



# THE IMPACT OF LOCATION AND BIO-STIMULANT COMBINATIONS ON THE MINERAL CONTENT OF LEAVES AND SOME FLOWERING INDICATORS OF THE BANANA PLANT, GRAND NAIN CULTIVAR

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Article history:	Abstract:
<p><b>Received:</b> 27<sup>th</sup> January 2024 <b>Accepted:</b> 20<sup>th</sup> March 2024</p>	<p>The research was carried out in two locations: Garmat Ali, Agricultural Experiment Station, College of Agriculture, University of Basrah, and Al-Hartha Research Station, Basrah Agriculture Directorate, for the two years 2021 and 2022. The aim was to study the impact of the location and bio-stimulant combinations on the mineral content of leaves and some flowering indicators of banana plants, Grand Nain cultivar. The experiment was designed as a factorial experiment with two factors according to a randomized complete block design (R.C.B.D) with three replications. The first factor is the cultivation location, which includes two locations: Garmat Ali and Al-Hartha. The second factor includes seven bio-stimulant combinations, between three concentrations of chitosan (0, 3, and 6 g L<sup>-1</sup>), two concentrations of Sitmplex (0 and 1.5 g L<sup>-1</sup>), and two concentrations of Biogrand (0 and 1.5 g L<sup>-1</sup>). The results showed that the location had a significant effect on the leaf mineral content. Al-Hartha's location was significantly superior in the percentage of nitrogen, phosphorus, and potassium in the leaves, amounting to 3.086, 0.2470, and 2.191%, respectively. As for the spraying combinations with the bio-stimulants, the S6 combination was significantly higher in the percentages of nitrogen, phosphorus, and potassium elements in the leaves, reaching 3.663, 0.3003, and 2.946%, respectively. The same combination also excelled in the number of days until flowering, 321.3 days, and in the number of flowers, it led to producing 49.3 flowers plant<sup>-1</sup>. The combination S6 was also significantly superior in the number of hands, as it gave the highest value of 7.33 hands plant<sup>-1</sup>.</p>

**Keywords:** Chitosan, Biogrand, Flowering, Nitrogen, Phosphorus, Potassium, Simplex.

## INTRODUCTION

The Banana (*Musa* spp.) is a monocot tropical plant that belongs to the Musaceae family and is believed to be native to southeastern Malaysia and the Philippines (Agha and Dawood, 1991). Bananas are one of the largest growing herbaceous plants that do not have a woody stem above the soil surface. Bananas are the most economically important fruit crop in the world after grape, citrus, and apple fruits. They are grown in 130 countries, mainly in the tropical and subtropical regions of the Southern Hemisphere. The global banana production reached 21 million metric tons in 2019 (FAO, 2023).

Plant tissue culture technology plays an important role in propagating plants to obtain many plants in a short period that are true to the type of mother plant (Al-Taha et al., 2014). The banana plant is one of the plants that has been propagated using this technique, and it is one of the modern techniques through which many plants are obtained that are free of viral diseases and genetically identical to the mother plant (Mohsen et al., 2021).

The banana plant blooms at any time of the year and needs a long day of 13 hours of light to stimulate the flowering process in the plant (Fortescue et al., 2011). The flowering stage is one of the important stages in the banana plant. The most important factors that affect flowering time are abiotic factors such as the length of the luminous intensity, temperature, and salinity. The biotic factors that affect banana flowers are hormone levels, the C/N ratio, and the age of the plant (Chaurasia et al., 2017).

Due to the different nature of banana plant growth and its large leaf area, its growth and production are affected by increased salt or heat stress conditions. There was a need to use some anti-biotic stress compounds that increase the flow of plant juices and stimulate the cytoplasm (Abdel et al., 2017). The most important bio-stimulant used in this

field is chitosan, a straight polymer, with units of the polymer chain reaching 2000-3000 units. It is a polysaccharide polymer; it is the second most common organic compound in nature after cellulose. This polymer is extracted from various natural sources and constitutes 20-30% of the waste from shrimp, crab, and lobster shells (Ahmed and Salim, 2020). The second bio-stimulant is Stimplex, which promotes leaf growth and stem elongation and enhances the process of photosynthesis and the formation of chlorophyll and protein. This bio-stimulant increases cell division, increases flowering rates, stabilizes fruit setting, and improves fruit quality. In addition, it increases plant resistance to stress (Spann and Little, 2010). As for the third stimulant, Biogrand is one of the compounds rich in amino acids and plant growth regulators that have a role in stimulating growth and development processes and resistance to abiotic stresses (Sun et al., 2010).

To promote this crop to meet the market needs, ensure the success of its cultivation in the atmosphere of Basra, and solve some of the problems facing its cultivation, such as weak growth and flowering. This research aims to study the effect of location on the success of the cultivation of banana, Grand-Nain cultivar, and the expansion of the cultivation of banana plants that are propagated by the in vitro culture method in the conditions of Basrah Governorate, Iraq. In addition to using combinations of the bio-stimulants, Stimplex, Biogrand, and chitosan to increase the efficiency of banana plants for vegetative and flowering growth.

### **MATERIALS AND METHODS**

The study was carried out in two sites: the first Garmat Ali, Agricultural Experiment Station, College of Agriculture, University of Basrah, and the second Al-Hartha, Basrah Agriculture Directorate, Iraq for the Autumn season 2021-2022 to investigate the effect of the planting site and some combinations of bio-stimulants on leaf mineral content and flowering growth indicators of *Musa* spp., Grand Nain cultivar. Random samples were taken from the field soil before planting at a depth of 0-30 cm, then mixed homogeneously, air-dried, ground, and sieved with a 2-mm sieve to estimate some physical and chemical properties of the soil and irrigation water in the laboratories of the Department of Soil and Water Sciences, College of Agriculture, University of Basrah, in both locations before starting of the study (Table 1 A, B).

The land was divided into three lines 10.5 m in length. The distance between one line and the other was 2 m, after which (7) holes were dug in each line, 60 cm deep and 60 cm in diameter. A mixture of decomposed animal manure and sand was added to each hole in a 1:1 ratio. The soil was sterilized with the systemic pesticide metalaxyl 5G at 4.6 g m<sup>-2</sup> and Velum pesticide of nematode worms at 10%. Then, 21 offshoots of homogeneous size, free of pathogens, and three months old from the Grand Nain banana cultivar were planted on 11/20/2021. The offshoots were treated with bio-stimulants on 3/20/2022 using the foliar spray application, with four sprays between one spray and another, one week in, respectively. It used a 15-liter hand pump for foliar application and carried out the process early in the morning until the treated offshoots were completely wet.

### **THE FACTORS**

The experiment included two factors:

- 1- The first factor: is the cultivation site, which includes two cultivation sites: Garmat Ali and Al-Hartha.
- 2- The second factor: It includes seven combinations of bio-stimulants, which are three concentrations of the bio-stimulant chitosan (0, 3, and 6 g L<sup>-1</sup>), two concentrations of Simplex (0, and 1.5 g L<sup>-1</sup>), and two concentrations of Biogrand (0, and 1.5 g L<sup>-1</sup>) as shown in Table (2).

### **Mineral content of leaves**

After the plant had fully grown, samples were taken from the middle below the growing tip of each banana leaf. They were placed in plastic bags (Zekri, 2012; Hasani et al. 2012). Then the samples were washed with tap water, acidified water (HCl) (0.1), and distilled water to remove any dust and pesticide residues stuck to them. After drying, they were placed in bags. Perforated paper and placed in an electric oven at a temperature of 70°C until the weight was constant (Al-Sahhaf, 1989). Samples of dry leaves were ground with an electric grinder. Then a weight of 0.2 g was taken from each sample and digested according to the method of Cresser and Parsons (1979). After completing the digestion process, the samples are now ready to estimate the elements N, P, and K in the leaves, as follows:

### **Nitrogen concentration in leaves (%)**

Nitrogen was estimated in the laboratory of the Soil and Water Sciences, College of Agriculture, University of Basrah, Iraq using the Micro-Kjeldhal device as described in Page et al. (1982).

### **Phosphorus concentration in leaves (%)**

The percentage of phosphorus was estimated in the laboratory of the Horticulture Department, College of Agriculture, University of Basrah Iraq using ammonium molybdate and ascorbic acid according to Murphy and Riley (1962).

### **Potassium concentration in leaves (%)**

Potassium element was estimated in banana leaves in the laboratory of the Soil and Water Sciences, College of Agriculture, University of Basrah, Iraq by using a flame photometer device, according to Page et al. (1982)

### **Flowering date**

The number of days was calculated from after offshoot planting in the field until the beginning of flowering.

### **Number of flowers in one inflorescence (flowers. inflorescence<sup>-1</sup>)**

The total number of flowers per inflorescence was calculated.

### **The number of hands per bunch (hands. bunch<sup>-1</sup>)**

The number of hands per bunch was counted manually.

### Statistical analysis

The experiment was designed as a factorial experiment with two factors according to a Randomized Complete Block Design (R.C.B.D) with three replications. The experiment included two factors: The planting site, which includes two, planting sites: Garmat Ali and Al-Hartha. The second factor included seven combinations of the bio-stimulants (Chitosan, Simplex, and Biogrand). An analysis of variance was conducted for each of the studied traits using the statistical program Genstat 2007. The least significant difference test was used to compare the means of the treatments at the 5% probability level based on Al-Rawi and Khalaf Allah (2000).

## RESULTS AND DISCUSSION

### Nitrogen content of leaves

Table (3) indicates a significant increase between planting sites in the percentage of nitrogen concentration in leaves. The Al-Hartha site showed the highest percentage of nitrogen concentration in the leaves, reaching 3.086%, compared to the Garmat Ali site, 2.923%.

The results also indicated a significant effect of bio-stimulants on the percentage of nitrogen content in leaves. The bio-stimulant combination (S6) gave the highest percentage of nitrogen content, reaching 3.432%, which was superior to the other combinations.

As for the interaction between site treatment and spray treatment with stimulants, it showed a significant effect on this characteristic. The interaction treatment between the Al-Hartha site and combination S6 was significantly superior. It recorded the highest nitrogen content in leaves at 3.663% compared to the lowest content of 2.727% for the interaction treatment between the Garmat Ali site and the control treatment.

### Phosphorus content of leaves

The Data of Table (4) shows that the Al-Hartha site was significantly superior to the Garmat Ali site in terms of the percentage of phosphorus in the leaves of 0.2470%.

The same table also shows that the S6 bio-stimulant combination was significantly superior in phosphorus content of leaves by 0.2792%.

It is clear from the same table that the interaction treatment between the Al-Hartha site and spraying with the S6 combination was significantly superior to the other interactions. It recorded the highest phosphorus content in the leaves, amounting to 0.3003%, compared to the lowest content, amounting to 0.1757% in the interaction treatment between the control treatment and the Garmat Ali site.

### Potassium content in leaves

The data in Table (5) showed a significant superiority of the Al-Hartha site over the Garmat Ali site in terms of the percentage of potassium content in the leaves of 2.191%.

The spray combination S6 was also significantly superior in potassium content in the leaves compared to the other combinations. It recorded the highest value of 2.856% compared to other combinations.

As for the interaction treatment, the table shows a significant superiority of the interaction treatment between the Al-Hartha site and the S6 combination at 2.946% compared to the lowest potassium content in the leaves, which reached 0.997% in the interaction treatment between the control treatment of the bio-stimulant and the Garmat Ali site.

### Flowering and fruiting growth

Figure (1) also shows the effect of bio-stimulant combinations on the number of days until flowering. The S6 combination excelled with the lowest number of days until the flowering reached 321.3 days, compared to the highest number of days until flowering, 369 days for the plant in the control treatment.

Figure (2) shows the effect of bio-stimulants on the number of flowers in an inflorescence. The significant superiority of the S6 combination compared to the other combinations. It recorded the highest number of flowers, which amounted to 49.3 flowers per inflorescence, compared to the lowest number of flowers per inflorescence in the S2 combination reaching 25.3 flowers per inflorescence.

Figure (3) shows the significant effect of the bio-stimulant S6 combination spraying on the number of fruit hands (Plate 1). The number of fruiting hands per plant when sprayed with combination S6 was about 7.33 hands per plant, compared to the lowest number of hands of 4.00 hands per plant in the control treatment.

Tables 3, 4, and 5 shows that the Al-Hartha site was a significant superior in the percentage of nutrients nitrogen, phosphorus, and potassium in the leaves compared to another site. The Al-Hartha site showed a significant effect on all traits. This may be due to the uniqueness of some soil characteristics in this site. The Al-Hartha site is represented by lower electrical conductivity than the Garmat Ali site. This reflects positively on plant growth through the plant's ability to absorb nutrients, especially potassium ions in contrast to sodium ions. Also, the components of the soil of the Al-Hartha site are silty sand, which is a better soil suitable for growing bananas compared to the Garmat Ali soil (clay-sand). The salinity of irrigation water also led to a significant improvement in plant growth and productivity. The electrical conductivity of irrigation water at the Al-Hartha site is  $3.1 \text{ dS.m}^{-1}$ . It is considered good for plant growth due to the ability of the plants to absorb elements from the soil perfectly compared to the Garmat Ali site where the salinity of the irrigation water was High  $6.10 \text{ dS.m}^{-1}$ . This may lead to salt stress on the plant and thus a decrease in absorption rates and the rate of carbon metabolism in the plant, which is evident in the concentration of elements in the leaves. The results of this study are consistent with other studies (Khalil, 2008; Al-Tamimi, 2012; Mustaffa and Kumar, 2012; Viskelis et al., 2019; Olivares et al., 2022).

The results of the same tables also show the significant superiority of the S6 bio-stimulant combination spray in all characteristics. This combination sprays included 6gm L<sup>-1</sup> chitosan + 1.5gm L<sup>-1</sup> Stimplex + 1.5gm L<sup>-1</sup> Biogrand. Chitosan is a carbohydrate complex that has a positive role in increasing the rate of carbon metabolism in plants because of its importance in building chloroplasts. It also has a role similar to the action of growth regulators because it increases the effectiveness of IAA, and enhances cell division (Chamnanmanoontham et al., 2015; Saharan and Pal 2016; Lopez-Moya et al., 2017). Stimplex is also considered an important bio-stimulant because it contains high levels of cytokinins. It also works as an antioxidant and contains betaine which has a role in preventing the decomposition and oxidation of the chlorophyll pigment (Kuwada et. al. 2006; Al-Alaywi et al., 2019). The bio-stimulant Biogrand contains many amino acids that play a role in protecting plants from stress. They act as osmotic substances that regulate the transfer of ions, opening and closing stomata, and increasing the effectiveness of enzymes (Rai, 2002). The significant effect of these bio-stimulants on increasing the percentage of nutrients (N, P, and K) in the leaves helps increase the plant's ability to tolerate stress. In addition to increasing the formation of chlorophyll pigment and increasing the effectiveness of photosynthesis, it reflects positively on the increased absorption of nutrients and their increased accumulation in the leaves of the banana plant. The results of this research are consistent with the results of the study conducted by AL-Hayany and Hathal (2021) on pomegranates.

Bio-stimulants have an impact on many physiological processes in plants, including respiration, photosynthesis, synthesis of nucleic acids, and increased absorption of elements. In addition to its role in improving plant tolerance to stress factors. It also increases its hormonal content, especially auxins (Pardiković et al., 2011). Therefore, these factors were reflected in the mineral content of the leaves (Tables 3, 4, and 5) and led to an earlier flowering date and an increase in the number of flowers per inflorescence and fruiting hands per bunch. These results are consistent with the results of other studies by Ravi et al. (2018) on bananas and Ali et al. (2022) on mangoes.

### CONCLUSIONS

Planting sites and bio-stimulants had a significant effect on the mineral content of N, P, and K elements in the leaves of the banana cultivar Grand Nian. The bio-stimulant has a positive effect on early flowering and increases the number of flowers per inflorescence and the number of fruiting hands per bunch.

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**Table (1, A): Some chemical and physical properties of the experimental soil and irrigation water of the Garmat Ali site**

Property		Value
pH of Soil		7.9
Electrical Conductivity (EC) of Soil		8.42 dS.m <sup>-1</sup>
Dissolved positive ions	Ca <sup>+2</sup>	10.30 mmol.L <sup>-1</sup>
	Mg <sup>+2</sup>	7.61 mmol.L <sup>-1</sup>
	Na <sup>+2</sup>	24.33 mmol.L <sup>-1</sup>
	K <sup>+</sup>	1.40 mmol.L <sup>-1</sup>
Organic matter		0.74%
Dissolved negative ions	SO <sub>4</sub> <sup>-2</sup>	22.60 mmol.L <sup>-1</sup>
	HCO <sub>3</sub> <sup>-</sup>	3.10 mmol.L <sup>-1</sup>
	Cl <sup>-</sup>	60.0 mmol.L <sup>-1</sup>
	CO <sub>3</sub> <sup>-2</sup>	0.0
Nitrogen uptake		191 ppm
Phosphorous uptake		74 ppm
Potassium uptake		215 ppm
Soil texture	Clay	26.4%
	Silt	10.6%

	Sand	63.0%
Soil classification	Clay sandy	
pH of Water irrigation		7.85
Electrical Conductivity of water irrigation		6.10 dS.m <sup>-1</sup>

**Table (1, B): Some chemical and physical properties of the experimental soil and irrigation water of the Al-Hartha site**

Property	Value
pH of Soil	7.9
Electrical Conductivity (EC) of Soil	5.22 dS.m <sup>-1</sup>
Dissolved positive ions	Ca <sup>+2</sup> 7.11 mmol.L <sup>-1</sup>

Symbol	Bio-stimulant Combination
<b>S0</b>	<b>0 Chitosan + 0 Stimplex + 0 Biogrand</b>
<b>S1</b>	<b>3 g L<sup>-1</sup> Chitosan + 0 Stimplex + 1.5 g L<sup>-1</sup> Biogrand</b>
<b>S2</b>	<b>3 g L<sup>-1</sup> Chitosan + 1.5 ml Stimplex + 0 Biogrand</b>
<b>S3</b>	<b>3 g L<sup>-1</sup> Chitosan + 1.5 ml Stimplex + 1.5 g L<sup>-1</sup> Biogrand</b>
<b>S4</b>	<b>6 g L<sup>-1</sup> Chitosan + 0 Stimplex + 1.5 g L<sup>-1</sup> Biogrand</b>
<b>S5</b>	<b>6 g L<sup>-1</sup> Chitosan + 1.5ml Stimplex + 0 Biogrand</b>
<b>S6</b>	<b>6 g L<sup>-1</sup> Chitosan + 1.5 ml Stimplex + 1.5 g L<sup>-1</sup> Biogrand</b>

	Mg <sup>+2</sup>	5.80 mmol.L <sup>-1</sup>
	Na <sup>+2</sup>	18.41 mmol.L <sup>-1</sup>
	K <sup>+</sup>	1.12 mmol.L <sup>-1</sup>
Organic matter		0.86%
Dissolved negative ions	SO <sub>4</sub> <sup>-2</sup>	19.30 mmol.L <sup>-1</sup>
	HCO <sub>3</sub> <sup>-</sup>	2.20 mmol.L <sup>-1</sup>
	Cl <sup>-</sup>	43.05 mmol.L <sup>-1</sup>
	CO <sub>3</sub> <sup>-2</sup>	0.0
Nitrogen uptake		216 ppm
Phosphorous uptake		82 ppm
Potassium uptake		260 ppm
Soil texture	Clay	14.9%
	Silt	14.0%
	Sand	71.1%
Soil classification	Clay sandy	
pH of Water irrigation		7.61
Electrical Conductivity of water irrigation		3.10 dS.m <sup>-1</sup>

**Table (2): Bio-stimulant combinations sprayed on offshoots of banana, Grand Nine cultivar**

**Table (3): The effect of the planting site and bio-stimulant combination and the interaction between them on the percentage of nitrogen (%) in the leaves of the Grand Nain banana cultivar**

Site	Bio-stimulant combination	Site Mean
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Site	Bio-stimulant combination							Site Mean
	S0	S1	S2	S3	S4	S5	S6	
<b>Garmat Ali</b>	2.727	2.817	2.817	2.917	2.901	3.082	3.200	<b>2.923</b>
<b>Al-Hartha</b>	2.833	2.857	2.918	3.014	3.117	3.203	3.663	<b>3.086</b>
<b>Bio-stimulant Mean</b>	2.780	2.837	2.867	2.966	3.009	3.142	3.432	
<b>L.S.D P ≤ 0.05</b>								
<b>Site</b>			<b>Bio-stimulant</b>			<b>Interaction</b>		
<b>0.063</b>			<b>0.118</b>			<b>0.168</b>		

**Table (4): The effect of the planting site and bio-stimulant combination and the interaction between them on the percentage of phosphorus (%) in the leaves of the Grand Nain banana cultivar**

	S0	S1	S2	S3	S4	S5	S6	
<b>Garmat Ali</b>	0.1757	0.2200	0.2213	0.2317	0.2327	0.2253	0.2580	<b>0.2235</b>
<b>Al-Hartha</b>	0.2213	0.2270	0.2240	0.2463	0.2530	0.2567	0.3003	<b>0.2470</b>
<b>Bio-stimulant mean</b>	0.1985	0.2235	0.2227	0.2390	0.2428	0.2410	0.2792	
<b>L.S.D P ≤ 0.05</b>								
<b>Site</b>	<b>Bio-stimulant</b>					<b>Interaction</b>		
<b>0.0081</b>	<b>0.0151</b>					<b>0.0214</b>		

Table (5): The effect of the planting site and bio-stimulant combination and the interaction between them on the percentage of potassium (%) in the leaves of the Grand Nain banana cultivar

Site	Bio-stimulant combination							Site Mean
	S0	S1	S2	S3	S4	S5	S6	
<b>Garmat Ali</b>	0.997	1.203	1.597	1.780	2.360	2.210	2.767	<b>1.845</b>
<b>Al-Hartha</b>	1.413	1.623	2.069	2.243	2.413	2.626	2.946	<b>2.191</b>
<b>Bio-stimulant mean</b>	1.205	1.413	1.833	2.011	2.386	2.418	2.856	
<b>L.S.D P ≤ 0.05</b>								
<b>Site</b>	<b>Bio-stimulant</b>					<b>Interaction</b>		
<b>0.147</b>	<b>0.276</b>					<b>0.390</b>		

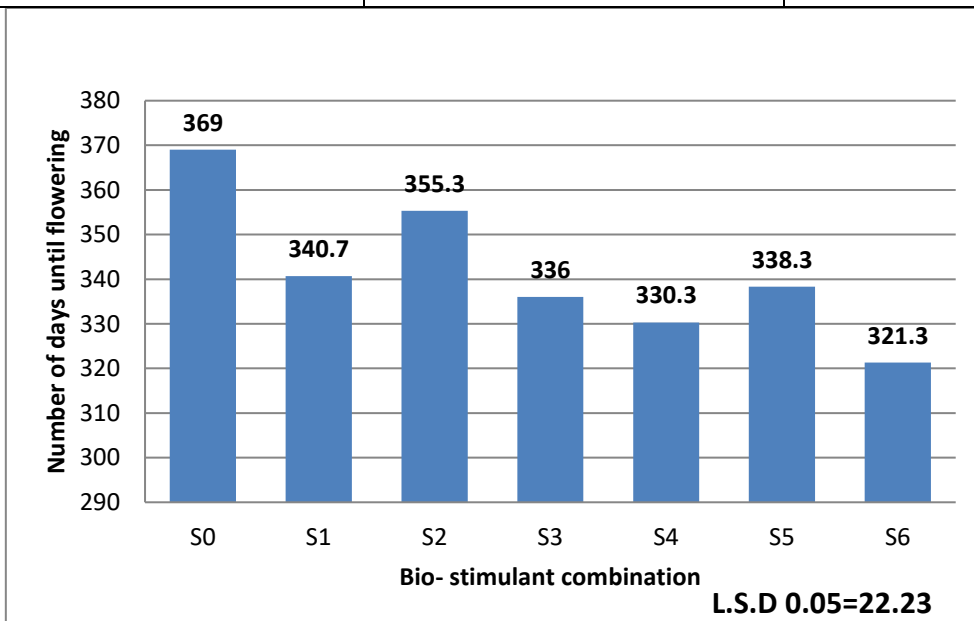


Figure (1): The effect of spraying with bio-stimulant combinations on the number of days until flowering



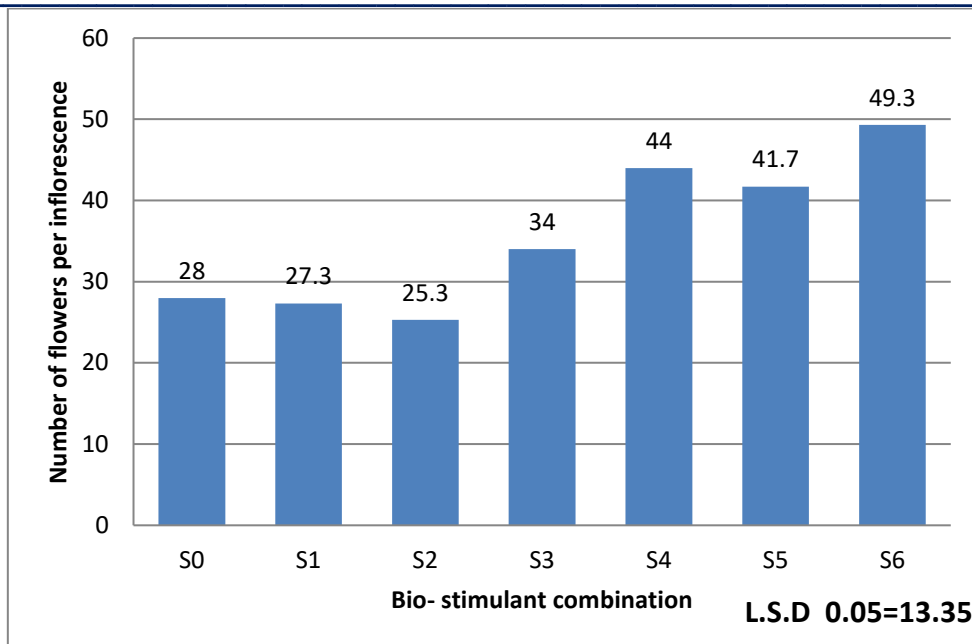


Figure (2): Effect of spraying with bio-stimulant combinations on the number of flowers in an inflorescence

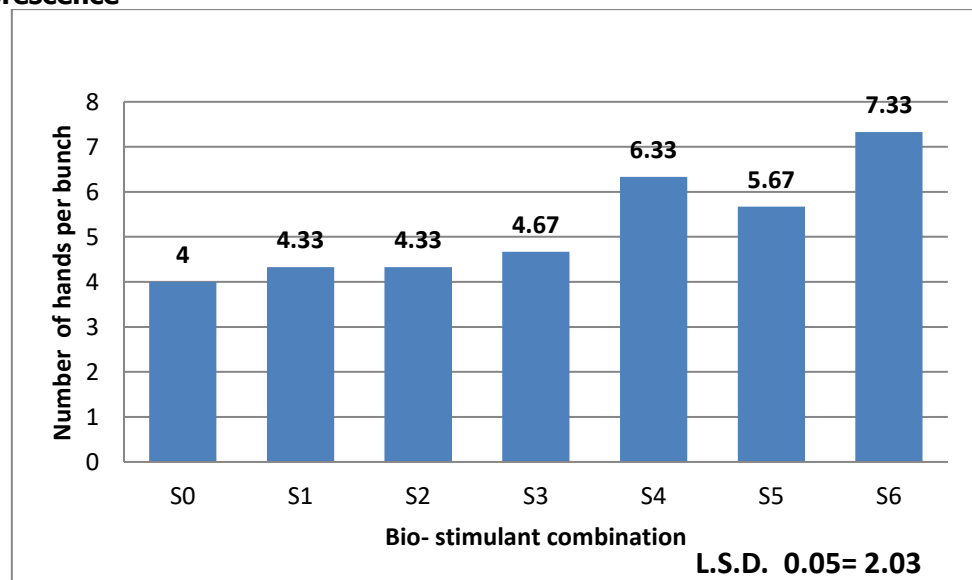


Figure (3): The effect of spraying with bio-stimulant combinations on the number of fruiting hands per bunch



**Plate (1): Fruit setting and the beginning of the formation of fruit hands on the bunch of the banana plant, Grand Nain cultivar**