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EFFECT OF BIO-FERTILIZATION ON THE GROWTH AND SOME FRUIT QUALITATIVE CHARACTERS OF THREE STRAWBERRY *FRAGARIA ANANASSA* DUCH VARIETIES

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ABSTRACT

This study was conducted in Shadow House in one of the private agricultural fields in the province of Najaf in the Abbasid district for the 2020 season for the purpose of studying the effect of biological fertilization with the bacteria *Bacillus subtilis* at four concentrations (0, 5, 10 or 15 ml.L^{-1}) on the vegetative growth and nutritional content and some specific fruit qualitative characteristics of three strawberry varieties (Regina, Robenson and Bulgariana) planted in plastic pots. The results showed that the strawberry cultivars significantly differed among each other in their response to bio-fertilization. Regina cultivar was significantly superior to the other cultivars in number of leaf.$plant^{-1}$, fruit content of TSS, anthocyanin stain and fruit content of vitamin C, while Bulgariana cultivar was significantly higher in leaf content of total chlorophyll, N, P and K compared to the other cultivars. On the other hand, the treatment with the bacterial vaccine led to a significant increase in all the above-mentioned traits, especially when the treatment was 15 ml.L^{-1}, which recorded the highest rates in the total number of leaves, the content of the leaves of chlorophyll and TSS and the fruit content of the anthocyanin stain, vitamin C, the ratio of N, P and K in the leaves. As for the interaction between the three varieties and the bacterial inoculum, the interaction treatment of Regina cultivar and *B. subtilis* at 15 ml.L^{-1} resulted in the highest rate of increase in the number of leaves, TSS, anthocyanin pigment and vitamin C, while the interaction of Bulgariana and the bio-fertilizer at same concentration (15 ml.L^{-1}) was the highest in the characteristic of leaf content of total chlorophyll and the proportion of nitrogen, phosphorus and potassium compared to the control treatment.

***Keywords*:** Strawberry, *Bacillus subtilis*, plant nutrition, fruits

Introduction

Strawberry is one of the smallest and most widespread fruit crops in the world. It belongs to the family Rosaceae and the genus *Fragaria* which includes more than 150 species that it is difficult to separate one from the other. All commercial varieties of strawberries resulted from the hybridization between *Fragaria virginiana* L. and *Fragaria chiloensis* L, which produced strawberries with a thick flesh similar to the pulp of a pineapple, including the name Fragaria x ananassa Duch (1966, Darrow) and all commercial varieties belong to these two types (Scott and Lawrence 2004) And (Khafaji 2000). America ranks first in the production of strawberries and strawberries have nutritional and medicinal importance because they contain anthocyanins necessary for the health of blood vessels, as well as the leaves contain tannic acids, flavones, pectin and sucrose, as well as vitamins (A, B, C Hassan, 2002) and Andrew (1996).

Strawberries contain 90% water and 37% calories, 0.7 g protein, 0.5 g oil, 10 g carbohydrates, 1.3 g fiber and contain vitamins 67 C international units and A 0.07 mg, vitamin B2, B1 0.3 mg, niacin 28 mg, and calcium 27 Amalgam Watt) and Merrill (1963.) Strawberry is one of the most popular soft fruits in the world because of its potential to grow well in different types of soils and suit environmental conditions, whether in tropical, subtropical or temperate regions, and it is produced in most countries of the world. Strawberries have a surface root system and medium spread, and the plant needs fertilization and nutrition in order to give a better yield (Kanani, 1988).

And bio-fertilization by adding biological vaccines is one of the methods that can be used to nourish plants, and it is a method commonly used in many countries such as India, America and Russia. Among the vital vaccines used are bacterial isolates with a highly specialized ability to supply plants with the necessary nutrients, including phosphorus. Bacillus bacteria is one of the most important of these isolates due to its high ability to dissolve phosphorous from insoluble sources, including phosphate rock. Bacteria dissolving phosphates, especially Bacillus megaterium, of interest in plant nutrition, as it is considered one of the vital fertilizers that increase the readiness of a number of nutrients. In addition to its role in reducing environmental pollution resulting from the use of chemical preparations, these bacteria formulations are considered an alternative to chemical fertilizers, which are considered an economic burden. *Pseudomonas, Aspergillus* and *Bacillus* are among the biota that increase the readiness of inorganic phosphorous and which have been shown to have the ability to dissolve calcium phosphate (Khan *et al*., 2007). Studies have proven the positive effect of biological fertilizers, and the biological product of B. subtilis has an effective effect on increasing the fresh and dry weight of the roots and stem of the sweet potato plant (Swain *et al*., 2007).

Zhang *et al*. (2008) showed the efficacy of the biological product of B. subtilis GBO3 in increasing the rate of photosynthesis of Arabidopsis thaliana and raising the level of glucose sugar in the stems and leaves of the plant and that the use of the biological product in treating the seeds led to a reduction in plant aging by reducing the activity of the glucose-destroying enzyme Hexokinase. The results of Al-Abbasi (2014) showed the positive effect of Bacillus bacteria in many vegetative and nutritional characteristics on citrus roots.

The root system and its ability to absorb nutrients from the soil varies between different strawberry varieties, so the variety must be taken into account when adding nutrients. It was found that the strawberry varieties differed in number of leaves, chlorophyll content, diameter of the crowns, root weight and the number of leaves, and this was attributed to the difference in the quality of the elements in the soil (Childers, 1965; Shewfelt, 1999). Other studies showed that different varieties of strawberries differed significantly in the average fresh and dry weight of both the shoot and root systems. This was attributed to the genetic variation among the varieties (Ibrahim, 1996; Borkowska, 2002; Khalifa, 2007; Beirut, 2008). This study aimed to evaluate the effect of biological fertilization with the use of bacteria with psyllus septalis on the growth, nutritional content, and some qualitative characteristics of the fruits in three different varieties of strawberries, namely Bulgariana, Robenson and Regina and to evaluate their success in the conditions of the region.

Materials and Methods

This experiment was conducted in the canopy of a private agricultural field in Al-Abbasiya district, Najaf governorate, for the 2020 agricultural season, to assess the effect of biological fertilization with Basil septalis on the growth and productivity of three varieties of strawberries. Seedlings were obtained from local nurseries for the propagation of commercial fruits, and they were grown in 10 kg of anvils containing soil and moss in a ratio of 1: 1. As for the bio-fertilizer, it includes inoculation with Bacillus subtilis bacteria obtained from the laboratories of the Faculty of Science/University of Kufa. The diagnosed isolation was chosen for which the ability to dissolve phosphorous compounds was carried out by cultivating it on Pikovskays's medium and creating transparent areas around the colonies. The experiment was based on a randomized complete block design (RCBD) and included 12 treatments consisting of three varieties of strawberries (Bulgariana, Robenson, Regina,) and bacterial inoculation with Bacillus subtilis at four levels (0, 5, 10, 15 ml as a soil injection treatment). With three replications for each treatment and the experimental unit consisted of 10 seedlings (360 total).

The experiment lasted for 4 months, from 15/2/2020 to 15/6/2020. At the end of the experiment, data were taken from four plants randomly selected for each experimental unit to study the vegetative and yield quality parameters including, 1) the total number of leaves (leaf.plant^{-1}), 2) leaf content of chlorophyll (mg. Chlorophyll (mg. Liter^{-1}) using the equation: total chlorophyll (mg. Liter^{-1}) = 20.2 x optical density at the wavelength of 645 + 8.02 x optical density at the wavelength of 663. 3) percentage of total dissolved solids (TSS) using Hand Refractometer measured for each harvest and the average was taken (Abbas and Abbas 1992). 4) fruit content Vitamin C (mg. 100 ml juice^{-1}) according to the color method described by Abbas and Abbas (1992). 5) Fruit content of red anthocyanin pigment (mg. 100 g^{-1} fruits) (Ranganna, 1977). 6) leaf content of total nitrogen (%) using Kjeldahl (Al-Sahaf, 1989). 7) leaf content of phosphorous (%) using a Spectrophotometer at a wavelength of 620 nm (Al-Sahaf, 1989). 8) leaf content of potassium (%) using a Flame photometer with a series of standard concentrations (Al-Sahaf, 1989).

Results and Discussion

The results in Table (2) showed the significant effect of the experimental factors that included strawberry varieties (Bulgariana, Robenson, Regina,) and biological fertilization with bacterial inoculation (Bacillus subtilis) and their interactions in all the vegetative growth and nutritional characteristics and specific qualitative traits of strawberry fruits. Regina cultivar recorded the highest rate in the number of total leaves, TSS, anthocyanin stain and vitamin C, with a significant difference from the other cultivars (Table 1). Whereas, Bulgariana cultivar recorded the highest rates for other traits including leaf content of total chlorophyll, nitrogen, phosphorous and potassium, compared with other cultivars that recorded significantly lower rates ($P \leq 0.05$).

As shown in Table 1, the significant effect of bacterial inoculation on the traits under study, and the rate of effect differed according to the concentration used. The biological fertilization treatment at a concentration of 15 ml-liter^{-1} recorded the highest rates in the number of total leaves, the content of the leaves of chlorophyll and T.S.S, the fruit content of anthocyanin and vitamin C and the percentage of nitrogen, phosphorus and potassium in the leaves, with a significant difference from the values obtained from other concentrations.

As for the interaction between the three strawberry cultivars and the bacterial inoculation, the results (Table1) showed that the interaction of cultivar Regina and bio-fertilization at concentration of 15 ml.L^{-1} resulted in the highest values in number of leaves, TSS, fruit content of anthocyanin and vitamin C, with a significant difference from all the other treatments. While the interaction treatment of Bulgariana and bio-fertilization at same concentration was significantly higher in leaf content of total chlorophyll, nitrogen, phosphorus and potassium compared to other treatments.

Findings showed that plant variety has a clear significant effect on the vegetative growth and qualitative indicators of fruits (number of total leaves, percentage of total soluble solids TSS, fruit content of anthocyanin pigment and fruit content of vitamin C). Perhaps the reason for the superiority of Regina variety over the other varieties under study is the presence of the phenomenon of the hybrid strength of this variety and the homogeneity of plant growth in the field. the increase in the percentage of nitrogen in the leaves generally was reflected in the increase in the percentage of chlorophyll and thus the increase in vegetative growth (Darrew, 1966) and (Jasim and Saadoun, 2012). The reason for the different growth measures from one variety to another may be attributed to the variation in genetics of the varieties responsible for the vegetative and specific characteristics of the strawberry plant. While the superiority of the variety (Bulgariana) in chemical indicators (leaf content of chlorophyll and percentage of leaf content of

NPK) may be attributed to the same reasons mentioned in addition to the difference of varieties in certain traits due to the ability to adapt to environmental conditions (Adai and Hamad, 2017). The superiority of Bulgariana in leaf content of nutrients (NPK) may be attributed to this difference in the genetic nature of the cultivars in their ability to absorb nutrients (N, P, K) and this is in agreement with the results of previous studies (Al-Sahaf, 1989; Kader, 1991; Ibrahim,1996; Shewfelt, 1999; Borkowska, 2002; Khalifa, 2007; Beirut, 2008).

The results also showed the clear effect of biological fertilization with bacterial inoculation (Bacillus subtilis) on increasing the measures of all studied traits. This is generally attributed to the role of Bacillus subtilis in increasing nitrogen concentrations and supplying plants with fixed nitrogen, which plays a fundamental role in increasing cell division, cell size and elongation, and increasing plant leaf area, thus increasing the effectiveness of physiological processes such as photosynthesis, which leads to an increase in the quantities of produced total carbohydrates. This will in turn improves plant vegetative growth and nutritional content in addition to qualitative characteristics of the resulting strawberry fruits (Al-Sahaf, 1989). The increase in the nutritional content of the fruits can also be attributed to the role of bacteria Bacillus subtilis in dissolving phosphorus during the period of plant growth and thus the bacteria play an important role in increasing the percentage of phosphorous avialabilty in the soil. The biosynthesis process due to the presence of bacteria increases phosphorous levels ready for plants because the bacteria need phosphorous compounds in process of synthesizing nucleic acids, phospholipids, phytates, energy units (ATP) and other organophosphorous compounds necessary for survival (Prasad and Power, 1997). The results of the study were in agreement with that of Hasan (2012), which showed that Bacillus subtilis increases phosphorous readiness in soil.

Table 1 : Effect of soil bio-fertilization with *Bacillus subtilis* on growth and productivity of three strawberry varieties

Cultivar	Bio-fertilizer B. subtillus $1x10^7.ml^{-1}$	Total number of leaves ($leaf.plant^{-1}$)	Leaf content of chlorophyll ($mg.g^{-1}FW$)	TSS (%)	Fruit content of anthocyanin ($mg.100g^{-1}$ fruit)	Fruit content of v. C ($mg.100ml^{-1}$ juice)	Leaf content (%) of		
							N	**P**	**K**
Regina	0	23.44	32.34	7.18	16.31	46.89	1.834	0.198	1.711
	5	23.59	33.97	7.22	16.48	49.44	1.907	0.207	1.859
	10	24.37	34.78	7.94	17.91	48.52	2.056	0.202	1.884
	15	28.98	36.55	8.80	19.34	52.53	2.023	0.218	1.948
Robenso n	0	21.19	33.89	6.21	14.24	46.94	2.143	0.220	1.928
	5	22.00	34.22	7.00	15.95	48.31	2.159	0.226	1.940
	10	22.56	35.00	6.82	15.45	50.51	2.042	0.249	2.023
	15	24.14	37.21	7.52	17.14	50.82	2.188	0.235	2.112
Bulgaria na	0	20.22	35.68	5.35	11.93	42.21	2.111	0.285	2.202
	5	21.23	36.17	6.14	13.11	43.16	2.007	0.299	2.164
	10	22.10	38.34	6.70	13.00	44.26	2.243	0.275	2.227
	15	22.98	39.89	6.55	14.67	44.65	2.254	0.304	2.316
L.S.D.$_{(P\leq0.05)}$ Interaction		3.16	3.22	1.46	3.53	4.21	0.190	0.021	0.255
Average of bio-fertilizer concentrations effect		21.62	33.97	6.25	14.16	45.35	2.029	0.234	1.947
		22.27	34.79	6.79	15.18	46.97	2.024	0.244	1.988
		23.01	36.04	7.15	15.45	47.76	2.114	0.242	2.045
		25.37	37.88	7.62	17.05	49.33	2.155	0.252	2.125
L.S.D.$_{(P\leq0.05)}$		1.82	1.86	0.84	2.03	2.44	0.110	0.013	0.148
Average of cultivar effect		25.01	34.41	7.79	17.51	49.34	1.955	0.206	1.851
		22.47	35.08	6.89	15.70	49.15	2.133	0.233	2.001
		21.63	37.52	6.19	13.18	43.57	2.154	0.291	2.227
L.S.D. $_{(P\leq0.05)}$		1.58	1.61	0.73	1.76	2.11	0.095	0.011	0.128

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