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Response of 'Rubygem' strawberry cultivar plants to balanced NPK fertilizer and humic acid application under greenhouse conditions

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ABSTRACT

Strawberry plants have high nutritional requirements for their tiny size; therefore, many experiments are carried out to determine the types of fertilizer and their concentrations that improve vegetative growth, yield, and fruits quality of strawberry. This experiment was conducted in the greenhouse belonging to Horticulture and Landscape dept./ College of Agriculture/ Tikrit University/ Iraq, to determine the effect of compound fertilizer (NPK, 20:20:20) and Humic acid on some characteristics of leaves and fruits of Ruby gem strawberry cultivar. NPK fertilizer was added to the soil with three levels (0, 50 and 100 g m^{-2}) and two times, the first before planting and the second was at the flowering stage. 100 ml per plant of humic acid solution was also added two times, the first, three days after planting and the second at the flowering stage, with three levels (0, 5 and 10 ml L^{-1}). The treatments were arranged into Randomized complete Block Design (RCBD) with three replicates. The results obtained that, the NPK fertilizer, Humic acid and their interactions especially the higher levels of them encouraged the studied characteristics and increased significantly leaves area per plant (cm^2), chlorophyll index (SPAD), number of fruits per plant, and TSS% and anthocyanin pigment concentration of fruit flesh. Meanwhile, the previous treatments decreased significantly titratable acidity of fruits. The more effective treatment was fertilizing the plants by 100 g of NPK plus 100 ml of humic acid solution of 10 ml L^{-1}, that it may be recommended to enhance the growth and fruit traits of Ruby gem cultivated under this experiment environment.

استجابة نباتات الشليك صنف Rubygem المزروعة تحت ظروف البيت الزجاجي للسماد المركب NPK وحامض الهيومك

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الخلاصة:

نباتات الفراولة ذات متطلبات غذائية عالية مقارنة بحجمها الصغير، لذا فان العديد من التجارب أجريت لتحديد نوع وكمية الأسمدة المؤثرة في النمو الخضري، الحاصل ونوعية ثمار هذه النباتات. هذه التجربة نفذت في البيت الزجاجي التابع لقسم البستنة وهندسة الحدائق– كلية الزراعة– جامعة تكريت، لتقييم تأثير السماد المركب NPK (20:20:20) وحامض الهيوميك في بعض صفات الأوراق والثمار لنباتات الفراولة صنف Rubygem. اضيف السماد المركب الى التربة بثلاثة مستويات (0، 50 و 100 غم م$^{-2}$) مرتين، الأولى قبل الزراعة والثانية عند التزهير. كما اضيف 100 مل من محلول حامض الهيوميك مرتين، الأولى بعد ثلاثة أيام من غرس الشتلات والثانية عند التزهير، وبثلاثة تراكيز (0، 5 و10 مل لتر$^{-1}$). نظمت المعاملات وفق تصميم القطاعات العشوائية الكاملة بثلاثة مكررات. أظهرت النتائج إن إضافة السماد المركب او حامض الهيوميك او تداخلهما لاسيما المستويات العالية منهما حسنت كثيرا من الصفات المدروسة، فزادت معنويا المساحة الورقية الكلية للنبات (سم^2)، محتوى الأوراق من الكلوروفيل النسبي (SPAD)، عدد الثمار للنبات، وتركيز كل من المواد الصلبة الذائبة الكلية وصبغة الانثوسيانين في الثمار، بينما قللت تلك المعاملات من نسبة الحموضة الكلية في الثمار. إن المعاملة الأكثر تأثيرا في الصفات المدروسة كانت إضافة السماد المركب NPK بمقدار 100 غم م$^{-2}$ مع 100 مل من محلول حامض الهيوميك بتركيز 10 مل لتر$^{-1}$، لذا يمكن التوصية بهذه المعاملة لتحسين نمو وإنتاج نباتات الفراولة صنف Rubygem المزروعة تحت ظروف التجربة والمشابهة لها.

الكلمات المفتاحية: الفراولة، الشليك، السماد المركب، NPK ، حامض الهيوميك.

INTRODUCTION

The strawberry (*Fragaria* × *ananassa*) is one of the most widespread small fruits in the northern zone of earth, sometimes heading north to cold regions. Strawberry varieties differ in their environmental requirements, as most of the commercial varieties are short-day plants. The roots of the strawberry plant are relatively superficial and medium spread, and extend horizontally and vertically for a distance of about 30 cm. The strawberry fruits have a high nutritional value, as it contains sugars, vitamins (especially vitamin C), and organic acids, that the most important is citric acid, proteins, anthocyanins, and mineral elements, in addition to water (Al-Saiedi, 2000; Samra *et al*., 2005).

The strawberry plants are voracious to nutrients, as they are high production plants compared to their small size, therefore they need a good growth media and rich in nutrients. The nutritional status of the soil during the initiation and development of floral buds greatly affects the number of fruits and yield as well as the quality of fruits (Khalil, 2014). Therefore, many studies have been conducted on the role of chemical fertilization to provide plants with the nutrients they need for their growth and production. Some of these studies showed that the strawberry plants respond to chemical fertilization especially NPK compound fertilizer. Medeiros *et al*. (2015)

mentioned that the characteristics of vegetative growth, yield and quality of strawberry plants respond positively to fertilization with nitrogen and phosphorus alone or in combination, and that the greatest effect is in the presence of potassium. The response to fertilization in general may vary depending on the cultivar, soil fertility, type of fertilizer, proportions of elements in it and its quantity, and this can be reached by looking at the results of studies conducted by Ali *et al*. (2003) on Tuff cultivar, Mahadeen (2009) on Ferscka cultivar, and Kumar *et al*. (2015) and Maurya *et al*. (2017) on Chandler cultivar. Al-Douri (2020) applied the NPK fertilizer to almond seedling with three doses (0.4, 0.8 and 1.2 g plant^{-1}) found that these treatments increased the leaves area per plant and the chlorophyll index (SPAD) in leaves compared to untreated seedlings.

In recent decades, many harmfully evidences caused by chemical fertilizers to the environment and humans have been noted, especially excessive fertilization with these fertilizers. Therefore, the focus has been on providing the nutritional needs of strawberry plants from organic sources, directly or indirectly. Decomposed animal residues and plant organic fertilizers are a good source of many nutrients, in addition to their role in improving the physical, chemical and biological properties of the soil. While the organic acids, especially humic and fulvic acids, improve the soil pH and maintain the cation exchange capacity of the soil, thus increasing the availability of the necessary nutrients in the soil solution, which prompted specialists in this field to use it as an attendant or alternative to mineral fertilizers, with the aim of improving plant growth and reducing high costs and the residual effect of nitrate and nitrite that are harmful to human, animal and plant health (Eman *et al*., 2008). A number of researchers used pure humic acid or as one of the components of commercial products with vary concentrations, spraying on the shoots, adding to the soil, or with irrigation water. A number of studies have demonstrated the positive effect of humic acid on the characteristics of vegetative growth, yield and quality of strawberry, including what was done by Al-Sinbol (2012) on Rubygem and Fern cultivars; Farahi (2013) on Aromas cultivar; Eshghi and Garashian (2015) and Rostami *et al*. (2022) on Poras cultivar; Izhar Ullah *et al*. (2017) on Chandler cultivar; Alkharpotly *et al*. (2017) on Festival cultivar; Hameed and Lattif (2018) on Ruby Gem cultivar, Kilic *et al*. (2021) on Albion cultivar, and Al-qadi and Alimam (2023) on the three varieties of strawberry, Festival, Ruby gem and Liberation D, Orleans, which encourages the producers of the strawberry to commercially use the humic acid as an organic fertilizer.

For all this and due to the lack of studies in the study region, the experiment was conducted to find out the magnitude of the response of the Rubygem strawberry plants to the neutral compound fertilizer NPK and humic acid and the interaction between the levels of the two factors.

MATERIALS AND METHODS

Strawberry plants, Rubygem cv., were planted in basins inside the greenhouse belonging to the Department of Horticulture and Landscape- College of Agriculture - University of Tikrit on November 1, 2017, with 40 x 20 cm apart. Every replicate contained nine experimental units, each with five plants grown in sandy soil. Decomposed manure was added at a rate of 5 dm 3 m^{-2}, and mixed with the soil at a depth of 20 cm. After that, the basins were covered with a layer of green saran (plastic net), and it was stretched to maintain a height of 5 cm above the soil surface, then the seedlings were planted through holes were made in this net to prevent the contact of leaves and fruits with the soil.

The experiment included two factors: the first is NPK compound fertilizer (20:20:20), which was added to the soil twice, the first before planting and the second at flowering stage, with three levels: Without the addition as control (C_0), 50 g m^{-2} of soil (C_1) and 100 g m^{-2} of soil (C_2). The second factor is a solution of humic acid, which was added to the soil twice as well, the first, three days after transplanting, and the second at flowering stage, at an amount of 100 ml for each plant, in three concentrations: distilled water as control (H_0), 5 ml L^{-1} (H_1) and 10 ml L^{-1} (H_2).At the end of the experiment, the characteristics were measured as follow: total leaves area of the plant (cm^2) according to Morsi *et al*. (1968), chlorophyll index (SPAD) measured by Minolta-chlorophyll meter (made in Japan), number of fruits (fruit plant-1), and the percentage of total soluble solids (TSS%) in the fruits with a hand refractometer, and total acidity (TA) by titration with sodium hydroxide in the presence of phenolphthalein dye based on citric acid according to Alighourchi *et al*. (2008), and anthocyanin pigment according to Ranganna (1986).

The collected data were analyzed according to the randomized complete block design (RCBD) using the SAS program, and the averages were compared by Duncan's multiple test at a probability level of 5% (Al-Rawi and Khalaf-Allah, 2000).

RESULTS AND DISCUSSIONS

The results in table (1) showed that, the NPK compound fertilizer treatments affect positively the studied characteristics. The addition 100 g m^{-2} of fertilizer gave the height values of leaves area of plant (1275 cm^2), chlorophyll index (46.00 SPAD), number of fruits (17.38 fruit $plant^{-1}$), TSS% (9.17%), and anthocyanin pigment in fresh fruit (18.67 mg 100 g^{-1}), whereas it gave the lowest value of acidity (0.47%). These values varied significantly onto control and the first level of additive fertilizer (50 g m^{-2}) treatment in all studied characteristics. On the other hand, the first level of NPK compound fertilizer (50 g m^{-2}) outperformed the control treatment with all studied characteristics except the total acidity.

Table (1): Effect of NPK fertilizer on leaves area, chlorophyll index, fruits count, and fruits' chemical traits of strawberry

NPK fertilizer (g m^{-2})	leaves area (cm^2 $plant^{-1}$)	Chlorophyll index (SPAD)	Number of Fruits per plant	TSS%	TA%	Anthocyanin (mg 100 g^{-1})
C_0: 0.0	884.00 c	32.00 c	16.58 b	8.27 b	0.59 a	16.68 c
C_1: 50.0	1142.00 b	44.67 b	17.26 a	8.89 a	0.56 a	17.60 b
C_2: 100.0	1275.00 a	46.00 a	17.38 a	9.17 a	0.47 b	18.67 a

Means followed by similar letter(s) are statistically at par with each at 5% levels of significance

The application of humic acid with two levels (5 and 10-ml L^{-1}) led to a significant increase of all estimated characteristics of strawberry plants, except titratable acidity which was decreased when humic acid was applied (Table 2). The two levels of humic acid didn't differ between them into all studied characteristics except the chlorophyll index in leaf. The highest values of the characteristics were at 100 ml L^{-1} humic acid treatment, that leaves area per plant (11346 cm^2),

chlorophyll index (46.67 SPAD), number of fruits (17.95 fruit plant^{-1}), TSS 9.42% and anthocyanin pigment (18.19 mg 100 g^{-1}), whereas this treatment gave the lowest value of total acidity of fruits (0.47%).

Table (2): Effect of humic acid on leaves area, chlorophyll index, Number of fruits per plant, and fruits' chemical traits of strawberry

Humic acid (ml L^{-1})	leaves area (cm^2 plant^{-1})	Chlorophyll index (SPAD)	Number of Fruits per plant	TSS%	TA%	Anthocyanin (mg 100 g^{-1})
H_0: 0.0	1050.00 b	32.00 c	15.61 b	7.62 b	0.63 a	16.66 b
H_1: 5.0	1116.33 a	43.00 b	17.67 a	9.28 a	0.52 b	18.09 a
H_2: 10.0	1134.67 a	46.67 a	17.95 a	9.42 a	0.47 b	18.19 a

Means followed by similar letter(s) are statistically at par with each at 5% levels of significance

The data in table (3) showed that, the interaction treatments between NPK compound fertilizer and humic acid affected significantly on the studied parameters of strawberry plants. The treatments mostly affected were C_2H_2 and C_1H_2. The first effective treatment was C_2H_2 that gave the highest values of responded characteristics were 55.00 SPAD, 18.25 fruit plant^{-1}, 9.80 % and 18.90 mg 100g^{-1} of chlorophyl index, number of fruits per plant, TSS% and Anthocyanin traits respectively, whereas this treatment gave the lowest titratable acidity of fruit (0.40%). The second effectively treatment was C_1H_2 that gave the highest value of leaves area (1300.00 cm^2 plant^{-1}) without significant difference with the C_2H_2 treatment. The two treatments exceeded the control treatment significantly, which was gave the lowest values of total leaves area of plant (870.00 cm^2 plant^{-1}), chlorophyll index (20.00 SPAD), number of fruits per plant, TSS% and anthocyanin content of fruit, and the maximum value of acidity.

Table (3): Effect of interaction treatments between NPK and humic acid on leaves area, chlorophyll index, number of fruits per plant, and fruits' chemical traits of strawberry.

Treatments	leaves area (cm^2 plant^{-1})	Chlorophyll index (SPAD)	Number of Fruits per plant	TSS%	TA%	Anthocyanin (mg 100 g^{-1})
C_0H_0	870.00 d	20.00 h	15.15 e	7.20 e	0.67 a	15.37 d
C_0H_1	1050.00 c	43.00 de	15.78 d	7.72 de	0.62 ab	16.30 c
C_0H_2	1230.00 b	33.00 gf	15.90 d	7.95 d	0.59 ab	18.30 a
C_1H_0	883.00 d	36.00 ef	17.10 c	8.70 c	0.58 ab	17.28 b
C_1H_1	1166.00 b	43.00 de	17.90 ab	9.40 ab	0.56 ab	18.20 a
C_1H_2	1300.00 a	50.00 ab	18.00 ab	9.75 a	0.42 c	18.80 a
C_2H_0	899.00 d	40.00 de	17.50 bc	8.92 bc	0.51 bc	17.38 b
C_2H_1	1210.00 b	48.00 bc	18.10 ab	9.55 a	0.51 bc	18.30 a
C_2H_2	1295.00 a	55.00 a	18.25 a	9.80 a	0.40 c	18.90 a

Means followed by similar letter(s) are statistically at par with each at 5% levels of significance

The values increment of studied characteristics (Leaves area of plant, chlorophyll index, number of fruits per plant, TSS% and anthocyanin content in fruit flesh), and decrement of TA%, referred to a positively response of strawberry plant (Rubygem cv.) to fertilization with NPK compound fertilizer and Humic acid which were added to the planting soil.The fertilization with NPK compound fertilizer means provide three important macro-elements to plants (Nitrogen, Phosphorus and Potassium), these three nutrients together, when they addition to a soil with a balanced amount may controlled vegetative growth, flowering, fruit set, fruits development and maturity, and enhance the chemical and quality characteristics of the fruits. Nitrogen is an essential constituent of amino acids, protein and chlorophyll which is imparts a green color of plant and a main pigment of photosynthesis process. Nitrogen promotes a rapid growth and development of leaves, stem and other vegetative parts of plant (Leghari *et al*., 2016). On the other hand, nitrogen stimulates root growth, so the nitrogen fertilization may be increasing the uptake of potassium and phosphorus and controls overall growth of plant (Bloom, 2015; Hemerly, 2016). Phosphorus is a second essential nutrient after nitrogen.

It is a building block for energy compounds e.g., ATP, ADP, NADP and NADPH. The energy in these compounds is used to produce carbohydrates by photosynthesis process and other energy-demand reactions in plant metabolism. Also, the structure of nucleic acids of DNA, RNA, genes, and chromosomes contain phosphorus. The carbohydrate utilization efficient and its movement in plant dependent on phosphorus adequate in a plant tissues, therefor, phosphorus plays a critical role into nutrient absorption against the forces of osmosis, cell division, leaf expansion and its area, root and shoots growth, fruits development and maturity (Suttenfuss and Doyle, 1991; Grant and Flaten, 2019). Potassium is very important nutrient for cell growth and plant development. it's an activator of dozens of serious enzymes, such as protein synthesis, sugar transport, N and C metabolism, and photosynthesis (Xu *et al*., 2020). K has potent mobility in plants and plays an important role in regulating cell osmotic pressure and balancing the cations and anions in the cytoplasm (Kaiser, 1982; Hu *et al*., 2016), so it is regulating stomatal opening and closing and cell elongation. Through these processes, K is one of regulators of the plant growth, yield and fruits quality (Marschner, 2012; Oosterhuis *et al*., 2014). Finally, the important roles N, P, and K nutrients combined contribute to the process of manufacturing living matter represented by protein in the cells (Jassim and Khuder, 2022), which means contributing to accelerating and increasing cell division and development and produce new tissues for plant growth. The results of the effect of NPK fertilizer on leaves area per plant and chlorophyll content (SPAD) are agreed for Al-douri (2020) study results on almond seedlings.

The improvement of the strawberry studied characteristics by humic acid addition, may be came out the direct and indirect impacts of humic acid and its component. Carbon, Hydrogen, Oxygen, and Nitrogen are the macro-nutrients in the composition of humic acid. The structure of humic acid includes some functional groups (Carboxylic, hydroxyl, amine, amide, and phenolic) that participate in a biological reaction in cells (Pena-Mendez *et al*., 2005). Low acidity of humic acid (3.8 – 5.8) affects the pH of soil and increase an availability and absorption of essential nutrients by roots when used in alkaline soil (Abbas *et al*., 2013). On the other hand, humic acid one of the organic matter components that is play an important role in accumulation and movement of elements cation such us essential nutrients of plant, and supply the plant roots with these cations subsequently (Aljumaily and Al-Hamandi, 2022). Zhang and Schmidt (2000) reported that humic acid contain cytokinins, and endogenous cytokinin and auxin levels will be increased after the strawberry plants are treated with humic acid which possibly led to improving growth and yield of the plants. On the other hand, humic acid compound addition increases a soil fertility by improves

its physico- chemical and biological properties, and encourage a root system of plants (Rengrudkij & Partida, 2003; Tan, 2003). These roles of humic acid in the soil or plant physiology led to improving the quantity and quality of strawberry fruits. According to Abbas *et al*. (2013), adding humic acid to the soil increases the pigments used for photosynthetic activity, which enhances photosynthesis process and plant growth. As photosynthesis increases, more incorporations emerge, which increases the fruit quality features (Abdel-Mawgoud *et al*., 2007). The results of the effect of humic acid on fruits traits were in a line of the results of Al-Qadi and Alimam (2023) when they added the humic acid of two concentrations (3 and 6 ml L^{-1}) for three varieties of straw berry which were included Raby gem.

The interaction treatments between the levels of NPK fertilizer and humic acid were more effectively on the studied characteristics compare to any one of them alone, and appeared the cooperation between them. The result agreed with Rostami *et al*. (2022) findings that, a best effect of humic acid when was take part with nitrogen fertilization, and the interaction treatment between nitrogen and humic acid enhanced the vegetative, roots, fruiting and fruit quality of "Poras" strawberry plants. Pinton *et al*. (1990) and Haghighi *et al*. (2012) suggested that, the humic acid play a role in modulating the absorption of nitrate through interaction with the plasma membrane H^+- ATPase, therefore nitrogen and nitrate absorption are accelerated, increasing nitrogen metabolism and protein production. This role may be benefit with the other essential nutrients of plant.

CONCLUSIONS

Strawberry plants responded well to fertilization with NPK fertilizer and humic acid solution individually or in combination. All treatments positively increased the leaves area and their chlorophyll content, that produced more carbohydrates by photosynthesis process which were reflected in the yield and fruits quality. So, the recommendation is fertilizing the strawberry plants (Rubygem cv.) with a balanced NPK compound fertilizer and humic acid together to achieve high yield with a preferable quality trait of fruits.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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