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The effect of Chlorella vulgaris and Spirulina platensis on enhancing the active compounds in basil (Ocimum basilicum L.) seeds and their role in supporting human health

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ABSTRACT

In light of the global trend towards organic and sustainable agriculture, there has been an increasing interest in using microalgae as bio-stimulants and natural fertilizers as alternatives to chemical fertilizers. Algae such as *Chlorella vulgaris* and *Spirulina platensis* are considered rich sources of bioactive compounds that promote plant growth and stimulate the production of active substances. This study aims to evaluate the effect of adding microalgal extracts (*Chlorella vulgaris* and *Spirulina platensis*) on the growth of basil plants (*Ocimum basilicum* L.) and to improve their content of active compounds. Taking place in 2024 in Alhindia city, Kerbala, Iraq, The aforementioned experiment was set up using a Randomized Complete Block Design (RCBD) with three replications and a factorial arrangement. Two components were used in the study: *Spirulina platensis* algae (control, 100, 200, 250 $mL.L^{-1}$) and *Chlorella vulgaris* (control, 100, 200, 250 $mL.L^{-1}$). After the active chemical components were measured, the plants were harvested when they reached full maturity. The results show that the effects of the spray treatments with the extracts of *Spirulina platensis* and *Chlorella vulgaris* $mL.L^{-1}$ resulted in notable variations in the active component content. Camphor, Linalool, Pinene, and Myercene mg g^{-1} at concentration (250 mL. L^{-1}) had the greatest averages of 2.97, 3.31 mg g^{-1}, 23.22, 24.97 mg g^{-1}, 0.23, 0.27 mg g^{-1}, 8.29, and 8.53 mg g^{-1}, respectively.

Keywords: *Chlorella vulgaris*, *Spirulina platensis*, Basil, supporting human health.

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Introduction

Algae are microscopic organisms that play an important role in many marine and freshwater ecosystems, and they have wide applications in various fields, including sustainable agriculture. Among these algae, *spirulina* and *chlorella* are considered well-known species that contain a rich array of nutrients and active compounds, making them excellent natural sources for stimulating plant growth and improving soil fertility [1] Basil (*Ocimum basilicum*) is one of the famous aromatic plants used in many culinary and medicinal applications [2] Basil is considered an aromatic plant rich in bioactive chemical compounds with diverse biological activities. Its medicinal importance lies in its content of essential oils, which constitute the main element of its therapeutic properties, in addition to a wide range of phenolic compounds and flavonoids [3]. Essential oils are among the most important components of basil, and they include compounds such as Eugenol It has anti-inflammatory and pain-relieving properties [4], and is used in dentistry. Linalool Known for its calming and anti-anxiety effects. Methyl chavicol: It is characterized by antifungal and antibacterial properties [5]. Basil also contains compounds with high antioxidant capacity such Rosmarinic acid, Quercetin and Apigenin [6], In addition to its active compounds, basil contains:Vitamins like A, C, and K Minerals like calcium, iron, and magnesium Essential amino acid [7] Studies indicate that basil extracts contain a high percentage of antioxidants [8] that combat free radicals and reduce the risk of chronic diseases such as cancer and heart disease [9,10], which contribute to its antibacterial, antifungal, and antiviral properties. However, environmental conditions and agricultural practices can significantly affect the concentrations of these active compounds[11,12]. It focus on studying how the use of algae as plant nutrients affects the growth of basil and increases the production of effective aromatic compounds that contribute to improving its nutritional and medicinal quality [13]. It also algae affect the overall health of the soil and the environmental balance. Spirulina algae (*Spirulina platensis*) has emerged as one of the natural sources rich in proteins, vitamins, minerals, and essential amino acids, making it an important nutritional element in promoting overall health [14]. Spirulina is widely used as a dietary supplement due to its proven health benefits, such as boosting immunity and improving overall health. Additionally, recent studies have shown that the benefits of spirulina algae are not limited to humans but also extend to plants, where they have demonstrated positive effects on plant growth and increased production of active compounds in some medicinal and aromatic plants. The use of spirulina in sustainable agriculture may improve the quality of agricultural crops and increase

the concentration of active compounds in plants like basil, enhancing their medicinal and aromatic value [15]. This study aimed to highlight the environmental and economic benefits of using algae in sustainable agriculture, with a focus on improving the quality of aromatic crops such as basil using these natural ingredients.

METHODS

Taking place in 2024 in Alhindia city, Kerbala, Iraq, the current study sought to ascertain the effects of fertilizer including *Spirulina platensis* and *Chlorella vulgaris* algae on the secondary metabolism compounds of basil (*Ocimum basilicum* L.). The aforementioned experiment was set up using a Randomized Complete Block Design (RCBD) with three replications and a factorial arrangement. Two components were used in the study: *Spirulina platensis* algae (control, 100, 200, 250 $mL.L^{-1}$) and Chlorella vulgaris (control, 100, 200, 250 $mL.L^{-1}$). After the active chemical components were measured, the plants were harvested when they reached full maturity [16].Twelve kilograms of soil were placed in plastic pots measuring 32 cm in diameter and 50 cm in height, using soil samples from the city of Karbala. On March 19, 2024, 40 basil seeds were sown in plastic pots , with each seed being buried 2 cm below the surface.

Essential oil extraction method

The basil seeds were extracted after the flower heads dried and turned brown Using a Soxhlet apparatus , Put the ground basil seeds inside the thimble , with the organic solvent hexane at a concentration of 70% so that it covers the plant material (basil seeds). Heat the solvent using a heat source, allowing the solvent to extract the essential oil from the seeds. This repetition (cycle) continues for several hours until the maximum amount of oil is extracted from the basil seeds .Separating the oil from the solvent, the oil is easily separated from the solvent by using evaporation apparatuses [17]. The extracted oil is stored in dark-colored bottles to protect it from light and heat. It is preferable to store it in a cool, dry place to maintain its quality, After the oil was stored in the refrigerator, it was taken to the Center for Science and Technology to examine the active compounds (Camphor, Linalool, Pinene, and Myercene mg. g^{-1}) using Gas chromatography mass-spectrometer (GC-MS) [18].

Statistical analysis

All the recorded data were analyzed according to randomized complete block design with factorial arrangement and three replications. The Least Significant difference ($LSD_{0.05}$) test was also used for comparison and separation of means [19].

RESULTS AND DISCUSSION

Camphor mg. g^{-1}

The results Indicate in Table (1) that there are significant differences in the content of the active compound Camphor (mg g^{-1})due to the effect of the spray treatments with the extract *Chlorella vulgaris* $mL.L^{-1}$. The spray treatment (250 $mL.L^{-1}$) achieved the highest average of 3.31 mg g^{-1}, followed by the spray treatments 200 and 100, which had averages of 2.87and 2.28 mg g^{-1}, respectively. Meanwhile, the control plants achieved the lowest average of 2.14 mg g^{-1}. And observe from the same table that the spray treatments with fertilizer *Spirulina platensis* $mL.L^{-1}$ significantly outperformed the control, with a concentration of (250 mL. L^{-1}) achieving the highest average for the trait at 2.97 mg g^{-1}, while the 200 and 100 mL $.L^{-1}$ treatments recorded the lowest average in the leaf content of Camphor at 2.73 , 2.51 mg g^{-1}, compared to the control treatment 2.38 mg g^{-1}, respectively. As observe from the results, there is a significant interaction. The plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* 250 mL. L^{-1}) interaction recorded the highest average of 3.93 mg g^{-1} compared to the plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* control $mL.L^{-1}$) interaction, which recorded the lowest average of 2.02 mg g^{-1}.

Table 1. Effects *Chlorella vulgaris* and *Spirulina platensis* algae on the Camphor mg g^{-1}.

Chlorella vulgaris $mL.L^{-1}$	*Spirulina platensis* $mL.L^{-1}$				Means
	0	100	200	250	
Control	2.02	2.12	2.18	2.24	2.14
100	2.13	2.19	2.29	2.51	2.28
200	2.54	2.75	2.99	3.23	2.87
250	2.86	2.99	3.47	3.93	3.31
Means	2.38	2.51	2.73	2.97	
$LSD_{0.05}$ *Chlorella vulgaris* = 1.76, $LSD_{0.05}$ *Spirulina platensis* = 1.53, $LSD_{0.05}$ Interaction =1.58					

Linalool mg. g^{-1}

Based on the results in Table (2), that there are significant differences in the content of the active compound Linalool (mg. g^{-1}) due to the effect of the spray treatments with the extract *Chlorella vulgaris* mL.L^{-1}. The spray treatment (250 mL. L^{-1}) achieved the highest average of 24.97 mg g $^{-1}$, followed by the spray treatments 200 and 100 mL. L^{-1}, which had averages of 21.93 and 20.42 mg g $^{-1}$, respectively. Meanwhile, the control plants achieved the lowest average of 18.09 mg g $^{-1}$. And observe from the same table that the spray treatments with fertilizer *Spirulina platensis* mL.L^{-1} significantly outperformed the control, with a concentration of (250 mL.L^{-1}) achieving the highest average for the trait at 23.22 mg g $^{-1}$, while the 200 and 100 mL. L^{-1} treatments recorded the lowest average in the leaf content of Linalool at 21.93, 20.89 mg g $^{-1}$, compared to the control treatment 19.40 mg g $^{-1}$, respectively. As observe from the results, there is a significant interaction. The plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* 250 mL. L^{-1}) interaction recorded the highest average of 26.84 mg g^{-1} compared to the plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* control mL.L^{-1}) interaction, which recorded the lowest average of 16.75 mg. g^{-1}.

Table 2. Effects *Chlorella vulgaris* and *Spirulina platensis* algae on the Linalool mg g^{-1}

Chlorella vulgaris mL.**L**$^{-1}$	***Spirulina platensis*** **ml.L**$^{-1}$				Means
	control	100	200	250	
Control	16.75	17.48	18.58	19.56	18.09
100	18.64	19.56	20.74	22.74	20.42
200	19.56	21.76	22.67	23.74	21.93
250	22.56	24.76	25.73	26.84	24.97
Means	19.40	20.89	21.93	23.22	
$LSD_{0.05}$ ***Chlorella vulgaris*** =1.75, $LSD_{0.05}$ ***Spirulina platensis*** 1.48=, $LSD_{0.05}$ Interaction =1.94					

Pinene mg. g^{-1}

Based on the results in Table (3), that there are significant differences in the content of the active compound Pinene mg. g^{-1} due to the effect of the spray treatments with the extract *Chlorella vulgaris* mL.L^{-1}. The spray treatment (250 mL. L^{-1}) achieved the highest average of 0.27 mg g $^{-1}$, followed by the spray treatments 200 and 100, which had averages of 0.24 and 0.19 mg g $^{-1}$, respectively. Meanwhile, the control plants achieved the lowest average of 0.15 mg g $^{-1}$, and

observe from the same table that the spray treatments with fertilizer *Spirulina platensis* mL.L^{-1} significantly outperformed the control, with a concentration of (250 mL. L^{-1}) achieving the highest average for the trait at 0.26 mg g $^{-1}$, while the 200 and 100 treatments recorded the lowest average in the leaf content of pinene at 0.23, 0.20 mg g $^{-1}$, compared to the control treatment 0.17 mg g $^{-1}$, respectively. As observe from the results, there is a significant interaction. The plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* 250 mL. L^{-1}) interaction recorded the highest average of 0.31mg .g^{-1} compared to the plants treated with the (*Spirulina platensis* + *Chlorella vulgaris* control mL.L^{-1}) interaction, which recorded the lowest average of 0.13 mg g^{-1}.

Table 3. Effects *Chlorella vulgaris* and *Spirulina platensis* algae on the pinene mg g^{-1} .

Chlorella vulgaris mL.L^{-1}	***Spirulina platensis*** mL.L^{-1}				Means
	control	100	200	250	
Control	0.13	0.14	0.16	0.19	0.15
100	0.15	0.17	0.20	0.25	0.19
200	0.19	0.22	0.27	0.29	0.24
250	0.23	0.28	0.29	0.31	0.27
Means	0.17	0.20	0.23	0.26	
$LSD_{0.05}$ ***Chlorella vulgaris*** =2.76, $LSD_{0.05}$ ***Spirulina platensis*** 2.86=, $LSD_{0.05}$ Interaction =0.73					

Myercene mg g^{-1}

Based on the results in Table (4), that there are significant differences in the content of the active compound Myercene mg .g^{-1} due to the effect of the spray treatments with the extract *Chlorella vulgaris* mL.L^{-1}. The spray treatment (250 mL.L^{-1}) achieved the highest average of 8.53 mg g $^{-1}$, followed by the spray treatments 200 and 100 mL. L^{-1}, which had averages of 7.57and 6.19 mg g $^{-1}$, respectively. Meanwhile, the control plants achieved the lowest average of 4.44 mg g $^{-1}$. And observe from the same table that the spray treatments with fertilizer *Spirulina platensis* mL.L^{-1} significantly outperformed the control, with a concentration of (250 mL. L^{-1}) achieving the highest average for the trait at 8.29 mg g $^{-1}$, while the 200 and 100 mL. L^{-1} treatments recorded the lowest average in the leaf content of Myercene at 7.15, 6.13 mg g $^{-1}$, compared to the control treatment 5.17

mg g $^{-1}$, respectively. Based on from the results, there is a significant interaction. The plants treated with the (*Spirulina platensis* mL.L^{-1} + *Chlorella vulgaris* mL.L^{-1} 250 mL. L^{-1}) interaction recorded the highest average of 10.12mg .g^{-1} compared to the plants treated with the (*Spirulina platensis* mL.L^{-1} + *Chlorella vulgaris* control) interaction, which recorded the lowest average of 3.75 mg g^{-1}.

Table 4. Effects *Chlorella vulgaris* and *Spirulina platensis* algae on the Myercene mg g^{-1} .

Chlorella vulgaris mL.L^{-1}	***Spirulina platensis*** mL.L^{-1}				Means
	control	100	200	250	
Control	3.75	3.96	4.23	5.84	4.44
100	4.85	5.38	6.83	7.73	6.19
200	5.76	6.74	8.34	9.45	7.57
250	6.34	8.43	9.23	10.12	8.53
Means	5.17	6.13	7.15	8.29	
$LSD_{0.05}$ ***Chlorella vulgaris*** =2.87, $LSD_{0.05}$ ***Spirulina platensis*** 2.67=, $LSD_{0.05}$ Interaction =1.65					

DISCUSSION

Microalgae such as *Chlorella vulgaris* and *Spirulina platensis* are considered rich sources of active compounds that contribute to enhancing plant health. When added to the soil or used as fertilizer, these algae may affect plant growth and the formation of their active compounds [20]. *Chlorella vulgaris* shows the presence of active biological compounds such as phenols, terpenoids, and alkaloids, which are known for their antioxidant and antibacterial properties. For example, Chlorella contains the compound β-1,3-glucan, which is considered a strong immunostimulant and has anti-tumor and cholesterol-lowering effects [21]. As for *Spirulina platensis*, it contains active compounds such as phenols and flavonoids, which exhibit antioxidant and anti-diabetic effects. For example, Spirulina extracts have shown inhibitory activity against the enzyme α-glucosidase, which contributes to reducing blood sugar levels [22]. When these algae are applied to basil plants, the active compounds present in the algae may contribute to enhancing the plant's growth and improving the quality of its active compounds. For example, the content of basil in antioxidant compounds such as phenols and flavonoids may be improved, enhancing its medicinal and nutritional properties [23]. These studies show that the use of *Spirulina platensis* may be more

effective than Chlorella vulgaris in enhancing the active compounds in basil, especially concerning phenolic and flavonoid compounds and antioxidant activity. However, it is recommended to conduct practical experiments to determine the appropriate doses and the best application methods to ensure optimal results [24,25].

CONCLUSIONS

Studies show that using *Chlorella vulgaris* and *Spirulina platensis* as agricultural additives may improve the quality of active compounds in basil, contributing to enhancing its health benefits. However, it is recommended to conduct practical experiments to determine the appropriate dosages and the best application methods to ensure optimal results.

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