Study the Effect of the Boron Nano and *Pseudomonas* on Chemical Parameters and the Active Compounds of Basil (*Ocimum basilicum* L.)

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ABSTRACT

Background: Basil (*Ocimum basilicum* L) is a fragrant medicinal herb belonged to the Lamiaceae family, cultivated as a important economic and ornamental plant. Its leaves are used as a spice in cooking as well as for food preservation. The plant has considerable nutritional and medicinal values. This study was carried out in Al-Najaf Al-Ashraf Province, Iraq, to explore how to improve the chemical and bioactive components of basil. The research aimed to evaluate the influence of soil inoculation with *Pseudomonas* bacteria and foliar application of nano-boron as a sustainable agricultural practice to improve the plant quality and its secondary metabolites.

Methods: The experiment with three replications, was designed as a factorial trial within a randomized complete block design (RCBD). Two main factors were studied. The first factor was the soil inoculation with the *Pseudomonas* bacteria at four different concentrations of control, 25, 125 and 200 mg/mL. The second factor involved the foliar application of nano-boron, also at four concentrations of control, 80, 150 and 250 mg/L. The study assessed the effects of these treatments on some chemical parameters, comprising nitrogen and carbohydrates and on important bioactive compounds in basil, such as linalool, camphor and pinene.

Result: The application of nano-boron and *Pseudomonas*, both separately as well as in combination, showed high improvements in all measured parameters. The most favorable results were found with the highest concentration levels of both treatments. Specifically, the combined application of 200 mg/mL of *Pseudomonas* and 250 mg/L of nano-boron produced the highest average values. This interaction led to camphor levels of 7.83 mg/g, pinene at 0.83 mg/g, linalool at 27.84 mg/g, carbohydrates at 21.74 mg/g and nitrogen content at 1.75 mg/g. These findings highlight a robust synergistic effect between the nano-fertilizer (nano-boron) and the biofertilizer (*Pseudomonas*), representing their potential to improve the productivity and phytochemical content of basil.

Key words: Basil, Bioactive compounds, Boron, Nano, *Pseudomonas*.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a green foliage fragrant medicinal herb in the Lamiaceae family. It is a tropical plant which is grown as an economically important crop plant and as an ornamental house plant in home gardens or containers (El-Shahat *et al.,* 2017). Its dried leaves are a spice and seasoning agent in cooking and food preservation. Basil is of great nutritional and medicinal importance, as it contains a high proportion of major elements, that is, Ca, K, P and N and minor elements, that is, Fe, Zn and Cu (Abdel-Hamid, 2020).

Nanofertilizers are important in agriculture to improve plant growth and productivity. Nanofertilizers are effective in managing micronutrients. They increase plant growth and may even inhibit it. Growth is due to the toxicity of nutrients, which provides space for metabolic reactions (Glotra and Singh, 2023; Muteab and AL-Abedy, 2025). They also increase the rate of photosynthesis. Boron is an essential nutrient for basil plants. Boron is added to the soil or sprayed onto the leaves or green parts of the plant, leading to increased plant growth and productivity (Roberts *et al.,* 2000). Among its functions are improving biological activity, enzymatic reactions and photosynthesis and it participates in protein synthesis and DNA formation (Niaz *et al.,* 2002).

The use of biofertilizers is one of the methods used to improve soil fertility and reduce production costs, in addition to reducing the risks associated with mineral fertilizers (Al-Yasssiry *et al.,* 2024). The casual use of chemical fertilizers has resulted in the accumulation of pollutant and metallic elements that have reduced the quality of soil and had negative effects on the health of plant production. To remedy this problem, scientists began to develop bio-fertilizers, which contribute to essential attendance of plants of nitrogen, phosphorous and potassium, as well as they secrete certain hormones and acids conscious-titled plant growth regulators (Al-Sudani and Al-Baldawi, 2018; Al-Masaoodi *et al.,* 2025). *Pseudomonas* is a non-commensal aerobic bacterium capable of atmospheric nitrogen fixation and producing a

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variety of regulators, primarily glacial rivers, cytokinins, vitamins, hormones and enzymes. It is among the microorganisms that produce nitrogen (Sharma, 2002). Due to its ability to increase nutrient availability in the soil, *Pseudomonas* is considered one of the best types of bacteria for promoting plant growth (Al-Rawi, 2010).

The aim of this study was to investigate the independent and interactive effects of Pseudomonas bacterial inoculation and foliar application of nano-boron on the levels of nitrogen and carbohydrate and on the accumulation of important secondary metabolites like camphor, linlaool and pinene (Al-Isawi, 2021).

MATERIALS AND METHODS

A field experiment was conducted in an agricultural field within Najaf Governorate during the 2024 growing season, using clay loam soil. The experiment followed a factorial arrangement in a randomized complete block design (RCBD) with three replicates, incorporating two factors. The first factor involved soil inoculation with *Pseudomonas aeruginosa* bacteria at four concentrations (control treatment: 25, 125 and 200 mg/mL). The second factor consisted of foliar spraying with nano-boron at three concentrations (control treatment: 80, 150 and 250 mg/L).

The prepared soil was placed into plastic pots, each 32 cm in diameter and 50 cm in height, containing 12 kg of soil and 50 seeds. Seeds were directly sown into the soil on May 1, 2024. The growth units were fertilized with *Pseudomonas aeruginosa* bacteria, with a one-centimeter-long incision made near the root zone and inoculated only once during the growing season.

Basil extraction was performed using a Soxhlet apparatus. Two hundred grams of ground basil were placed in the thimble, then subjected to 70% hexane alcohol at 75°C for 24 hours. The oily extract was then separated from the alcohol using a rotary evaporator.

Determination of active compounds in basil plants

HPLC model SYKAM (Germany) It was used to analyses, The mobile phase was an isocratic acetonitrile : D.W : (75 : 25) at flow rate at 0.7 mL/min , column was C18 - ODS (25 cm * 4.6 mm) and the detector UV- 280 nm (Jedlicka and Klimes, 2005). Twenty-five microliters of the prepared solution were injected into a high-performance liquid chromatography (HPLC) system for the identification and quantification of the target active compounds (camphor, linalool and pinene) in basil leaves. Compounds were separated and identified by comparing them to standard materials on the separation column, under the same conditions and concentrations as the separated substances in the tested sample.

Nitrogen content

Total nitrogen was determined using a micro-Kjeldahl apparatus, as described by Al-Ghazali *et al.* (2023).

Carbohydrate content in seeds (mg/g)

Carbohydrates in the seeds were quantified for each treatment using a single method, as outlined by Malarkodi *et al.* (2021).

Statistical analysis

The experiments were analyzed using the randomized complete block design (RCBD) as factorial experiments with three replicates, with the least significant difference (L.S.D) calculated at a probability level of 0.01, following the methodology of Alakashy and Al-Bedairi (2020).

RESULTS AND DISCUSSION

Camphor (mg gm^{-1})

The results in Table (1) indicated a significant effect of *Pseudomonas aeruginosa* on camphor (mg/g^{-1}). The *Pseudomonas aeruginosa* injection treatment at a concentration of (200 ml^{-1}) achieved the highest average camphor (5.84 mg g^{-1}), significantly different from all other treatments, which yielded averages of (4.09, 2.79 and 2.23 mg g^{-1}) respectively.

Similarly, foliar application of nano-boron at the concentration of 250 mg L^{-1} resulted in the highest camphor content of 5.05 mg g^{-1}, showing a significant difference compared to other concentrations, which recorded averages of 4.03, 3.41 and 2.46 mg g^{-1} respectively.

The interaction between *Pseudomonas aeruginosa* (200 mg ml^{-1}) and nano-boron (250 mg L^{-1}) produced the highest camphor content of 7.83 mg g^{-1}, whereas the control interaction (no *Pseudomonas* and no nano-boron) yielded the lowest average camphor content of 1.45 mg g^{-1}.

The observed increase in camphor content might be attributed to the effect of the treatment, which could be

Table 1: Effect of *Pseudomonas* and foliar spray boron nano on camphor (mg.gm^{-1}).

Pseudomonas ml L^{-1}	Boron nano mg/L^{-1}				Mean of *pseudomonas*
	Control	80	150	250	ml L^{-1}
Control	1.45	1.99	2.52	2.93	2.23
25	1.96	2.58	2.89	3.73	2.79
125	2.58	3.68	4.38	5.72	4.09
200	3.83	5.37	6.34	7.83	5.84
Mean of boron nano	2.46	3.41	4.03	5.05	
LSD 0.05	*Pseudomonas*	Boron nano	Interaction		
	7.03	1.53	2.53		

responsible for the elevated active components in the sweet basil plant. The formation of free radical scavenging and the efficient absorption of nutritional components are highly effective for uptake. This is reflected in the nutritional components, growth and proliferation of mitochondrial cells. Additionally, these components expand the leaf surface area, provide nutrient-rich protein and carbohydrate from the leaves and transport them to areas of need. Furthermore, plant tissues must develop, as evidenced by the increased dry weight (Rahimi *et al.,* 2009; Zahwan *et al.,* 2013).

Linalool (mg gm^{-1})

The results in Table (2) indicated a significant effect of *Pseudomonas aeruginosa* on linalool (mg g^{-1}). The *Pseudomonas aeruginosa* injection treatment at a concentration of (200 ml^{-1}) achieved the highest average linalool concentration (mg g^{-1}) (25.98 mg g^{-1}), significantly different from all other treatments, with averages of (24.78, 22.36 and 19.68 mg g^{-1}).

Similarly, foliar application of nano-boron at the concentration of 250 mg L^{-1} produced the highest average linalool content of 25.20 mg/g^{-1}, showing a significant increase compared to other concentrations, which recorded averages of 24.24, 22.81 and 20.56 mg/g^{-1}, respectively.

The interaction between *P. aeruginosa* (200 mg mL^{-1}) and nano-boron (250 mg L^{-1}) resulted in the maximum linalool content of 27.84 mg g^{-1}. In contrast, the control treatment (without *P. aeruginosa* or nano-boron) showed the lowest mean linalool content (17.56 mg g^{-1}).

The observed increase in active compounds in basil oil may be attributed to enhanced chlorophyll content in leaves, consequently improving photosynthetic efficiency (Al-Balkhi, 1990). These results align with previous findings reported by Al-Sudani *et al.* (2018), supporting the positive influence of these treatments on secondary metabolite production in basil plants.

Pinene (mg g^{-1})

The results in Table (3) indicated a significant effect of *Pseudomonas aeruginosa* on pinene (mg g^{-1}). The *Pseudomonas aeruginosa* injection treatment at a concentration of (200 ml^{-1}) achieved the highest average pinene concentration (0.57 mg g^{-1}), significantly different from all other treatments, with averages of (0.37, 0.27 and 0.18 mg g^{-1}) respectively. Nanoboron at a concentration of (250 mg L^{-1}) also achieved the highest average pinene concentration (0.50 mg g^{-1}), significantly different from all other treatments, with averages of (0.41, 0.29, and 0.21 mg g^{-1}) respectively. While the interaction between *Pseudomonas* bacteria and nanoboron (200 ml^{-1} and 250 mg L^{-1}) achieved the highest average pinene at 0.83 mg g^{-1}, the interaction between the control (*Pseudomonas* bacteria and nanoboron) achieved the lowest average pinene at 0.11 mg g^{-1}.

Given its microbiological role in directly supplying plants with the nitrogen component NH4, which is directly represented by amino acids, after their structural and biological role within the plant cell, amino acids enter a catabolic pathway in the cytosol to produce pyruvate, which in turn is converted into two units of acetyl-CoA. Acetyl-CoA enters the mevalonate acetate pathway to produce isopentenyl pyrophosphate. This is an efficient method for building active compounds (Jilani, 1997).

Boron plays a role in increasing active compounds by contributing to vegetative growth and exerting a physiological effect on plant growth and development. It also contributes to cell wall formation and the transport of sugars across

Table 2: Effect of *Pseudomonas* and foliar spray boron nano on linalool (mg.gm^{-1}).

Pseudomonas ml L^{-1}	Boron nano mg/L^{-1}				Mean of *pseudomonas*
	Control	80	150	250	ml L^{-1}
Control	17.56	18.95	20.47	21.75	19.68
25	19.45	21.76	23.86	24.35	22.36
125	21.65	24.76	25.85	26.85	24.78
200	23.56	25.76	26.76	27.84	25.98
Mean of boron nano	20.56	22.81	24.24	25.20	
LSD 0.05	*Pseudomonas*	Boron nano	Interaction		
	1.74	1.71	2.17		

Table 3: Effect of *Pseudomonas* and foliar spray boron nano on pinene (mg.gm^{-1}).

Pseudomonas ml L^{-1}	Boron nano mg/L^{-1}				Mean of *pseudomonas*
	Control	80	150	250	ml L^{-1}
Control	0.11	0.17	0.20	0.25	0.18
25	0.15	0.20	0.33	0.39	0.27
125	0.22	0.32	0.44	0.51	0.37
200	0.34	0.46	0.66	0.83	0.57
Mean of boron nano	0.21	0.29	0.41	0.50	
LSD 0.05	*Pseudomonas*	Boron nano	Interaction		
	1.57	1.38	2.43		

Table 4: Effect of *Pseudomonas* and foliar spray boron nano on nitrogen.

Pseudomonas ml L^{-1}	Boron nano mg L^{-1}				Mean of *Pseudomonas*
	Control	80	150	250	ml L^{-1}
Control	0.65	0.99	1.21	1.32	1.04
25	0.88	1.11	1.35	1.49	1.21
125	1.25	1.39	1.48	1.57	1.42
200	1.38	1.54	1.63	1.75	1.58
Mean of boron nano	1.04	1.26	1.42	1.53	
LSD 0.05	*Pseudomonas*	Boron nano	Interaction		
	2.67	1.96	3.66		

Table 5: Effect of *Pseudomonas* and foliar spray boron nano on carbohydrate (mg g^{-1}).

Pseudomonas ml L^{-1}	Boron nano mg L^{-1}				Mean of *Pseudomonas*
	Control	80	150	250	ml L^{-1}
Control	12.65	14.76	15.84	16.36	14.90
25	13.65	15.36	16.94	18.45	16.1
125	15.64	17.45	18.45	19.73	17.82
200	17.45	19.45	20.46	21.74	19.78
Mean of boron nano	14.84	16.76	17.92	19.07	
LSD 0.05	*Pseudomonas*	Boron nano	Interaction		
	1.74	1.57	2.63		

cellular membranes (Howard *et al.,* 2000). Additionally, boron facilitates the translocation of sugars from roots to leaves, enhances pollination and germination processes, and supports seed formation and the production of active oils (Howard *et al.,* 2000).

Nitrogen content (mg g^{-1})

The results in Table (4) indicated a significant effect of *Pseudomonas aeruginosa* on nitrogen (mg g^{-1}). The *Pseudomonas aeruginosa* injection treatment at a concentration of (200 ml^{-1}) achieved the highest average nitrogen (mg g^{-1}) (1.58 mg g^{-1}), significantly different from all other treatments, which yielded averages of (1.42, 1.21, and 1.04 mg g^{-1}).

Nanoboron (250 mg L^{-1}) also reached the highest average nitrogen (1.53 mg g^{-1}), which was quite different from the mean value of any other treatment (1.42, 1.26 and 1.04 mg g^{-1}). The interaction between *Pseudomonas* bacteria and nanoboron (200 and 250 mg L^{-1}) produced the highest average nitrogen of 1.75 mg g^{-1} while the interaction between the control (*Pseudomonas* bacteria and nanoboron) produced the lowest average nitrogen of 0.65 mg g^{-1}.

Biofertilization leads to the synthesis of nutrients within plant tissue from carbohydrates, leading to an increase in active compounds through the carbohydrates' involvement in sugar decomposition, producing pyruvic acid and, subsequently, mevalonic acid, which is key to the production of isoprene, the basic unit for volatile oil formation (Mohammadreza *et al.,* 2014).

Carbohydrate

The results in Table (5) indicated a significant effect of *Pseudomonas aeruginosa* on carbohydrates. The treatment injected with 200 ml l of *Pseudomonas* achieved the highest average carbohydrate content (19.78 mg g^{-1}), significantly different from all other treatments, which yielded averages of 17.82, 16.1 and 14.90 mg g^{-1}. Nanoboron at a level of 250 mg l^{-1} obtained the highest average carbohydrate content (19.07 mg g^{-1}), being significantly different from all other treatments with averages of 17.92, 16.76 and 14.84 mg g^{-1}. The interaction of *Pseudomonas* bacteria and nanoboron (200 ml^{-1} and 250 mg L^{-1}) were efficient and recorded the highest mean of the carbohydrate content of 21.74 mg g^{-1}, which was observed for the interaction of *Pseudomonas* bacteria and nanoboron control, were least efficient and recorded the lowest mean of carbohydrate content of 12.65 mg g^{-1}.

In a laboratory experiment, inoculation of sweet basil (*Ocimum basilicum* L.) seeds with *Bacillus subtilis* stimulated the production of eugenol and R-terpineol, which are influential in oil production (Banchio *et al.,* 2009). Similarly, inoculation of coriander (*Coriandrum sativum* L.) with *Azotobacter* bacteria enhanced the production of volatile oil and the percentage of volatile oil content (Chandrakala *et al.,* 2024). In a field experiment, Darzi *et al.* (2012) found that foliar application of boron led to an increase in chemical properties, as boron plays a crucial role in protein synthesis and the formation of plant hormones, thereby affecting nutrient content, chemical compounds, carbohydrates and proteins.

CONCLUSION

Findings of this study reveal that the *P. aeruginosa* soil inoculation and nano-boron foliar application significantly

improved the chemical as well as biochemical constituents of basil (*O. basilicum* L.). Maximum nitrogen, carbohydrate and bioactive compounds (camphor, linalool, pinene) were recorded with *Pseudomonas* at 200 mg/mL in combination with nano-boron of 250 mg L^{-1}, revealing the potential synergistic effect of biofertilizers and Nanofertilizers for increase in plant quality and secondary metabolites, which can be a promising sustainable practice in agriculture to improve the productivity and phytochemical constituents of aromatic and medicinal plants.

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Disclaimers

The views and conclusions expressed in this research are solely those of the author and do not necessarily reflect the official policies or positions of the affiliated institution, sponsor, or publisher. The authors bear full responsibility for the content of this work.

Informed consent

Informed consent is not required for this research study, as it was carried out on basil plants and does not include any human participants.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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