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BIOFERTILIZERS EFFECTS ON THE ACTIVE COMPOUNDS OF SWEET BASIL (*OCIMUM BASILICUM* L.)

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SUMMARY

The progressive study aimed to determine the effects of biofertilizers (*Azotobacter* and *Pseudomonas*) on active chemical compounds of sweet basil (*Ocimum basilicum* L.), carried out in 2023 at the Afghan City, Kerbala, Iraq. The experiment set out in a randomized complete block design (RCBD) with a factorial arrangement and three replications. The study comprised two factors: the first was *Azotobacte*r (control, 50, 100, 150 g/bacteria), and the second factor was *Pseudomonas* (control, 50, 100, 150 g/bacteria). Both biofertilizers attained mixing with seeds before planting. Results revealed significant differences among the various concentrations of *Azotobacter and Pseudomonas* and their interactions. *Azotobacte*r and *Pseudomonas* treatment with same dilution (150 g bacteria^{-1}) provided the highest mean values for active chemical compounds in the essential oil, i.e., camphor (3.70 and 4.56 mg g^{-1}), linalool (24.83 and 24.90 mg g^{-1}), pinene (1.09 and 1.38 mg g^{-1}), myrcene (13.64 and 12.84 mg g^{-1}), and limonene (18.16 and 17.76 mg g-^{1}), respectively.

Keywords: Sweet basil (*Ocimum basilicum* L.), *Pseudomonas*, *Azotobacter*, active compounds

Key finding: Biofertilizers (*Azotobacter* and *Pseudomonas*) and their interactions enunciated considerable differences for active chemical compounds. *Azotobacter* and *Pseudomonas* with same concentration (150 g/bacteria) produced the highest mean values in the essential of sweet basil (*Ocimum basilicum* L.) for camphor (3.70 and 4.56 mg g^{-1}), linalool (24.83 and 24.90 mg g^{-1}), pinene (1.09 and 1.38 mg g^{-1}), myrcene (13.64 and 12.84 mg g^{-1}), and limonene (18.16 and 17.76 mg g^{-1}), respectively.

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INTRODUCTION

The sweet basil (*Ocimum basilicum* L.) plant is a member of the family Lamiaceae, commonly known as mint, and a healing plant family because it contains about 150 aromatic species that differ in leaf size, flower color, outward characteristics, and flavor. Africa and Asia are the original home of basil (Al-Ibrahemi *et al*., 2023); however, it is also present in the Middle East, America, and all countries worldwide. Many family members have widespread cultivation, not only for their aromatic qualities but also their ease of cultivation, which is ready for propagation by stem cuttings.

In addition to propagating, its seeds grow in well-drained soil and less water. The pH range of the dirt should be between 6.0 and 7.5. Commercial fertilizer can help fertilize the ground once or twice during the growing season. Basil is also a plant sensitive to environmental conditions, and cold weather can kill these plants; thus, the right timing for planting is crucial (Bączek *et al.,* 2019).

Plant growth biofertilizers are one of many essential requirements vital in reducing environmental pollution and maintaining biological balance, improving soil properties, root water absorption, and enhancing organic matter content for physiological activities (Sharma, 2002). Biofertilizers help develop growth hormones, such as, gibberellins, auxinates, and cytokinins, contributing directly to plant growth and development (Vessey, 2003). Biofertilizers, whether bacterial, fungal, or both, are beneficial after their isolation, expansion, and preparation as a vaccine added to the soil or used with seed treatment (Berg *et al*., 2002).

Some microorganisms confirmed nitrogen symbiotically, estimated to be about 10^{11} N g on land per year, and about 61% of the nitrogen reached the soil, as verified by the bacteria characterized of the presence of nitrogen enzymes (Jarak *et al*., 2006; Al-Tamimi and Farhood, 2022; Makenova *et al*., 2023). Biomagnifications consist of the isolation, purification, and characterization of various microorganisms added to the central growth of plants to enhance their nutrient absorption as the microbial content in the rhizosphere area changes. However, its success depends on the efficiency of the microorganisms used, their compatibility with the vegetarian family, and their competitiveness with the organisms found in the soil, with the viability of the biota in the rhizosphere area (Kader *et al*., 2002).

The process involves maximizing the use of microorganisms that help improve the soil's physical, chemical, and vital properties and maintain balanced nutrients in agricultural lands, converting them into ready-made nutrients for plant feeding (Al-Haddad, 1998). Antibiotics help to resist some endemic diseases in the soil and benefit the plant and its production. Bioaccumulation can aid in reducing the dependence on mineral fertilizers, providing a large part of the nutrients required for plant feeding, resulting in lower production costs and environmental pollution rates (Al-Rawi, 2010).

One of the microorganisms that establish nitrogen is *Azotobacter*, an independent aerobic bacterium that can stabilize atmospheric nitrogen and produce several beneficial organic compounds, i.e., glaciers, cytokines, vitamins, hormones, and enzymes (Al-Balkhi, 1990). *Pseudomonas* is one of the best species of bacteria to stimulate plant growth, considerably enhancing the availability of soil nutrients (Sharma, 2002). Medicinal and aromatic plants have eminent economic values due to the high demand for their products, whether from local or foreign markets.

Basil is one of the most remarkable herbs in these aromatic and medicinal plants (Alakashy and Al-Bedairi, 2020a, b). Basil has many uses as a medicinal plant to treat many diseases, such as, headaches, coughs, worms, gastroenteritis, malaria, and obesity, and to reduce infections and colds. Also, basil can enhance food flavors, with its leaves eaten as an appetizer and salad. Basil can serve industrial purposes to make spices, vinegar, and pickles (Al-Ibrahemi *et al*., 2020; Abdel-Hamid, 2020). The prevailing study determined the effects of biofertilizers (*Azotobacter* and *Pseudomonas)* to activate the chemical compounds in sweet basil (*Ocimum basilicum* L.).

MATERIALS AND METHODS

The present study aimed to determine the effects of biofertilizers (*Azotobacter* and *Pseudomonas*) on active chemical compounds of sweet basil (*Ocimum basilicum* L.), carried out in 2023 at the Afghan City, Kerbala, Iraq. The experiment set out in a randomized complete block design (RCBD) with factorial arrangement and three replications. The two factors comprised the *Azotobacte*r (control, 50, 100, 150 g/bacteria), while the second factor was *Pseudomonas* (control, 50, 100, 150 g/bacteria). The basil seeds' planting ensued after mixing them with biofertilizers. The plants' harvesting at full maturity continued after completing the active chemical compounds' measurement.

The samples of the blended soil of Kerbala City, Iraq, served as planting media, placed in plastic pots with a 32 cm diameter and a 50 cm height, containing 12 kg of the soil. Estimating the soil's vital physical and chemical characteristics employed the procedures outlined in Table 1. The Basil Sweet seeds commenced planting in the plastic pots with soil on March 24, 2023, with 50 seeds placed in each pot at a depth of 3 cm.

Bacterial inoculation using *Azotobacter* and *Pseudomonas* transpired once throughout the growing season. The procedure involved creating a 1-cm incision near the rhizosphere region, followed by the subsequent application of bacterial inoculation. The harvesting procedure progressed 40–55 days after the crop's complete maturation, as documented by Hussain *et al*. (2023).

Essential oil extraction method

After the complete harvest collecting the leaves, a drying process continued within a well-aired environment. Subsequently, the dried leaves sustained careful grinding to a desired consistency. Using a Soxhlet apparatus, 100 g of finely powdered leaves went into a thimble, later combined with 300 ml of ether solution containing 80% ether concentration. Transfer of the mixture ensued into a round-bottom flask with a volume of 1000 ml. The extraction process ran for 20 h to effectively separate alcohol from the essential oil. The extract sustained evaporation at a temperature of 45 °C using the rotary evaporation equipment (AL-Ibrahemi *et al*., 2022).

Statistical analysis

Analyzing all the recorded data was according to the RCBD with factorial arrangement and three replications. The Least Significant Difference ($LSD_{0.05}$) test also helped compare and separate means.

RESULTS AND DISCUSSION

Camphor (mg g^{-1})

Results revealed significant differences occurred among the biofertilizers (*Azotobacter* and *Pseudomonas*) concentrations and their interactions with camphor in sweet basil (*Ocimum basilicum* L.) (Table 2). *Azotobacter*

Table 1. Chemical properties of the soil used for the experiment on sweet basil (*Ocimum basilicum* L.).

Chemical Attributes	Values
pH	7.54
K	2.27 mm L^{-1}
Ca	7.47 mm L^{-1}
Na	2.73 mm L^{-1}
S	2.49 mm L-1
Mg	2.63 mm L^{-1}
NH_4N	24.57 mg kg^{-1}
Cl	4.88 mm L^{-1}
Sand (g kg^{-1} soil)	310
Silt (g kg^{-1} soil)	340
Clay (g kg^{-1} soil)	300

Table 2. Effect of *Azotobacter*, *pseudomonas*, and their interaction on Camphor (mg g^{-1}) in sweet basil (*Ocimum basilicum* L.).

Azotobacter (g $bacteria^{-1}$)	*Pseudomonas* (g $bacteria^{-1}$)				Means
	0	50	100	150	
0	1.07	2.39	2.97	3.87	2.58
50	1.45	2.75	3.65	4.32	3.04
100	1.67	2.96	3.85	4.58	3.27
150	1.87	3.25	4.38	5.29	3.70
Means	1.56	2.84	3.71	4.56	

$LSD_{0.05}$ *Pseudomonas* = 0.11, $LSD_{0.05}$ *Azotobacter* = 0.11, $LSD_{0.05}$ P × A Interaction =1.76

Table 3. Effect of *Azotobacter* and *pseudomonas* and their interaction on Linalool (mg g^{-1}) in sweet basil (*Ocimum basilicum* L.).

Azotobacter (g $bacteria^{-1}$)	*Pseudomonas* (g $bacteria^{-1}$)				Means
	0	50	100	150	
0	18.87	20.45	21.45	22.76	20.88
50	19.67	21.76	22.45	23.63	21.88
100	20.74	22.74	23.45	24.74	22.92
150	21.45	23.65	25.76	28.45	24.83
Means	20.18	22.15	23.28	24.90	

$LSD_{0.05}$ *Pseudomonas* = 1.02, $LSD_{0.05}$ *Azotobacter* = 1.02, $LSD_{0.05}$ P × A Interaction =1.16

treatment at the concentration 150 g/bacteria provided the highest camphor content (3.70 mg g^{-1}) compared with other concentrations, i.e., 100, 50, and the control (3.27, 3.04, and 2.58 mg g^{-1}, respectively). The *pseudomonas* treatment at 150 g/bacteria concentration produced the highest camphor content (4.56 mg g^{-1}) compared with the 100, 50, and control treatments (3.71, 2.84, and 1.56 mg g^{-1}, respectively). The interaction among both biofertilizers indicated the superiority of the *Azotobacter* and *pseudomonas* at the same dose (150 g/bacteria) in producing camphor (5.29 mg g^{-1}). However, the interaction of the control (*Azotobacter* and *pseudomonas*) showed the lowest estimate of the camphor (1.07 mg g^{-1}). An increase in the active chemical compounds of the sweet basil plant may be due to the effect of the venom. The efficient absorption of food elements and the construction of a radical sum are highly efficient in absorption. The food elements influenced the division and growth of the mitochondrial cells. Also, they increase the surface area of the leaves, the supply of food manufactured in carbohydrates and proteins, and their transfer to other plant parts where needed. Therefore, the necessity to build the plant tissues vigorously will reflect in enhancing dry matter (Zahwan *et al*., 2013). Alakashy and Al-Bedairi (2020a, b) suggested that treating *Ocimum basilicum* L. with the bacteria *Azotobacter* reflected a significant effect on the oil ratio (Camphor, Linalool, Pinene, Myrcene, and Limonine).

Linalool (mg g^{-1})

In the sweet basil, the biofertilizer *Azotobacter* treatment (150 g/bacteria) provided the highest mean of linalool (24.83 mg g^{-1}) compared with two other concentrations (100 and 50 g/bacteria), and the control treatment (22.92, 21.88, and 20.88 mg g^{-1}, respectively) (Table 3). The *pseudomonas* treatment (150 g/bacteria) also produced the highest content of linalool (24.90 mg/g^{-1}) compared with other lower concentrations (100 and 50 g/bacteria), and the control treatment (23.28, 22.15, and 20.18 mg/g^{-1}, respectively). The interaction of both biofertilizers (*Azotobacter* and *pseudomonas*) with the same concentration (150 g/bacteria) boosted the linalool content (28.45 mg g^{-1}). However, the interaction of the

control (*Azotobacter* and *pseudomonas*) treatments showed the lowest estimate of linalool (18.87 mg g^{-1}) in sweet basil. The observed elevation in the said active chemical within the oil could be attributable to heightened chlorophyll levels and improved effectiveness of the photosynthetic process (Befrozfar *et al.,* 2013). By treating the *Linum usitatissimum* L. with biofertilizer *Pseudomonas* bacterium, a significant effect appeared on the oil ratio with different active chemical compounds like linoleic acid, linolenic acid, and oleic acid (Al-Sudani and Al-Baldawi, 2018).

Pinene (mg g^{-1})

The biofertilizer *Azotobacter* treatment at the concentration of 150 g/bacteria produced the highest mean of pinene (1.09 mg g^{-1}) versus other lower concentrations (100 and 50 g/bacteria) and the control treatment (0.80, 0.68, and 0.50 mg g^{-1}, respectively) in the sweet basil (Table 4). The *pseudomonas* treatment at 150 g/bacteria also showed the highest content of the active compound pinene (1.39 mg g^{-1}) compared with the lowest doses of the same biofertilizer (100 and 50 g/bacteria) and control treatment (0.92, 0.45, and 0.32 mg $g^{-1,}$ respectively). The interaction of *Azotobacter* and *Pseudomonas* indicated the superiority with the same dose (150 g/bacteria) (1.76 mg g^{-1}). However, the reactions of the control treatments showed the lowest estimate of the pinene (0.15 mg g^{-1}) in the sweet basil. Through the microbiology role that directly equips the plant from its nitrogen component NH_4, which amino acids precisely represent, and after its structural and biological part within the plant cell, amino acids enter a destructive route in the cytosol to produce a pyruvate compound, which turns into two units of Acetyl-CoA. The Acetyl-CoA enters the acetate-mevalonate pathway to produce an Isopentenyl pyrophosphate, effectively building the active chemical compounds (Daoudi, 1991). The findings of Hellal *et al*. (2011) also revealed a significant increase in oil yield and active chemical components when treating *Anethum graveolens* with *Azotobacter* and *Pseudomonas.*

Myrcene (mg g^{-1})

In sweet basil, the *Azotobacter* treatment at 150 g/bacteria gave the highest mean of myrcene (13.64 mg g^{-1}) compared with its lowest concentrations (100 and 50 g/bacteria), and the control treatment (10.34, 8.56, and 6.47 mg g^{-1}, respectively) (Table 5). The *Pseudomonas* treatment at the concentration of 150 g/bacteria also provided the highest content of myrcene (12.84 mg g^{-1}) versus its two other concentrations (100 and 50 g/bacteria), and the control treatment (10.79, 8.93, and 6.43 mg g^{-1}, respectively). The interchange of *Azotobacter* and *Pseudomonas* (150 g/bacteria) also implied superiority (17.98 mg g^{-1}); however, the interaction of the control treatments showed the lowest estimate of the myrcene (4.34 mg g^{-1}) in sweet basil (*Ocimum basilicum* L.). Biofertilization leads to the manufacture of foodstuffs within the plant fabric of carbohydrates, leading to an increase in the active compounds through the entry of carbohydrates into sugar degradation and the production of pyruvic acid, which is the key to producing isoprene units, which work as basic units for the formation of volatile oil (Muráriková *et al*., 2017).

Table 4. Effect of *Azotobacter and pseudomonas* and their interaction on Pinene (mg g^{-1}) in sweet basil (*Ocimum basilicum* L.).

Azotobacter (g bacteria^{-1})	*Pseudomonas* (g bacteria^{-1})				Means
	0	50	100	150	
0	0.15	0.21	0.66	0.99	0.50
50	0.20	0.33	0.84	1.35	0.68
100	0.41	0.55	0.77	1.45	0.80
150	0.51	0.69	1.41	1.76	1.09
Means	0.32	0.45	0.92	1.39	

$LSD_{0.05}$ *Pseudomonas* = 1.45, $LSD_{0.05}$ *Azotobacter* = 1.45, $LSD_{0.05}$ P × A interaction =2.75

Table 5. Effect of *Azotobacter, pseudomonas*, and their interaction on Myrcene (mg g^{-1}) in sweet basil (*Ocimum basilicum* L.).

Azotobacter (g $bacteria^{-1}$)	*Pseudomonas* (g $bacteria^{-1}$)				Means
	0	50	100	150	
0	4.34	5.56	6.98	8.98	6.47
50	5.87	7.85	9.76	10.74	8.56
100	6.56	9.45	11.67	13.67	10.34
150	8.94	12.86	14.76	17.98	13.64
Means	6.43	8.93	10.79	12.84	

$LSD_{0.05}$ *Pseudomonas* = 1.07, $LSD_{0.05}$ *Azotobacter* = 1.07, $LSD_{0.05}$ P × A interaction =1.79

Table 6. Effect of *Azotobacter*, *pseudomonas,* and their interaction on Limonine (mg g^{-1}) in sweet basil (*Ocimum basilicum* L.).

Azotobacter (g $bacteria^{-1}$)	*Pseudomonas* (g $bacteria^{-1}$)				Means
	0	50	100	150	
0	4.87	6.98	10.86	12.85	8.89
50	7.87	8.67	12.87	14.87	11.07
100	8.23	13.98	16.67	19.56	14.61
150	11.76	16.87	20.26	23.76	18.16
Means	8.18	11.63	15.17	17.76	

$LSD_{0.05}$ *Pseudomonas* = 1.56, $LSD_{0.05}$ *Azotobacter* = 1.56, $LSD_{0.05}$ P × A interaction =2.03

Limonine (mg g^{-1})

The *Azotobacter* treatment (150 g/bacteria) revealed the highest mean of limonine (18.16 mg g^{-1}) compared with the other concentrations (100 and 50 g/bacteria), and the control treatment (14.61, 11.07, and 8.89 mg g^{-1}, respectively) in sweet basil (*Ocimum basilicum* L.) (Table 6). The *Pseudomonas* treatment at 150 g/bacteria also showed the maximum content of limonine (17.76 mg g^{-1}) compared with its lowest doses (100 and 50 g/bacteria), and the control treatment (15.17, 11.63, and 8.18 mg g^{-1}, respectively). The interaction of both biofertilizers (*Azotobacter* and *Pseudomonas*) with the same dilution (150 g/bacteria) indicated the dominance of limonine content (23.76 mg g^{-1}). However, the interchange of the control treatments showed the lowest estimate of the limonine (4.87 mg g^{-1}). In a laboratory experiment, the sweet basil (*Ocimum basilicum* L.) seeds' treatment with bacteria *Bacillus subtilis* stimulated the production of oil-efficient compounds Eugenol and R-Terpineol (Banchio *et al*., 2009). The coriander (*Coriandrum sativum* L.), according to Heidari and Golpayegani (2012), when applied with the *Azotobacter*, the seeds, demonstrated notable impacts on the proportion of volatile oil. Darzi *et al*. (2012) also enunciated that by treating the *Coriandrum sativum* plant with *Azotobacter*, *Pseudomonas,* and *Azopirillum,* there emerged a significant increase in active chemical components.

CONCLUSIONS

The growth of the sweet basil (*Ocimum basilicum* L.) was considerably better by treating its seeds with biofertilizers. *Azotobacter* treatment at the concentration of 150 g/bacteria gave the highest mean of chemical compounds, limonine and myrcene, while *Pseudomonas* treatment at 150 g/bacteria provided the highest content of camphor, linalool, and pinene.

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