Tikrit Journal for Agricultural Sciences (2023) 23 (1): 120-129
https://doi.org/10.25130/tjas.23.1.15

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

Effect of some iron and zinc sources on the vegetative growth characteristics of lemon seedlings (Citrus lemon L.)

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KEY WORDS:

lemons, iron sources, zinc sources, vegetative growth.

ARTICLE HISTORY:

Received: 03/07/2022

Accepted: 25/07/2022

Available online: 31/03/2023

ABSTRACT

The experiment was conducted in the lath house of Hawija Horticultural Station/ Horticulture Department/ Ministry of Agriculture, during the 2021 growing season on one-year-old of the lemon Transplants. (local species). This study conducted to compare the effect of some sources of iron and zinc and their interaction on the vegetative growth characteristics of lemon Tranplants. The experiment included two factors: iron sources at four levels (control, ferrous sulfate, chelated iron, nano-iron), and zinc with four levels as well (control, zinc sulfate, chelated zinc, and nano-zinc).The Transplants were sprayed three times during the growing season with the two components from their different sources at a concentration of 50 mg L-1. The experiment was carried out according to Randomized Complete Block Design (RCBD) with four replicates. The data were collected and analyzed statistically and the averages were compared by Duncan's multiple range test at 0.05 probability level. The results showed that spraying with ferrous sulfate led to a significant increase in percent of seedling height,and spraying with chelated iron gave significant superiority in increase percent of stem diameter, and leaf thickness,and spraying with nano iron achieved the highest values in the one leaf area, and the total leaves area which outperformed significantly compared with control treatment. Spraying with chelated zinc gave the largest total leaves area of the seedling, while spraying with nano-zinc gave the highest percentage of increase in stem diameter, seedling height, one leaf area of the seedling, and leaf thickness. Regarding for the interaction,Transplant treated with chelated iron and nano-zinc gave the highest values in the percentage of increase in stem diameter,single leaf area,and leaf thickness.The treatment of the interaction between ferrous sulfate and nano-zinc gave the highest value for the percentage of increase in the seedling height, while the highest value of the total leaves area of the seedling was with the interaction treatment for both nanoparticles of iron and zinc.

INTRODUCTION

Lemon (*Citrus limon* L.) belongs to the genus Citrus belonging to the Rutaceae family, which grows in tropical and subtropical areas (Ibrahim and Khalif, 1995). Lemon is one of the most important and widespread citrus fruits in the world after orange, in Iraq the central region is one of the areas where lemon cultivation is most widespread. It ranks third among the cultivated citrus fruits after orange and bitter orange. The estimated number of lemon trees planted in Iraq, according to the data of the Central Statistical Organization (2020) about 291487 trees and production amounted to 5375 tons, and the average yield 18.4 kg tree^{-1}. The slow growth of fruits and the l Transplant ong duration to reach the stage that suitable for grafting or transporting to the permanent place leads to an increase in the costs of its production, and this leads to use other means to

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accelerate the arrival of the seedling to the appropriate size for grafting, such as spraying the vegetative part of these Transplants with micro-nutrients ,these elements have an important role in all stages of plant development and are essential for growth mainly because of their function as necessary elements ,since lemons are sensitive to the deficiency of microelements such as iron and zinc, and that lemon are not a Transplant ble to obtain an sufficient amount of microelements, whether because of their low quantity in the soil or not being available to the plant, and this is due to many factors, including low temperature, low soil moisture, salinity and high content of calcium carbonate. Therefore, the foliar fertilization process with micro-nutrients, including iron and zinc is an effective way to improve growth by filling in the shortage of these essential elements for the plant (Nasiri et al., 2010 and Fageria et al. 2014). Iron is one of the essential nutrients for plant growth and stimulates the vegetative growth of treated Transplants for its role in the formation of chlorophyll and cytochromes which is important in the process of respiration and photosynthesis as well as its importance in the formation of protein and the synthesis of DNA, which is part of the protein ferredoxin, and has a significant role in carbohydrate metabolism (Jundia**,** , 2003 and Focus, 2003 and Al-Maraqi, 2005). Zinc is also one of the essential micro-elements for optimal plant growth and plays a role in many biochemical reactions inside the plant. It is also important in the formation of auxin growth hormone, cell division, maintaining the structure of cell membranes, and increasing plant height (Cakmak, 1988 and Hassan, 2000 Kessel, 2006). Providing a certain nutrient element requires obtaining it from a chemical or organic source, which is considered a certified fertilizer for this element. Iron sulfate and zinc sulfate were initially used as sources of these two elements in plants fertilization. Then, studies turned to the use of chelating materials because of their known advantages. Finally, attention turned to the use of nano-fertilizers on the basis that small amounts of them can fulfill the purpose of providing plants with these elements (Al-Shalit, 2006).

There are many researchers who have been interested in studying the effect of iron and/or zinc fertilization on vegetative growth and mineral content of different fruit trees and Transplants . Al-Zuhairi et al. (2021) showed that spraying lemon Transplant with three concentrations of Fe_3SO_4 and chelated iron Fe-EDTA (15, 30 mg L^{-1} nano and 30 mg L^{-1} chelated), that a concentration of 30 mg L^{-1} nano led to a significant increase in plant height, number of leaves, and total leaves area. Al-Shammari and Al-Obaidi (2018) found a significant increase in stem length, leaf area, chlorophyll content, and stem diameter after they sprayed grape vines, Helwani specis with ferrous sulfate $FeSO_4.7H_2O$ at a concentration of (100 mg Fe L^{-1}). Al-Kaabi (2006) also obtained a significant increase in the content of total chlorophyll in local orange leaves, one leaf area, and the contents of nitrogen, phosphorous, potassium and zinc when the Transplant sprayed with zinc sulfate ($ZnSO_4.7H_2O$) at levels (0.56 and 1.13). Due to the absence of a comparative study of iron and zinc sources in the vegetative growth characteristics of local lemon Transplant in Iraq, this study was conducted.

MATERIALS AND METHODS

The experiment was conducted in the lath house of Hawija horticultural station, southwest of Kirkuk (43.775180, 35.319498) during the 2021 growing season, on one year old of local variety of lemon Transplant grafted on bitter orange (*Citrus aurantium*) stock, almost homogeneous in size, with an average length of 50 cm and a diameter of 4 mm, the Transplant were brought from one of the private nurseries in al Hawija, planted in two- $dasam^2$ plastic bags on February 15, 2021, turned into eight-$dasam^2$ plastic bags filled with river soil, their characteristics are shown in table (1), the Transplant were distributed in four rows with a distance of 1 m between the rows and 0.5 m between the Transplants , so that each row represented a whole block. Service operations were carried out for all Transplant of irrigation, fertilization, weeding and control with an insecticide (Top Seller) on 1 Transplant 4/9. A balanced compound fertilizer NPK (Russian origin) (15:15:15) was added at the rate of 5 g to each seedling once on 3/4/2021 and urea fertilizer 2 g pot^{-1} 17/4/2021 three times with an interval of three weeks.

Table (1): Some physical and chemical properties of the soil used in the bags

Properties	Value	Measurement units
available nitrogen	9.667	mg kg-1
available phosphorous	28.4	mg kg-1
available potassium	30.48	mg kg-1
Fe	1.494	mg kg-1
Zn	0.475	mg kg-1
TDS	390	mg kg-1
EC	0.56	ds.m-1
pH	7.65	
Clay	10	%
Silt	2	%
Sand	88	%
Organic matter	0.634	%
Soil texture	Loamy Sand	%

Soil analyzed in Kirkuk Agriculture Directorate lab

The experiment included two factors, the first one was iron sources with four levels (control iron (comparison), ferrous sulfate, chelated iron, and nano iron), and the second factor was zinc sources with four levels also (control zinc (comparison), zinc sulfate, chelated zinc, and nano-zinc). Both iron and zinc from all their sources were sprayed at a concentration of 50 mg L^{-1} on the basis of the pure element, three times during the growing season with an interval of three weeks in the early morning with the use of the solubilizing agent (Tween 20) until complete wetness and covering the planting bags to prevent the spray solution from reaching to the soil, the first spray of iron was conducted on March 9, A day later, zinc has sprayed for the first time. The experiment was carried out according to a Randomized Complete Block Design (RCBD) with four replicates (Al-Rawi and Khalaf Allah, 2000). After data collection, statistical analysis was carried out according to the design used by the computer and SAS program, and the averages were compared with Duncan's multiple range test at 0.05 probability level (SAS, 2012).

Measurements

1- Percentage of increase in seedling height (%):

The height of the seedling was measured from the grafting area to the tip of the sapling using a metal tape measure twice, the first before spraying on 3/5/2021 and the second on 10/20/2021. The percentage increase in the height of the seedling was calculated according to the following equation:

Seedling height in the end of experiment (cm) - Seedling height in the beginning of experiment (cm)

100 = ———————————————————— × (%) Percentage of increase in seedling height

Seedling height in the end of experiment (cm)

2- Percentage of increase in stem diameter (%):

The diameter of the stem was measured at a height of 5 cm from the grafting place by electronic Venire for two times, the first measuring was before spraying on 4/3/2021 and the second time on 18/10/2021. The percentage of increase in stem diameter was calculated according to the following equation:

Stem diameter in the end of experiment (mm) – Stem diameter in the beginning of experiment (mm)

100 = ———————————————————— × (%) Percentage of increase in stem diameter

Stem diameter in the end of experiment (cm)

3- Area of leaf cm^2 :

On 22/10/2021, (10) full expansion leaves were collected, then cleaned with a cloth to get rid of dust, they were weighed using a sensitive scale, known area discs were cut, then weighed with an electronic scale, and the area of the leaf (cm^2) was calculated on the basis of wet weight as stated by Morsi et al. (1968) and according to the following equation:

$$\text{One leaf area (cm}^2\text{)} = \frac{\text{Leaves weight (g) x Disc area (cm}^2\text{)}}{\text{Disc weight (g)}}$$

4- Total leaf area of the seedling (cm^2):

The total leaf area of the seedling was calculated by multiplying the area of one leaf x the number of leaves on the seedling.

5- Leaf thickness (mm):

The thickness of 8 leaves was measured from different locations on the seedling, except from the apical top, using the electronic Venire, and the average thickness of these leaves was calculated in the last week of October 2021.

RESULTS AND DISCUSSION

Percentage of increase in seedling height (%):

It is noted from Table (2) that spraying treatment with ferrous sulfate achieved the highest percentage increase in the height of the local lemon Transplants (20.26%), followed insignificantly by spraying with nano iron (19.51%) and that the two treatments significantly outperformed compared with control treatment that gave the lowest value for this trait 13.60%. For spraying zinc treatments, it is noted from the same table that the three treatments of spraying zinc caused an increase in the increase percentage Transplants height and outperformed significantly on control which gave the lowest percentage of increase (9.69%), while the nano zinc treatment gave the highest increase (24.39%) which was superior significantly on both treatments; spraying zinc sulfate and chelated zinc (which did not differ significantly from each other) as well as the control treatment. It is clear from the same table that the interaction treatments between the two factors have achieved positive results in this trait. The interaction treatment between ferrous sulfate and nano-zinc gave the highest percentage of increase (30.57%) and significantly outperformed a number of treatments, including the control, and the interaction of chelated iron without zinc which gave the lowest percentage of increase in seedling height (7.36%) and it did not differ significantly from the control treatment.

Table (2): Effect of spraying with iron and zinc sources and their interaction on the percentage of increase in of local lemon Transplant height (%)

Iron sources	Zinc sources				Iron sources averages
	Control	Zinc sulfate	Chelated zinc	Nano-zinc	
Control	7.74f	13.22c-f	10.50ef	22.95ab	13.60b
Iron sulfate	11.85def	17.01b-e	21.60abc	30.57a	20.26a
Chelated iron	7.36f	22.32ab	16.94b-e	23.39ab	17.50ab
Nano- iron	11.79def	22.89ab	22.69ab	20.67bcd	19.51a
Zinc sources averages	9.69c	18.86b	17.93b	24.39a	

The values of the averages and their interactions with similar letters do not differ from each other significantly according to Duncan's multiple range test at 0.05 probability level.

Ratio of increase in stem diameter (%):

It is clear from Table (3) that the treatments of spraying with iron from its three sources were significantly superior compared with control treatment, and the treatment of chelated iron gave the highest percentage of increase in stem diameter (18.15%) and was significantly superior to all other treatments, while the control treatment gave the lowest increase rate of 7.32% . Spraying zinc treatments also had a significant effect, as all zinc treatments caused a significant increase in the percentage of stem diameter increase compared with control treatment, but the spray nano zinc treatment achieved the highest increase in stem diameter (16.87%) and significantly outperformed all other treatments, while the control treatment gave the lowest percentage for this trait 8.74%. For the interaction treatments between the levels of the two factors, the treatment of the interaction between chelated iron with nano-zinc gave the highest percentage of increase in main stem diameter of the seedling (30.20%) and significantly outperformed compared with all treatments, while the interaction treatment of without iron with chelated zinc gave the lowest percentage of diameter increase which amounted 4.40% and did not differ significantly with control treatment which achieved an increase rate of 6.13%.

Table (3): Effect of spraying iron and zinc sources and their interaction on the percentage of increase in stem diameter of local lemon Transplant (%)

Iron sources	Zink sources				Iron sources averages
	Control	Zinc sulfate	Chelated zinc	Nano-zinc	
Control	6.13 ef	10.41c-f	4.40f	8.36def	7.32c
Iron sulfate	10.21c-f	16.70bc	6.20ef	15.48bcd	12.15b
Chelated iron	12.04cde	13.33cde	17.03bc	30.20a	18.15a
Nano- iron	6.60ef	13.26cde	22.32b	13.47cde	13.91b
Zinc sources averages	8.74c	13.42b	12.49b	16.87a	

The values of the averages and their interactions with similar letters do not differ from each other significantly according to Duncan's multiple range test at 0.05 probability level.

Area of leaf of seedling (cm^2):

The results showed in Table (4) that spraying iron from the three sources was significantly increasing the leaf area compared with control treatment, and spraying nano iron gave the highest value for the area of one leaf (24.33 cm^2) and significantly outperformed on ferrous sulfate and control treatments that achieved the smallest area per leaf for lemon Transplant (21.07 cm^2). Spraying the three sources of zinc were significantly superior compared with control treatment and did not differ from each other significantly, and spraying with nano-zinc gave the largest area per leaf (24.27 cm^2). Whereas, control treatment gave the least one leaf area 20.94 cm^2. The results of the interaction between iron and zinc sources show that the interaction between chelated iron and nano-zinc gave the highest value of one leaf area for local lemon Transplant which amounted 25.98 cm^2, and did not differ significantly with most other treatments, but significantly superior with control which gave lowest value 20.37 cm^2.

Table (4): Effect of spraying iron and zinc sources and their interaction on one leaf area(cm^2) of local lemon Transplant

Iron sources	Zink sources				Iron sources averages
	Control	Zinc sulfate	Chelated zinc	Nano-zinc	
Control	20.37d	21.17cd	20.90d	21.82bcd	21.07c
Iron sulfate	21.27cd	23.51abc	23.89ab	23.45abc	23.03b
Chelated iron	20.88d	23.48abc	25.56a	25.98a	23.97ab
Nano- iron	21.24cd	24.79a	25.49a	25.83a	24.33a
Zinc sources averages	20.94b	23.24a	23.96a	24.27a	

The values of the averages and their interactions with similar letters do not differ from each other significantly according to Duncan's multiple range test at 0.05 probability level.

Total leaves area (cm^2):

The results in Table (5) showed that spraying iron, whether in the form of ferrous sulfate, chelated iron, or nanoparticles were significantly superior on control treatment in total leaf area of lemon Transplant, and did not differ from each other significantly. Also, spraying nano iron gave the highest value for this trait (875.03 cm^2), while the control treatment gave the lowest value of lemon leaf area Transplant which was 676.81 cm^2. The results in the same table show that spraying zinc treatments from different sources achieved a significant increase compared with control treatment, spraying chelated zinc gave the highest value in total leaf area of lemon seedling (862.05 cm^2), and distinguished significantly compared with control treatment which gave the lowest value for this trait 716.45 cm^2. The interaction treatments between iron and zinc had positive results, as the interaction between nano-iron and nano-zinc gave the highest value for this trait (968.67 cm^2) and it was significantly superior to some treatments including control which show the lowest total of seedling leaf area (623.02 cm^2).

Table (5): Effect of spraying iron and zinc sources and their interaction on total leaf area(cm^2) of local lemon Transplant

Iron sources	Zink sources				Iron sources averages
	Control	Zinc sulfate	Chelated zinc	Nano-zinc	
Control	623.02g	714.98efg	723.41efg	645.84g	676.81b
Iron sulfate	687.84fg	909.73ab	925.87ab	895.46abc	854.72a
Chelated iron	786.22def	807.43cde	938.69ab	929.44ab	865.44a
Nano- iron	768.75def	902.44abc	860.25bcd	968.67a	875.03a
Zinc sources averages	716.45b	833.64a	862.05a	859.85a	

The values of the averages and their interactions with similar letters do not differ from each other significantly according to Duncan's multiple range test at 0.05 probability level.

Leaf thickness(mm):

The results in Table (6) showed that there were no significant differences between the iron spraying treatments, and spraying chelated iron gave the highest value of this trait 0.40 mm, and was significantly superior on control, and distinguished significantly compared with control which shows the lowest leaf thickness (0.34 mm). On the other hand, spraying with the three sources of zinc did not differ from each other significantly, but all of them excelled significantly compared with control which led to lowest leaf thickness 0.31 mm, while spraying nano-zinc gave the largest leaf thickness 0.40 mm. Also, the results of the two factors interaction showed that treat with chelated iron and nano-zinc as foliar application gave the highest value for this trait (0.49 mm), and outperformed significantly compared with number of treatments, including nano-iron and without zinc treatment that shows the lowest leaf thickness (0.30 mm) and with control treatment which did not differ from each other significantly.

Table (6): Effect of spraying iron and zinc sources and their interaction on leaf thickness (mm) of local lemon Transplant

Iron sources	Zink sources				Iron sources averages
	Control	Zinc sulfate	Chelated zinc	Nano-zinc	
Control	0.33bcd	0.37bcd	0.35bcd	0.32cd	0.34b
Iron sulfate	0.31cd	0.40abc	0.39bcd	0.38bcd	0.37ab
Chelated iron	0.32cd	0.37bcd	0.42ab	0.49a	0.40a
Nano- iron	0.30d	0.39bcd	0.39bcd	0.40abc	0.37ab
Zinc sources averages	0.31b	0.38a	0.39a	0.40a	

The values of the averages and their interactions with similar letters do not differ from each other significantly according to Duncan's multiple range test at 0.05 probability level.

DISCUSSION

The treatment of spraying with ferrous sulfate led to a significant increase in the character and height of the Transplants (Table 2) that the height of the Transplant resulted from unlimited growth in the stems from the addition of new cells from the division of the terminal meristematic tissues (Abdul-Azim, 1985) that may be due to the role of iron in building chlorophyll in the leaves The higher the content of chlorophyll in the leaves, the higher the photosynthesis process, as well as its entry into the synthesis of many important compounds such as cytochromes and ferrodoxin, which participate in the process of respiration and photosynthesis (Al-Sahhaf, 1989). Ferrous sulfate also contains a percentage of sulfur that enters the composition of amino acids, proteins, carbohydrates and some Coenzymes and its representation in metabolic processes in the plant (Abu Dahi and Al-Younis, 1988 and Kazem, 2016) agrees with Al-Shamri and Al-Obaidi (2018) on the grape vines of the Halawani Cultivar, the treatment of spraying with chelated iron was achieved The highest value in the thickness of the leaf and the percentage increase in the diameter of the stem Table (3, 6) The increase in the diameter of the stem occurs as a result of pressure on the epidermis when secondary abrasion occurs by adding wood to the inside and bark to the outside, and the number of branches results from the growth of interstitial meristem tissues in addition to the role of Chelated iron in preserving the element in a way that facilitates its absorption and transmission by the plant (Al-Shalit, 2006) He agrees with Al-Araji and Al-Hamdani (2012) on peach Transplant , and Raja and Salman (2013) on local orange Transplants. The nano iron gave the largest area per leaf and total leaf area (Table 4, 5). Leaves are exogenous, they arise as superficial protrusions from the developing apical and the increase in the leaf area is the result of the expansion and elongation of cells, which makes the cell wall and the plasma membrane expand by increasing the biological activities due to the presence of some plant hormones and then the entry of water to fill the voids arising in the cell and the swelling pressure has a role in the expansion of the cell wall and membranes (Abdul Azim, 1985).

The reason for this may be due to the small size of the iron nanoparticles whose size is less than 100 nm and thus leads to an increase in the surface area, which facilitates their entry directly into the leaf parts and vital interactions occur inside the cells (Qureshi, 2018) agrees with Abou El-Nasr (2015) on pear Transplant .

Spraying with chelated zinc gave the largest total leaf area in Table (5) for the role of chelated zinc in preserving the element from precipitation in the nutrient solution. This agrees with Imam and Al-Jubouri (2008) on pistachio Transplants and does not agree with him regarding the area of one leaf. Spraying with nano-zinc gave the largest area per leaf. The highest value for leaf thickness, seedling height and stem diameter ,as in table (2, 3, 4, 6). Nano zinc has the ability to increase metabolic processes and the fact that the nanomaterials have diameters ranging between (1-100) nanometers and is characterized by a large surface area that leads to a high reaction speed and increases the absorption of elements with their slow release and leads to an increase in the speed of growth by activating photosynthesis processes and is reflected It encourages vegetative growth (Navarro et al., 2008) Zinc may be considered one of the main components of the plant compound Tryptophane, which is composed of Auxin, which is responsible for plant length through its role in increasing cell division and elongation (Jawad et al., 1988 and McCll et al., 2000) agrees with El-said et al. (2019) on Flame grape vines. seedless and does not agree with him in the content of leaves of chlorophyll and dry weight.

Conclusions:

1- Local lime Transplants respond well to spraying with iron or zinc or both.

2- The effect of foliar spraying with iron or zinc sources did not differ much, whether in the form of sulfate, chelating materials or nanoparticles, on the basic vegetative growth characteristics of the studied Transplants

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مقارنة تأثير بعض مصادر الحديد والزنك في صفات النمو الخضري لشتلات الليمون الحامض (Citrus lemon L.)

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الخلاصة

الكلمات المفتاحية:
الليمون الحامض، مصادر الحديد، مصادر الزنك، النمو الخضري.

أجريت التجربة في الظلة التابعة لمحطة بستنة الحويجة/ دائرة البستنة/ وزارة الزراعة، خلال موسم النمو 2021 على شتلات الليمون الحامض Citrus lemon L.صنف محلي بعمر سنة واحدة. لمقارنة تأثير بعض مصادر الحديد والزنك وتداخلها في صفات النمو الخضري لشتلات الليمون الحامض. تضمنت التجربة عاملين هما مصادر الحديد بأربعة مستويات (بدون، كبريتات الحديدوز، الحديد المخلبي، الحديد النانوي)، والزنك بأربعة مستويات أيضا (بدون، كبريتات الزنك، الزنك المخلبي، والزنك النانوي)، اذ رشت الشتلات ثلاث مرات خلال موسم النمو بالعنصرين من مصادرهما المختلفة بتركيز 50 ملغم لتر-1 . نفذت التجربة وفق تصميم القطاعات العشوائية الكاملة بأربعة مكررات. جمعت البيانات وحللت احصائيا وقورنت المتوسطات باختبار دنكن متعدد الحدود عند مستوى احتمال 0.05، فأظهرت النتائج ان معاملة الرش بكبريتات الحديدوز أدت الى زيادة معنوية في نسبة الزيادة في ارتفاع الشتلة ، وأعطت معاملة الرش بالحديد المخلبي تفوق معنوي في صفة الزيادة في قطر الساق وسمك الورقة، ومعاملة الرش بالحديد النانوي حققت اعلى القيم في مساحة الورقة الواحدة والمساحة الورقية الكلية وتفوقت معنويا على معاملة المقارنة. أعطت معاملة الرش بالزنك المخلبي اكبر مساحة ورقية كلية للشتلة ، بينما أعطت معاملة الرش بالزنك النانوي اعلى نسبة الزيادة في قطر الساق وزيادة في ارتفاع الشتلة ومساحة الورقة الواحدة للشتلة وسمك الورقة، أما بالنسبة للتداخل فقد أعطى التداخل بين الحديد المخلبي والزنك النانوي اعلى القيم في نسبة الزيادة في قطر الساق ومساحة الورقة الواحدة وسمك الورقة ، وأعطت معاملة تداخل كبريتات الحديدوز والزنك النانوي اعلى قيمة لنسبة الزيادة في ارتفاع الشتلة ، في حين كانت اعلى قيمة للمساحة الورقية الكلية للشتلة عند معاملة تداخل الحديد والزنك والنانويين.