cm.plant^{-1}, 12.6 cm.leaf^{-1}, 10.62 leaves.plant^{-1}, 129.61 cm^2.leaf^{-1}, 8.007%.leaf^{-1}, 7.75 kg.plot^{-1} and 25.843 ton.ha^{-1}) respectively. While it gave the lowest value of leaf content of oxalate, nitrate percentage in the leaf where reached (68.28 mg.100 gm^{-1} fresh weight, 0.2%.leaf^{-1}) respectively. The highest values were showed clearly in F2 treatment due to the positive effects of folic acid on the photosynthesis efficiency, activity of meristematic tissues, cell division and cell enlargement which led to increasing vegetative growth and then total yield and those results were agreed with Paucean et al. (2018) and AL-maliky et al. (2019).

***Table 2**. Effect of folic acid on growth, yield, and oxalate in spinach*

Treatments	Plant height (cm)	Leaf width (cm)	Leaves number (leaf.plant^{-1})	Leaf area (cm^2.leaf^{-1})	Dry matter %	Oxalate (mg.100 gm^{-1} fresh weight)	Nitrate %	Yield per plot (kg)	Total yield (ton.ha^{-1})
F0	39.1	11.93	9.63	117.21	7.553	73.66	0.25	6.94	23.132
F1	42.06	12.4	10.36	124.9	7.707	69.72	0.22	7.48	24.937
F2	42.98	12.6	10.62	129.61	8.007	68.28	0.2	7.75	25.843
LSD 5%	0.633	0.147	0.095	1.091	0.1142	1.215	0.021	0.153	0.5077
SD	3.825	1.083	1.148	18.67	0.54	7.623	0.0404	0.781	2.604

The results in *Table 3* showed the superiority and significant effects of yeast foliar spraying at Y1 treatment (5 gm.l^{-1}) for plant height, leaf width, number of leaves, leaf area, yield per plot and total yield which reached (45.02 cm.plant^{-1}, 13.15 cm.leaf^{-1}, 11.13 leaf.plant^{-1}, 141.51 cm^2.leaf^{-1}, 7.96 kg.plot^{-1} and 26.533 ton.ha^{-1}) respectively. While Y2 treatment showed the highest value in leaf content of dry matter and the lowest value in nitrate percentage of leaves which reached (8.24%. leaf^{-1}, 0.19%. leaf^{-1}) respectively. The mentioned characters were increased as compared with control

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treatment due to increasing the dry matter content of leaves and decreasing the nitrate content of leaves by protein synthesis where nitrate was reduced to ammonia which reacted with the keto acids which were the products of carbohydrate metabolism during photosynthesis and that was agreed with Al-Dhalimi (2017).

***Table 3**. Effect of yeast on growth, yield, and oxalate in spinach*

Treatments	Plant height (cm)	Leaf width (cm)	Leaves number (leaf.plant^{-1})	Leaf area (cm^2.leaf^{-1})	Dry matter %	Oxalate (mg.100 gm^{-1} fresh weight)	Nitrate %	Yield per plot (kg)	Total yield (ton.ha^{-1})
Y0	37.16	10.9	8.77	100.06	7.093	80.36	0.27	6.49	21.622
Y1	45.02	13.15	11.13	141.51	7.933	65.13	0.22	7.96	26.533
Y2	41.96	12.88	10.72	130.14	8.24	66.17	0.19	7.73	25.757
LSD 5%	0.633	0.147	0.095	1.091	0.1142	1.215	0.021	0.153	0.5077
SD	3.825	1.083	1.148	18.67	0.54	7.623	0.0404	0.781	2.604

The results in *Table 4* showed the superior effects of F2Y1 treatment (spraying 150 mg. l^{-1} of folic acid and 5g.l^{-1} of yeast) on the plant height, leaf width, the number of leaves, leaf area, yield per plot and the total yield reached (46.47 cm.plant^{-1}, 13.45 cm.leaf^{-1}, 11.54 leaf.plant^{-1}, 147.35 cm^2.leaf^{-1}, 8.29 kg.plot^{-1} and 27.63 ton.ha^{-1}) respectively. While it showed the lowest value of leaf content of oxalate reached 61.36 mg.100 gm^{-1} fresh weight. Also, F2Y2 treatment (spraying 150 mg. l^{-1} of folic acid and 10 g.l^{-1} of yeast) showed the highest percentage of dry matter 8.43% and the lowest percentage of nitrate 0.17% in leaves. While control treatment of interaction gave the least values for all the growth and yield indicators. The increasing values of studied characters by interaction treatments refer to the positive and significant effect of both folic acid and yeast in promoting and increasing enzyme activities and having an essential role in amino and nucleic acid synthesis, and similar results were obtained by Shalaby and El-Nady (2008), AL-Ansari (2014), Salman and Alewi (2017), Paucean et al. (2018) and Mohammed and Al-Ubaidy (2020).

***Table 4.** Effect of folic acid and yeast interaction on growth, yield, and oxalate in spinach*

Treatments	Plant height (cm)	Leaf width (cm)	Leaves number (leaf.plant^{-1})	Leaf area (cm^2.leaf^{-1})	Dry matter %	Oxalate (mg.100 gm^{-1} fresh weight)	Nitrate %	Yield per plot (kg)	Total yield (ton.ha^{-1})
F0Y0	34.45	10.6	8.11	93.54	6.91	83.67	0.29	5.82	19.387
F0Y1	42.76	12.7	10.61	134.36	7.72	69.8	0.24	7.55	25.16
F0Y2	40.09	12.5	10.18	123.72	8.03	67.52	0.22	7.46	24.85
F1Y0	38.1	10.95	8.96	101.7	6.99	79.12	0.26	6.66	22.18
F1Y1	45.83	13.3	11.23	142.81	7.87	64.22	0.22	8.04	26.81
F1Y2	42.25	12.95	10.9	130.18	8.26	65.81	0.19	7.75	25.82
F2Y0	38.92	11.15	9.25	104.95	7.38	78.28	0.25	6.99	23.3
F2Y1	46.47	13.45	11.54	147.35	8.21	61.36	0.19	8.29	27.63
F2Y2	43.54	13.2	11.07	136.53	8.43	65.19	0.17	7.98	26.6
LSD 5%	1.096	0.255	0.164	1.889	0.1978	2.104	0.036	0.264	0.8793
SD	3.825	1.083	1.148	18.67	0.54	7.623	0.0404	0.781	2.604

Table 5 referred to the positive correlation of plant height, leaf width, number of leaves, leaf area, dry matter content of leaves with yield per plot where the highest value was showed between the number of leaves and yield per plot which reached (0.9697).

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Also, all the characters were correlated negatively with both oxalate concentration and nitrate percentage in leaves. While the correlation among oxalate and nitrate was positive. The results showed the ability to decrease the leaf content of oxalate and nitrate by improving the vegetative growth characters.

Table 5. *Correlation analysis of growth and yield among studied indicators of spinach*

Plant height (cm)								
Leaf width (cm)	0.9231							
Leaves number (leaf.plant^{-1})	0.957	0.9802						
Leaf area (cm^2.leaf^{-1})	0.9569	0.9786	0.9812					
Dry matter %	0.7561	0.888	0.8673	0.8365				
Nitrate %	-0.7202	-0.8008	-0.805	-0.7607	-0.839			
Oxalate (mg.100 gm^{-1} fresh weight)	-0.92	-0.976	-0.9674	-0.9609	-0.9089	0.8002		
Yield per plot (kg)	0.9543	0.9314	0.9697	0.9429	0.8368	-0.7905	-0.9414	
	Plant height (cm)	**Leaf width (cm)**	**Leaves number (leaf.plant^{-1})**	**Leaf area (cm^2.leaf^{-1})**	**Dry matter %**	**Nitrate %**	**Oxalate (mg.100 gm^{-1} fresh weight)**	**Yield per plot (kg)**

Conclusion

According to the obtained results in the current study, we could conclude that exogenous spraying with yeast at (5 g.l^{-1}), Folic acid at (150 mg. l^{-1}) and their interaction affected positively and significantly growth, yield, and quantitative characters. Treatments effectively decreased the nitrate and oxalate content of leaves, and in addition increased the growth and yield indicators. Therefore, we recommend future studies to investigate the different levels of yeast and folic interactions to improve the qualitative and quantitative characters of spinach.

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