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Effect of Mineral, Bio-Fertilizer and Brassinolide Spraying on the Growth and Oil Percentage of Peanut (Arachis Hypogaea L.)

R. H. Mohammed * **B. Sh. J. Alobaidy**

College of Agriculture, University of Anbar

***Correspondence to:** Reem H. Mohammed, College of Agriculture, University of Anbar, Iraq.
Email: ree21g3008@uoanbar.edu.iq

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Abstract

A field experiment was conducted in one of the agricultural fields located on the right bank of the Euphrates River in Heet District, Anbar Governorate, during the 2022 agricultural season to study the effect of mineral and bio-fertilizers and the growth regulator brassinolide on growth traits and oil percentage of peanut (Arachis hypogaea L.). The experiment was arranged using a Randomized Complete Block Design (RCBD) in a split-plot arrangement with three replications. The main factor included brassinolide spraying stages (no spray, one spray at the beginning of flowering, and two sprays—first at the beginning of flowering and the second at the beginning of peg formation). The sub-factor included fertilization treatments (full mineral fertilizer recommendation, bio-fertilizer only, 25% mineral fertilizer + bio-fertilizer, 50% mineral fertilizer + bio-fertilizer, and 75% mineral fertilizer + bio-fertilizer). The results showed that the full mineral fertilizer recommendation achieved the highest number of branches and leaf chlorophyll content (4.72 branches plant^{-1} and 41.39 SPAD, respectively). Meanwhile, the treatment with 50% mineral fertilizer + bio-fertilizer recorded the highest values for shoot dry weight and oil percentage (368.9 g and 52.37%, respectively.

Keywords: Growth, Oil Percentage, Brassinolide, Peanut.

تأثير السماد المعدني والحيوي ورش البراسينولايد في نمو الخضري ونسبة الزيت لفستق الحقل

ريم هاشم محمد * **بشرى شاكر جاسم العبيدي**

قسم المحاصيل الحقلية، كلية الزراعة، جامعة الانبار، العراق

***المراسلة الى:** ريم هاشم محمد، قسم المحاصيل الحقلية، كلية الزراعة، جامعة الانبار، العراق.

البريد الالكتروني: ree21g3008@uoanbar.edu.iq

الخلاصة

نفذت تجربة حقلية في احد الحقول الزراعية الواقعة في الضفة اليمنى لنهر الفرات في قضاء هيت التابع لمحافظة الانبار للموسم الزراعي 2022 بهدف دراسة تأثير السماد المعدني والحيوي ومنظم النمو البراسينولايد في صفات النمو ونسبة الزيت لمحصول فستق الحقل استعمل تصميم القطاعات العشوائية الكاملة (RCBD) بترتيب الألواح المنشقة Split plots بثلاثة مكررات. شمل العامل الرئيسي مراحل رش منظم النمو هي (بدون رش، رشة واحدة في بداية التزهير، ورشتين الاولى في بداية التزهير والأخرى في بداية تكوين المهاميز). والعامل الثانوي شمل معاملات التسميد (التسميد حسب التوصية السمادية والتسميد الحيوي فقط و25% من التوصية بالسماد المعدني + تسميد حيوي و50% من التوصية بالسماد المعدني + تسميد حيوي و75% من التوصية بالسماد المعدني + تسميد حيوي). اظهرت النتائج تفوق معاملة التوصية السمادية في صفتي عدد التفرعات ومحتوى الاوراق من الكلوروفيل (4.72 فرع نبات$^{-1}$ 41.39 spad)، في حين تفوقت معاملة 50% من التوصية بالسماد المعدني +تسميد حيوي في صفتي الوزن الجاف للمجموع الخضري ونسبة الزيت في البذور (368.9 غم و52.37%).

كلمات مفتاحية: النمو، الزيت، البراسينولايد، فستق الحقل.

Introduction

The rapid increase in the world's population has doubled the need to find suitable means to increase productivity per unit area and improve seed quality at relatively low costs. As a result, researchers have recently turned their attention to bio-fertilization technologies as alternatives to chemical fertilizers that have harmful effects on the environment. These fertilizers produce biological compounds that help fix nitrogen and dissolve phosphorus and potassium, in addition to producing growth-promoting hormones such as auxins, gibberellins, amino acids, vitamins, and decomposing crop residues to release nutrients.

Fertilizer management is one of the most critical factors for the successful cultivation of crops, as it ultimately affects the quantity, quality, and type of yield. Since the use of chemical fertilizers leads to soil and environmental degradation, the idea of using bio-fertilizers emerged due to their important role in improving the physical, chemical, and biological properties of the soil. They serve as reservoirs of essential nutrients for plant growth and help maintain the regulatory capacity of certain soils, which encourages their use as substitutes for mineral fertilizers.

Rhizobium inoculation in leguminous plants improves plant growth and total nitrogen yield, which is one of the most essential elements for soil fertility (2 and 17). The success of the biofertilizer used as an inoculant in the plant growth medium depends on the efficiency of the added microorganism, its ability to coexist with the host plant, and its competitiveness with native microorganisms in the root zone (4 and 13). Microorganisms differ in their nature, behavior, activity, and the type of nutrients they supply to the plant (16). It has been shown (6) that the use of bacterial inoculation increased plant growth and the efficiency of nitrogen fixation in a symbiotic manner, resulting in increases in most of the studied traits, such as plant height, dry weight of the root and shoot systems, number of branches per plant, number and dry weight of nodules, number of pods per plant, and nitrogen concentration in the shoot system. These results varied according to the bacterial strain and the faba bean variety used. Therefore, there is a pressing need for an in-depth understanding of the response of peanut crops to various fertilization treatments and the possibility of replacing part of the mineral fertilizers with bio fertilizers to reduce agricultural production costs and environmental pollution. Preliminary studies have shown that brassinosteroids play a prominent role in increasing the total number of flowers, and there is evidence that brassinosteroids stimulate flowering by reducing the levels of flowering-inhibiting compounds (8 and 15).

Materials and Methods

A field experiment was conducted in one of the agricultural fields located on the right bank of the Euphrates River in Heet District, Anbar Governorate, during the 2022 agricultural season to study the effect of mineral and bio-fertilization and the growth regulator brassinolide on some growth and quality traits of the local peanut variety. The experiment was arranged using a Randomized Complete Block Design (RCBD) in a split-plot arrangement with three replications. The main plots included the stages of brassinolide application: (control – sprayed with water only, one spray at the beginning of flowering, and two sprays – the first at the beginning of flowering and the second at the beginning of peg formation). The subplots involved fertilization treatments as follows: mineral fertilization according to recommendations, bio-fertilization, 25% of the recommended mineral fertilizer + bio-fertilization, 50% of the recommended mineral fertilizer + bio-fertilization, and 75% of the recommended mineral fertilizer + bio-fertilization. The area of the experimental unit was (3×3) meters. Sowing was done in holes on ridges, with a spacing of 30 cm between holes and 60 cm between rows, resulting in a plant density of 55,555.55 plants per hectare. Sowing took place on April 1, 2022, and harvesting on October 1, 2022. Urea fertilizer (46% N) was applied at a rate of 80 kg N ha^{-1} and triple superphosphate at a rate of 80 kg P_2O_5 ha^{-1}. Rhizobia were obtained from root nodules of peanut plants previously grown in pots. The roots were placed in a mesh sieve and gently washed under running water to remove soil. Nodules were separated from the roots, then surface-sterilized by immersing them in alcohol for 10 seconds, followed by sodium hypochlorite (3%) for 6 minutes, then rinsed several times with sterile water. Using sterile forceps, nodules were transferred to Petri dishes containing 1 ml of sterile distilled water and crushed to obtain a suspension containing the root nodule bacteria. The inoculant was then prepared following Vincent's method (14) and stored at 4°C until use in the Soil Science Department's microbiology laboratory. The brassinolide growth regulator solution was prepared at the required concentration (1.5 mg L^{-1}) by converting the active ingredient concentration to ppm. One gram of the compound (U.S.-made) was dissolved in distilled water with 2.5 ml of 50% ethanol as a solvent, and the volume was completed to one liter to obtain a stock solution of 1000 mg L^{-1}. The desired concentration was prepared using the dilution formula:

$C_1V_1 = C_2V_2$

Where:

C_1 = concentration of the stock solution

V_1 = volume of the stock solution

C_2 = desired concentration

V_2 = final volume

Ten randomly selected and pre-marked plants were taken from each experimental unit to calculate the number of branches on the main stem. Dry weights of the root and shoot systems were recorded. Chlorophyll content was measured using a SPAD meter. Oil percentage in seeds was also analyzed.

Statistical analysis was conducted using analysis of variance (ANOVA), and the least significant difference (LSD) test was used to compare means at the 5% significance level.

Results and Discussion

Number of Branches (branches plant^{-1}): Table 1 shows significant differences among fertilization treatments and their interactions with brassinolide spraying, while the spraying factor alone did not show a significant effect on this trait. The treatment with full recommended mineral fertilization recorded the highest average number of branches (4.72 branches per plant), although it was not significantly different from the treatments with 25%, 50%, and 75% of the recommended mineral fertilizer combined with bio-fertilization, which recorded averages of 4.64, 4.39, and 4.62 branches per plant, respectively. In contrast, the bio-fertilization-only treatment gave the lowest mean (3.76 branches per plant), which was significantly different from the other treatments. This may be due to the synergistic effect between mineral and bio-fertilizers, which increases nutrient availability and transport into the plant, stimulating bud formation that later develops into branches. This process may occur through a mechanism known as Rhizocoenosis, where cell membranes are regulated to promote the surface absorption of nutrients by root cortex cells, positively affecting bud development and increasing the number of branches. This finding agrees with studies by (1, 2 and 14), which indicated the significant impact of bio-fertilizers on vegetative growth in peanut plants.

The results also revealed a significant interaction between fertilization treatments and spraying stages, as fertilization treatments with higher levels of mineral fertilizer (25%, 50%, and 75%) responded more positively with increased spraying frequency, while the full recommendation and bio-fertilization treatments showed a better response when sprayed twice.

Table 1: Effect of fertilization and brassinolide on the number of branches (branches plant⁻¹) of peanut.

Spraying / Treatments	Recommended Fertilization	Bio-Fertil.	2Bio+ %25 Mineral	Bio + %50 Mineral	Bio +75% Mineral	Mean
Control (Water only)	3.27	4.07	3.80	3.33	4.07	3.71
One spray	5.73	4.00	4.20	5.00	4.60	4.71
Two sprays	4.87	3.20	5.87	5.60	5.20	4.55
Mean	4.72	3.76	4.39	4.64	4.62	

LSD 0.05: Fertilization: 0.459 | Spraying: NS | Interaction: 0.959.

Shoot Dry Weight (g plant^{-1}): Table 2 shows significant differences among fertilization treatments and brassinolide spraying treatments, while their interaction did not have a significant effect on shoot dry weight. The treatment with 50% of the recommended mineral fertilizer + bio-fertilizer recorded the highest average shoot dry weight (368.9 g plant^{-1}), followed by the 75% mineral + bio-fertilizer treatment (267.8 g plant^{-1}). The lowest average was observed in the bio-fertilization-only treatment (193.9 g plant^{-1}). This could be attributed to the synergy between the added mineral fertilizer and the action of bio-fertilizers in enhancing the availability of essential nutrients in the rhizosphere, which supports plant growth and is reflected in higher branching (Table 1). These findings are consistent with (3, 10 and 11) in their studies on mung bean and faba bean. The average shoot dry weight was similar between the one-spray and two-spray treatments (273.0 and 275.7 g plant^{-1}, respectively), both significantly higher than the control (spraying with water only), which recorded the lowest average (242.7 g plant^{-1}). This may be due to the functional similarity of brassinolide to other plant hormones such as gibberellins and cytokinins, which play vital roles in plant development and in delaying leaf senescence, contributing to increased biomass accumulation (12).

Table 2: Effect of fertilization and brassinolide on the shoot dry weight (g plant⁻¹) of peanut.

Spraying / Treatments	Recommended Fertilization	Bio-Fertil.	Bio+ %25 Mineral	Bio + %50 Mineral	Bio +75% Mineral	Mean
Control (Water only)	203.30	156.70	236.70	360.00	256.70	242.70
One spray	260.00	201.70	260.00	366.70	276.70	273.00
Two sprays	278.30	223.30	226.70	380.00	270.00	275.70
Mean	247.30	193.90	241.10	368.90	267.80	

LSD 0.05: Fertilization: 24.03 | Spraying: 14.53 | Interaction: NS.

Root Dry Weight (g plant^{-1}): As shown in Table 3, there were significant differences among fertilization treatments and their interaction with brassinolide spraying, while the spraying factor alone did not have a significant effect. The 75% mineral + bio-fertilizer treatment gave the highest average root dry weight (16.45 g plant^{-1}), not significantly different from the 50% mineral + bio-fertilizer and full recommendation treatments (15.89 and 15.67 g plant⁻¹, respectively). The lowest value was recorded in the 25% mineral + bio-fertilizer treatment (9.67 g plant⁻¹). The increase may be due to enhanced root nodulation and nitrogen fixation, improved nutrient availability, and the production of growth-promoting substances by rhizobacteria, such as ethylene, which promotes root biomass accumulation. These results are in agreement with (5) on peanut and (7, 9 and 10) on other crops. The interaction between fertilization and spraying treatments showed that the highest root dry weight (19.00 g plant⁻¹) was obtained in the 75% mineral + bio-fertilizer treatment with one brassinolide spray, while the lowest value (6.67 g plant⁻¹) was observed in the 25% mineral + bio-fertilizer treatment with one spray.

Table 3: Effect of fertilization and brassinolide on the root dry weight (g plant⁻¹) of peanut.

Spraying / Treatments	Recommended Fertilization	Bio-Fertil.	Bio+25% Mineral	Bio+50% Mineral	Bio+75% Mineral	Mean
Control (Water only)	16.33	12.67	12.33	18.00	13.67	14.60
One spray	17.67	11.00	6.67	17.67	19.00	14.40
Two sprays	13.00	10.67	10.00	12.00	16.67	12.47
Mean	15.67	11.44	9.67	15.89	16.45	

LSD 0.05: Fertilization: 2.887 | Spraying: NS | Interaction: 5.043.

Chlorophyll Content in Leaves (SPAD units): Table 4 reveals that brassinolide spraying had no significant effect on chlorophyll content, while fertilization treatments and their interaction with spraying did show significant effects.

The highest chlorophyll content was recorded with the 50% mineral + bio-fertilizer treatment (42.33 SPAD), which was not significantly different from the full mineral recommendation (41.39 SPAD). The lowest average was recorded with the 25% mineral + bio-fertilizer treatment (38.74 SPAD). The increase in chlorophyll may be due to improved nutrient supply, particularly nitrogen, as well as the role of bio-fertilizers in synthesizing organic compounds such as proteins, nucleic acids, and enzymes essential for chlorophyll formation.

The interaction effect was also significant, where the combination of 50% mineral + bio-fertilizer and two brassinolide sprays recorded the highest value (45.38 SPAD), while the combination of 25% mineral + bio-fertilizer and the control treatment (no spray) recorded the lowest value (38.07 SPAD).

Table 4: Effect of fertilization and brassinolide on chlorophyll content in leaves (SPAD) of peanut.

Spraying / Treatments	Recommended Fertilization	Bio-Fertil.	Bio+25% Mineral	Bio+50% Mineral	Bio+75% Mineral	Mean
Control (Water only)	42.10	41.87	38.07	41.87	41.50	41.08
One spray	41.83	40.80	40.00	39.30	39.03	40.19
Two sprays	40.23	38.30	38.17	45.83	38.20	40.15
Mean	41.39	40.32	38.74	40.32	40.14	

LSD 0.05: Fertilization: 0.86 | Spraying: NS | Interaction: 1.576.

Oil Percentage in Seeds: According to Table 5, significant differences were found among fertilization treatments, while brassinolide spraying alone had no significant effect. However, the interaction between fertilization and spraying was significant.

The highest oil percentage was observed in the 50% mineral + bio-fertilizer treatment (52.37%), which was not significantly different from the bio-fertilizer-only treatment (52.20%). These treatments were significantly superior to the 25%, 75%, and 100% mineral fertilizer treatments, which recorded close averages (49.87%, 49.66%, and 49.10%, respectively).

The increase in oil content is likely due to the positive role of bio-fertilizers in enhancing nutrient availability and uptake, particularly nitrogen, and increasing chlorophyll content (as shown in Table 4). The interaction analysis showed that the combination of 50% mineral + bio-fertilizer with the control spraying treatment resulted in the highest oil percentage (53.16%), while the combination of full mineral fertilization and one spray resulted in the lowest (47.48%).

Table 5: Effect of fertilization and brassinolide on oil percentage in peanut seeds (%).

Spraying / Treatments	Recommended Fertilization	Bio-Fertile.	Bio+ %25 Mineral	Bio + %50 Mineral	Bio +75% Mineral	Mean
Control (Water only)	48.04	51.60	48.12	53.16	50.80	50.34
One spray	47.48	52.00	51.38	51.00	50.29	50.43
Two sprays	51.77	53.00	50.10	52.94	47.89	51.14
Mean	49.10	52.20	49.87	52.37	49.66	

LSD 0.05: Fertilization: 1.161 | Spraying: NS | Interaction: 1.939.

Conclusions

The results of this study indicate the importance of using integrated fertilization involving both mineral and bio-fertilizers, particularly the treatment of 50% of the recommended mineral fertilizer combined with bio-fertilizer. This combination positively influenced key traits such as shoot dry weight and oil percentage in seeds, contributing to a reduction in the use of mineral fertilizers and hence lowering production costs and minimizing environmental pollution.

Additionally, spraying with brassinolide had a positive impact on plant growth traits, especially when applied twice (at the beginning of flowering and at the start of peg formation), as it functionally resembles natural plant growth hormones, contributing to enhanced vegetative growth and improved physiological performance.

Therefore, it is recommended to adopt an integrated fertilization strategy and growth regulator spraying to improve the quantitative and qualitative traits of peanut crops.

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Author 1; methodology, writing—original draft preparation, Author 2 writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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