UPGRADING GROWTH, YIELD, AND FOLATE LEVELS OF LETTUCE VIA SALICYLIC ACID AND SPIRULINA, VERMICOMPOST AQUEOUS EXTRACTS

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ABSTRACT

The aim of this study is evaluating the impact of salicylic acid and Spirulina algae, vermicompost aqueous extracts, on lettuce (*Lactuca sativa* L.) traits. Field experiment was implemented at vegetable field of College of Agricultural Engineering Sciences - University of Baghdad during fall season 2021. The experiment was conducted using factorial arrangement within Randomized Complete Block Design with two factors and three replicates (3X3X3). Foliar spraying of salicylic acid represented the first factor (0, 150, 300 µmol.L^{-1}), which symbolized (S_0, S_1, S_2). The second factor was foliar application of distilled water, aqueous extracts of Spirulina (5g.L^{-1}) and vermicompost (2:1 v/v) which symbolized (W, P, V) respectively. The results indicated the significance of interaction treatment S_1P in producing the highest outer, inner leaves, head weight, folate content (24.67, 42.44, 916.7g, 142 µg.100 g^{-1} F.W.) respectively, in compare with control treatment S_0W (15.33, 32.67, 550g, 96.3 µg.100 g^{-1} F.W.) respectively.

Keywords: elicitation; algae; foliar application; vitamin B_9; nitrate

مجلة العلوم الزراعية العراقية -2023 :54(1):235-241 الخفاجي والجبوري

تحسين نمو وحاصل ومستويات الفولات لنبات الخس بالمعاملة بحامض السالسيلك والمستخلصات المائية للسبيرولينا وسماد الدود

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المستخلص

هدفت الدراسة الى تقييم تاثير حامض السالسيلك والمستخلص المائي لكل من طحالب السبيرولينا وسماد الدود في نمو وحاصل نبات الخس (*Lactuca sativa* L.) ، اجريت تجربة حقلية في حقول كلية علوم الهندسة الزراعية/جامعة بغداد للموسم الخريفي 2021. نفذت التجربة باستعمال تصميم القطاعات الكاملة المعشاة حسب ترتيب التجارب العاملية بعاملين وثلاث مكررات (3X3X3)، فيما يخص العامل الاول فتم رش حامض السالسيلك بثلاث مستويات (0، 150، 300 مايكرومول.لتر$^{-1}$) والذي رُمز له (S_0 وS_1 و S_2)، اما العامل الثاني فتضمن الرش بالماء المقطر، المستخلص المائي للسبيرولينا (5 غم.لتر$^{-1}$)، المستخلص المائي لسماد الدود (2:1 حجم/حجم) والذين رمز لهم (W وP و V) على التتابع، أوضحت النتائج التفوق المعنوي لمعاملة التداخل S_1P في زيادة عدد الاوراق الخارجية والداخلية ووزن الرأس ومحتوى الفولات (24.67، 42.44، 916.7 غم، 142 مايكروغم.100 غم$^{-1}$ وزن طري) على التتابع، مقارنة بمعاملة القياس S_0W (15.33، 32.67، 550 غم، 96.3 مايكروغم.100 غم$^{-1}$ وزن طري) على التتابع.

الكلمات المفتاحية: حث، طحالب، تغذية ورقية، فيتامين ب9، نترات

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INTRODUCTION

Leafy greens characterized by their exceptional properties which have been insisted by nutritionists to maintain a high level of human health (19). In compare with other leafy greens; Lettuce (*Lactuca sativa* L.) which related to Compositae family has elevated popularity all over the world (16). Lettuce is an optimal source of fibers, iron, vit. C. vit E, and carotenoids (22). Exceedingly; lettuce considered a distinctive source of folate (vit. B_9) (33). Folate deficiency very common in developing countries and this causes an evident imbalance in the human health major functions. (18) .in fact; B_9 has a vital role in DNA, protein synthesis and intact neural tube formation in human being (17). In parallel with that; there are serious scientific suspicions about the bioavailability and carcinogenicity of synthetic form of folate (folic acid) for the long term intake (15). As a result; many researches emphasized on increasing the natural bioavailable form of folate (5-MTHF) in plants (24, 25, 26, 35). Salicylic acid ($C_7H_6O_3$) is a prominent elicitor that induces genes expression for different biosynthetic pathways of phytochemical compounds. It showed significant results in phytonutrient biosynthesis in many researches (24, 29). Puthusseri et al (24) reported that spraying Salicylic acid (250 µmol) increased folate in coriander plant. Another research (25) showed that foliar application with Salicylic acid (300 µmol) improved folate content in Arabidopsis foliage. Moreover; salicylic acid increased lettuce vegetative and yield traits in many researches (7, 32). Recently; intense researches focused on natural aqueous extracts due to their safety and diversity of use in agriculture, such as using them as a safe alternative to pesticides (1, 11, 13) or as seeds invigorators (14) or as plant growth and fitness stimulators (3, 4, 28) Spirulina algae considered a plant biostimulant. It is regarded significant source of minerals, amino acids, polypeptides, phytohormones, and vitamins (20). Furthermore; Spirulina has many antioxidants such as phycocyanin (10). Hassan et al (12) noticed significant increases in growth and yield traits of arugula plant when it sprayed with Spirulina aqueous extract. Yassen et al (36) investigated that spraying Spirulina aqueous extract (2 $g.L^{-1}$) caused increases in growth and yield of lettuce traits. Vermicompost is an economical ecofriendly fertilizer that has proved its effectiveness in various agricultural crops (5, 27, 30). Vermicompost aqueous extract contains water extractable minerals and hormones that received by plants them (28). Zaller (37) results demonstrated that spraying vermicompost extract had a significant impact on fruit circumference and glucose content of tomato. Pant (21) mentioned that vermicompost extract enhanced plant yield and carotenoid content of pak choi (*Brassica Rapa*). Consequently; this study was aimed to improve growth, quality, yield traits, and folate accumulation of lettuce.

MATERIALS AND METHODS

This experiment was carried out during fall season 2021 at researches station (A) College of Agricultural Engineering Sciences, University of Baghdad (Al-Jadiryah). Table 1 shows field soil properties. The seedlings of lettuce Fajr hybrid were transplanted in four rows on terraces in 1st/November, 2021. The spacing between rows and plants was 0.3m. The plot area was $2.52m^2$.

Table 1. Physical and chemical Characteristics of the soil

Character	Values Fall 2021
Ph	7.35
$EC_{1:1}$ ($ds.m^{-1}$)	3.00
Total N ($mg\ kg^{-1}$)	35.2
P ($mg\ kg^{-1}$)	11.7
K ($mg\ kg^{-1}$)	31.8
Ca ($mg\ kg^{-1}$)	385.6
Mg ($mg\ kg^{-1}$)	222.1
Fe ($mg\ kg^{-1}$)	2.22
Na ($Meq\ L^{-1}$)	95.1
Cl^- ($Meq\ L^{-1}$)	77.2
SO_4^{-2} ($Meq\ L^{-1}$)	411
HCO_3^- ($Meq\ L^{-1}$)	495
O.M. (%)	6.99
Gypsum (%)	333
Sand (%)	28.27
Silt (%)	31.45
Clay (%)	40.28
Texture	Clay soil

Drip irrigation was set up. The recommended dose of mineral fertilizer was added for lettuce plants to all plots before planting (2). All plots harvested after 99 days of the seedlings

transplanting.The experiment was designed by using factorial arrangement within Randomized Complete Block Design with two factors and three replicates (3X3X3). Spraying three levels of salicylic acid represented the first factor (0, 150, 300 µmol.L^{-1}), which symbolized (S_0, S_1, S_2). The second factor was foliar application of distilled water, aqueous extracts of Spirulina (5g.L^{-1}) and vermicompost (2:1 v/v) which symbolized (W, P, V) respectively. The plants sprayed three times interval of 15 days. The first spraying was after one month from transplanting.

Aqueous extracts of Spirulina was prepared from *Spirulina platensis* algae according to Pise and Sabale (23) method. For the preparation of aqueous extracts of vermicompost; the method of Zaller (37) was followed. The variables that were investigated; plant height (cm), number of outer and inner leaves, leaf area (cm^2), fresh and dry head weight (g), total yield (MT.ha^{-1}), folate content (µg.100 g^{-1} F.W.) (34), nitrate content (mg.g^{-1}) (9). The obtained data were statistically analyzed by analysis of variance and the means were compared using L.S.D. test (5%probability).

RESULTS AND DISCUSSION

Plant height (cm), outer leaves number, and leaf area (cm^2): Results in Table 2 show that S_1 treatment is significantly the highest in plant height, outer leaves number, and leaf area (40.89 cm, 21.78. 272.1 cm^2) respectively, when compare with S_2 (36.21 cm, 19.11. 213.4 cm^2) and S_0 (37.78 cm, 16.89. 202.6 cm^2) respectively. The mentioned vegetative traits were superiored in response to aqueous extract of Spirulina (P) (42.22 cm, 21.67. 202.6 cm^2) respectively, and those are more than the aqueous extract of vermicompost (V) (38.22 cm, 18.67. 225.8 cm^2) and control (W) (37.11 cm, 17.22. 199.7 cm^2) (Table 2). Interaction treatments; plant height did not affected by the treatments. S_1P treatment produced the highest outer leaves number (24.67) when compare with the control (S_0W) (15.33). While the highest leaf area produced by S_1V treatment (269.0), when compare with S_0W (199.7) (Table 2).

Table 2. Effect of salicylic acid and aqueous extracts of Spirulina and vermicompost on vegetative growth traits of lettuce

traits / treatments	Plant height (cm)	outer leaves number	Leaf area (cm^2)
S_0	37.78	16.89	202.6
S_1	42.22	21.67	281.6
S_2	36.21	19.11	213.4
L.S.D.$_{(0.05)}$	0.82	0.81	12.1
W	37.11	17.22	199.7
P	40.89	21.78	272.1
V	38.22	18.67	225.8
L.S.D. $_{(0.05)}$	0.82	0.81	12.1
S_0W	36.34	15.33	169.3
S_0P	40.67	19.67	246.7
S_0V	36.33	15.68	191.6
S_1W	40.34	19.60	240.0
S_1P	44.33	24.67	235.7
S_1V	42.00	20.61	269.0
S_2W	34.67	16.66	189.7
S_2P	37.67	21.00	234.0
S_2V	36.30	19.68	216.5
L.S.D. $_{(0.05)}$	N.S.	1.41	21.0

Inner leaves number, head weight (F.W.g^{-1}), and total yield (MT.ha^{-1}): Plants that sprayed with salicylic acid (150 µmol.L^{-1}) (S_1) developed highest inner leaves number (42.44) when combare with S_2 (37.11) and S_0 treated plants (37.11, 36) respectively. S_1 produced

the highest head weight (789.9 g) and total yield (87.59 MT.ha^{-1}) in compare with S_2 (605.6 g, 67.26 MT.ha^{-1}) and S_0 (608.3 g, 67.54 MT.ha^{-1}) respectively (Table 3). Table 3 also shows that the two tested aqueous extracts (P and V) exhibit significant effect to inner leaves (41.33, 38.00), head weight (775, 633.3g), and total yield (86.04, 70.33 MT.ha^{-1}) respectively, when compare with control treatment (W) (33.29, 594.4g, 66.01 MT.ha^{-1}) respectively. It is clear from interaction treatments that all the second factor terms (W, P, and V) show superiority with salicylic acid (S_1) over control for the entire yield traits. Even more; all salicylic acid concentrations (S_0, S_1, and S_2) demonstrate superiority with aqueous extract of Spirulina (P) when compare with control for the mentioned traits (Table 3). The treatment S_1P shows the most significant influence on inner leaves, head weight, and total yield (45.35, 916.7g, 101.77 MT.ha^{-1}) respectively, in compare with control S0W (32.67, 550g, 61.07 MT.ha^{-1}) respectively (Table 3).

Table 3. Effect of salicylic acid and aqueous extracts of Spirulina and vermicompost on yield traits of lettuce

traits treatments	Inner leaves number	Head weight (fw.g^{-1})	Total yield (MT.ha^{-1})
S_0	36.00	608.3	67.54
S_1	42.44	789.9	87.59
S_2	37.11	605.6	67.26
L.S.D.$_{(0.05)}$	1.68	25.3	2.80
W	36.29	594.4	66.01
P	41.33	775.0	86.04
V	38.00	633.3	70.33
L.S.D. $_{(0.05)}$	1. 68	25.3	2.80
S_0W	32.67	550.0	61.07
S_0P	38.66	691.9	76.80
S_0V	36.67	583.3	64.77
S_1W	39.33	683.5	75.87
S_1P	45.35	916.7	101.77
S_1V	42.67	766.7	85.13
S_2W	37.00	550.5	61.10
S_2P	40.00	716.7	79.57
S_2V	34.33	550.0	61.10
L.S.D. $_{(0.05)}$	2.91	43.8	4.86

Head weight (g), folate content (µg.100 g^{-1} F.W.) and nitrate content (mg.g^{-1}): Table 4 shows that the most accumulation of dry head weight and folate is found in S_1 treatment (31.22 g, 138.2 µg.100 g^{-1} F.W.) respectively when compare with S_1 (23.78 g, 126.6 µg.100 g^{-1} F.W.), and S_0 (24.38 g, 113.1 µg.100 g^{-1} F.W.) respectively. Aqueous extract of Spirulina was produced the largest amounts of head dry weight and folate (31.22 g, 134.6 µg.100 g^{-1} F.W.) respectively, in compare with V (24.78 g, 124.2 µg.100 g^{-1} F.W.) and W (23.38 g, 119.1 µg.100 g^{-1} F.W.) respectively (Table 4). For interaction treatments; S_1P produced the highest amount of dry head weight and folate (36.67 g, 142 µg.100 g^{-1} F.W.) over the lowest amount of head dry weight that found in S_2V (21g), and lowest amount of folate that produced by control S_0W (96.3 µg.100 g^{-1} F.W.) (Table 4). In regard to nitrate content; neither salicylic acid nor interaction between salicylic acid and aqueous extracts significantly affected nitrate content. However; aqueous extracts as a single factor exhibited differences in nitrate content. Spirulina aqueous extract produced the highest content (1.657mg.g^{-1}), while the lowest content found in vermicompost aqueous extract (1.133 mg.g^{-1}) (Table 4).

Table 4. Effect of salicylic acid and aqueous extracts of Spirulina and vermicompost on quality traits of lettuce

traits treatments	Dry head weight (g^{-1})	Folate content (µg.100 g^{-1} F.W.)	Nitrate content (mg.g^{-1})
S_0	24.38	113.1	1.264
S_1	31.22	138.2	1.333
S_2	23.78	126.6	1.344
L.S.D.$_{(0.05)}$	1.14	4.0	N.S.
W	23.38	119.1	1.152
P	31.22	134.6	1.657
V	24.78	124.2	1.133
L.S.D. $_{(0.05)}$	1.14	4.0	0.164
S_0W	22.13	96.3	1.123
S_0P	28.00	128.3	1.603
S_0V	23.00	114.6	1.067
S_1W	26.67	137.6	1.167
S_1P	36.67	142.0	1.700
S_1V	30.33	135.0	1.133
S_2W	21.33	123.3	1.166
S_2P	29.00	123.0	1.667
S_2V	21.00	133.6	1.200
L.S.D. $_{(0.05)}$	1.98	7.0	N.S.

The results in tables 2, 3, and 4 that emphasize the superiority of spraying salicylic acid (150 µmol.L^{-1}) on the study traits could be due to its remarkable role in regulating plant growth and development via many process such as, thermogenesis, nutrient absorption, membrane permeability, ethylene production, stomata activity, enzymatic activity and photosynthesis (8). This led to increase plant growth and yield traits upon treatment with salicylic acid. Also; the improvements noted in folate accumulation could be due to salicylic acid work as elicitor. In fact; salicylic acid affected folate accumulation in many plants by affecting the genes expression and proteins that responsible of its biosynthesis (6). Spirulina aqueous extract was more effective and produce better results from vermicompost aqueous extract on lettuce vegetative, yield, and quality variables. This impact of Spirulina aqueous extract could result from its components such as organic nitrogen (65%) and amino acids which have a fundamental role in chlorophyll synthesis vitamins metabolism, nutrients absorption, accumulation of metabolites, and preserving the necessary protein for cellular division (31). However; nitrate accumulation in inner leaves of Spirulina treated plants could be due to Spirulina high content of nitrogen, which led to its accumulation in leaves in the form of nitrates (NO_3). The interaction between salicylic acid and Spirulina aqueous extract could be trigger synergetic effect in producing better results. In fact; salicylic acid works as a phytonutrients booster by inducing annual plants to synthesize beneficial chemicals (29). In turn; aqueous extract of Spirulina supports salicylic acid work by having polysaccharides and Lipopolysaccharides which have the same eliciting effect (6). In summary, the results of salicylic acid and Spirulina and their interaction are very promising, but not exceeding the concentrations 150 µmol.L^{-1} and 5g.L^{-1} to obtain the highest amount from folate in lettuce inner leaves, and by that; we are averting irreversible damage of common folate deficiency by consuming them.

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