EFFECT OF BIOFERTILIZER, TECAMIN MAX AND BENZYL ADENINE ON LEAVES MINERALS OF SWEET ORANGE SEEDLINGS

A. S. Abdulrhman **S. M. Al-Atrushy**
Assist. Prof. **Prof.**
Dept. Hort. Coll. Agric. Engi. Sci. University of Duhok, Iraq
amira.salih@uod.ac **ShawkaT.Mustafa@uod.ac**

ABSTRACT

This research was aimed to study the effect of soil application of Bio health and foliar application with Tecamin max and Benzyl adenine on leaves minerals content of sweet orange (*Citrus sinensis* L.) seedlings during two successive seasons (2020 and 2021) using Randomized Complete Design with four replications. Bio health was added with three concentrations (0, 6 and 12 g.L^{-1}) to the soil and foliar application of Tecamin max with three concentration (0, 5 and 10 ml.L^{-1}) and Benzyl adenine with three concentration (0, 100 and 200 mg. L^{-1}) on the sweet orange seedlings which were brought from private nursery in Duhok city and have two-years old and almost uniform in growth vigor. The results showed that all parameters such as, N, P, K, Ca and Fe leaves content in both seasons were increased significantly as compared with control, accept proline and Na was significantly decreased with increasing application of the bio-health, Tecamin max and Benzyl adenine, Furthermore, combination among high concentration of Bio health, Tecamin max and Benzyl adenine improved all parameters in comparison with the control.

Keyword: Bio health, proline, Cytokinin, nutrition, Citrus, Na, Fe.
Part of PhD dissertation of the first author.

تأثير السماد الحيوي والتيكامين ماكس والبنزل ادينين في محتوى الاوراق من العناصر المعدنية لشتلات البرتقال الحلو

عامرة صالح عبدالرحمن شوكت مصطفى محمد الاتروشي
استاذ مساعد استاذ
قسم البستنة/ كلية علوم الهندسة الزراعية/ جامعة دهوك

يهدف هذا االبحث الى دراسة تاثير اضافة السماد الحيوي الى التربة والرش بالتيكامين ماكس والبنزل ادينين على صفات النمو الخضري لشتلات البرتقال الحلو (*Citrus sinensis* L.) خلال موسمي النمو (2020–2021) باستخدام تصميم قطاعات العشوائية الكاملة وباربع مكررات. اضيف السماد الحيوي الى التربة بثلاث تراكيز (0 و6 و12) غم.لتر$^{-1}$ والرش بالحامض الاميني تيكامين ماكس بثلاث تراكيز (0 و 5 و10 مل.لتر$^{-1}$ وثلاث تراكيز من البنزل ادينين (0 , 100, 200) ملغ.لتر $^{-1}$ على شتلات البرتقال الحلو التي جلبت من مشتل اهلي في مدينة دهوك، عمرها سنتين ومتماثلة تقريبا في قوة النمو. اظهرت النتائج ان اغلب الصفات مثل محتوى الاوراق من (Ca, K, P,N Fe,) في كلا الموسمين ازدادت معنويا مقارنة بمعاملة المقارنة، ماعدا البرولين وNa التي انخفضت معنويا باضافة السماد الحيوي والرش بالتيكامين ماكس والبنزل ادنين في كلا الموسمين، اضافة لذلك فان التداخل بين التركيز العالي من السماد الحيوي والحامض الاميني والبنزل ادينين حسنت كل الصفات مقارنة بالكونترول.

الكلمات الافتتاحية: بايوهيلث، برولين،سايتوكانين، تغذية، حمضيات،صوديوم،حديد.
***البحث جزء من اطروحة دكتوراه للباحث الاول**

Received: 25/3/2022, Accepted:27/7/2022, Published:30 June.

INTRODUCTION

Citrus (*Citrus sinensis* L) fruits are an evergreen fruit tree belonging to the Rutaceae family, which includes a number of genera, Citrus, Poncirus and Fortunella, species belonging to the genus of Citrus are widespread throughout the world because of their adaptation to a wide range of environmental conditions, so citrus cultivation is distreputed in areas under Tropical (arid), subtropical and equatorial regions between latitude of 40^o north and 40^o south equator (7, 10, 24). Citrus genus includes four groups: orange, mandarin, lemon and acid group. Each group includes a number of species that include many varieties and strains (18, 26). The quality of nursery seedling to be planted in the orchard is a major factor determining not only the rate at which the orchard becomes established, but also the level of productivity in the early years (17, 31). The use of well-branched nursery trees for orchard planting can shorten the time to commercial fruit production (11, 28). Thus, by increasing the number of actively-growing shoots, allotted space is filled early; more leaf surface is available for photosynthetic activity, generally enhancing tree canopy development (25) Bio-fertilizers are supposed to be safe alternatives to chemical fertilizers to minimize the ecological disturbance, which produce significant results in many crops (4, 5, 8) Bio-fertilizers are cost effective, eco-friendly and when they are required in bulk could be generated at the farm itself. There is no need of application of bio-fertilizers because partial inoculums are sufficient for growth and manipulation. They improve soil texture, pH and other properties of soil (32). Ennab (14) carried out a field experiment to study the effect of farmyard manure and bio-fertilizers with NPK dose on leaf nutrient content of Eureka lemon trees. Results revealed significant differences amongst various growth attributes, leaf mineral content to farmyard manure and bio-fertilizers application. Moreover, this treatment improved the nutritional status of the trees, through the beneficial effects of organic and bio-fertilizers which enhanced the availability of most nutrients in the soil. 1. AL-Marsoumi and AL-Hadethi.(1) Mentioned that application of biofertilizers on stimulated vegetative growth characteristics in orange transplants. Amino acids (Tecamin Max) have various prominent functions in plants. Besides their usage during protein biosynthesis, they also represent building blocks for several other biosynthesis pathways and play essential roles during signaling processes as well as in plant stress response. In general, the 20 amino acids differ strongly and change dynamically depending on the developmental and physiological state of the plant cell. Besides amino acid biosynthesis, which has already been investigated in great detail, the catabolism of amino acids is of central importance for adjusting their pool sizes but so far has drawn much less attention. The degradation of amino acids can also contribute substantially to the energy state of plant cells under certain physiological conditions, e.g. carbon starvation (19, 27). Alalaf *et al.,* (2) investigate spraying two organic fertilizers viz. Delfan plus and Vegeamino, which contain amino acids, on the vegetative growth of pomelo seedlings using concentrations of 0, 1, 2, 3, and $4ml.l^{-1}$. The results showed that the application of organic containing amino acids especially at the levels of 3 and 4 $ml.L^{-1}$ of Vegeamino, achieved a significant increase in all the studied traits; nitrogen, phosphorous, potassium, protein and iron content in the leaves of pomelo seedlings, except the zinc content of the leaves. Plant growth regulators (PGRs) can be well integrated into orchard production systems. Benzyl adenine, also called 6-benzylaminopurine, is synthetic cytokinin that stimulates cell division in plants. Among other actions, it spurs plant growth, sets blossoms, and improves fruit quality (3). Cytokines stimulate biological processes and inhibit apical dominance and increase lateral branching, as it stimulates the production of nucleic acids RNA synthesis and nitrates production (7and 15). This study was aimed to investigate order effect of combinations of bio-fertilizers, Amino acids and Benzyl adenine on improving the minerals content of sweet orange seedlings and preserving the soil and environment from pollution by using environmentally friendly fertilizers.

MATERIALS AND METHODS

This research was performed during two successive seasons 2020 and 2021 in the lath house of the College of Agricultural Engineering Sciences, Duhok province, Kurdistan region, Iraq, to study the effect of soil application of Bio health with three concentrations (0, 6 and 12 $g.L^{-1}$), and foliar application of Tecamin max with three concentration (0, 5 and 10 $ml.L^{-1}$), Benzyl adenine with three concentration (0, 100 and 200 $mg.L^{-1}$) and their interaction on leaf mineral content of Sweet orange seedling. The selected experimental seedlings of sweet orange were bought from a certified source from the private nursery in Duhok city and have two-year-old, having nearly uniform vigor and health. All seedlings were transferred from plastic bags to the pots with a capacity of 10 kg containing growing medium which prepared by mixing of 1:3 organic manure and river sand. The decided concentration Bio health was dissolved in a liter of water, and then the solution was added to the soil around the seedlings in 20 March for both seasons and was repeated one month later. The decided concentrations of the previous Tecamin max and Benzyl adenine were prepared, then added as foliar application three times per season: first, 24/3 /2020-2021 for Tecamin max and 27 /3 / 2020-2021 for Benzyl adenine and repeated twice in interval one month for the two growing seasons, the spraying was done till the runoff, Tween-20 was used to increase the surface tension of leaves, the control plants were sprayed only by distilled water with Tween-20. Treatments were arranged in a randomized complete block design with four replications, each replicate represented by three seedlings. All the data were analyzed statistically by using SAS programs (29). Duncan‘s multiple range test (DMRT) at 5% level of portability was used to compare the treatments means according to the (6). During the study period, all seedlings received the recommended uniform management practices including irrigation, insecticide, pesticide applications, and removal of weeds. A lath house structure was covered with transparent nylon during the cold winter months from December until March, to provide frost and seedlings cold protection. In the end of each growing season the following parameters were measured; Proline leaf content ($mg.g^{-1}$ Dry Matter), Leaf chemical composition (N, P, K, Fe, Ca, Na).

RESULTS AND DISSCUION

Proline content ($mg.Kg^{-1}$ D. M.): It’s clear from Table (1) that soil application of bio-health in first season lead to increase proline content in leaves of sweet orange seedling, the highest value of proline ($5.033mg.kg^{-1}$) resulted in seedling treated with 6 $g.L^{-1}$ compare to the lowest value ($4.705\ mg.kg^{-1}$) in leaves of seedling treated with 12 $g.L^{-1}$ bio-health which is differ significantly from other concentration. While, in second season there are no significant differences among the concentration of bio-health. Same Table also indicate that foliar application of Tecamin max had no significant effect in first season, whereas in second season, foliar application of Tecamin max at 10 $mg.l^{-1}$ caused a significant decreases in proline content which was ($2.887\ mg.kg^{-1}$) compare to highest proline content ($3.898\ mg.kg^{-1}$) in leaves of un sprayed seedling. Foliar application of benzyl adenine at all concentrations had no significant effect in both seasons. Concerning the interaction effect of soil application of bio-health and foliar application of Tecamin max and benzyl adenine, Table (1) notice that the supreme content of proline ($7.678\ mg.kg^{-1}$) was resulted from application of 0 bio-health +10 $mg.l^{-1}$ Tecamin max + 200 $mg.l^{-1}$ benzyl adenine in first season while the supreme content of proline ($6.055\ mg.kg^{-1}$) in second season was resulted from application 0 bio-health +5 $mg.l^{-1}$ Tecamin max + 200 $mg.l^{-1}$ benzyl adenine.

Table 1. Effect of bio-health, Tecamin max and benzyl adenine on proline content (mg.kg^{-1}) in leaf of sweet orange seedlings

Treatments		2020 season				2021 season			
Bio health	Tecamin max	Benzyl adenine (BA)mg.L^{-1}			Bio × T.	Benzyl adenine (BA)mg.L^{-1}			Bio × T
g.L^{-1}	mg.L^{-1}	0	100	200		0	100	200	
0	0	4.487 bc	4.714 bc	5.317 a-c	4.839 ab	4.731 ab	4.200 a-c	3.881 a-c	4.271 ab
	5	3.599 c	3.532 c	5.350 a-c	4.160 b	3.327 bc	2.671 bc	6.055 a	4.018 ab
	10	5.322 a-c	5.086 a-c	7.678 a	6.029 a	2.234 bc	3.689 a-c	1.911 c	2.612 c
6	0	5.296 a-c	5.261 a-c	5.234 a-c	5.264 ab	6.081 a	4.571 a-c	3.883 a-c	4.845 a
	5	6.804 ab	3.602 c	5.903 a-c	5.436 ab	3.958 a-c	3.578 a-c	2.187 bc	3.241 bc
	10	4.317 bc	4.364 bc	4.520 bc	4.400 b	2.161 bc	2.991 bc	2.336 bc	2.496 c
12	0	5.520 a-c	4.611 bc	4.371 bc	4.834 ab	2.550 bc	2.683 bc	2.499 bc	2.577 c
	5	4.979 a-c	3.659 c	5.079 a-c	4.572 ab	3.260 bc	3.826 a-c	3.266 bc	3.451 bc
	10	3.800 c	4.976 a-c	5.354 a-c	4.710 ab	3.450 bc	3.881 a-c	3.327 bc	3.553 a-c
Bio-health		Bio × BA			Main E of Bio.	Bio× BA			Main E of Bio.
0		4.470 b	4.444 b	6.115 a	5.009 a	3.431 a	3.520 a	3.949 a	3.633 a
6		5.472 ab	4.409 b	5.219 ab	5.033 a	4.067 a	3.713 a	2.802 a	3.527 a
12		4.766 ab	4.416 b	4.934 ab	4.705 b	3.087 a	3.463 a	3.031 a	3.193 a
Tecamin		Tecamin × BA			Main E of Tecamin	Tecamin × BA			Main E of Tecamin
0		5.101 a	4.862 ab	4.974 ab	4.979 a	4.454 a	3.818 ab	3.421 ab	3.898 a
5		5.127 a	3.598 b	5.444 a	4.723 a	3.515 ab	3.358 ab	3.836 ab	3.570 ab
10		4.480 ab	4.809 ab	5.850 a	5.046 a	2.615 b	3.520 ab	2.525 b	2.887 b
Main effect of Benzyl adenine		4.903 a	4.423 a	5.423 a		3.528 a	3.566 a	3.261 a	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Nitrogen content in leaves (%):

It's quite evident from Table (2) that soil application of different concentrations of bio health to sweet orange seedlings have significant effect when compared with the untreated seedlings in both seasons, where the highest value of N% (3.184 and 2.841) respectively in both seasons recorded in seedlings treated with 12 g.L^{-1} and the lowest value of N% (2.303, 1.33) respectively for both seasons recorded in untreated soil seedlings. As for the effect of Tecamin max on seedlings, data in same Table showed that have a significant effect compared with the untreated seedlings. The highest value in first season (2.923%) recorded when treated with 10 ml.L^{-1} Tecamin Max, while in the second season the highest value (2.372%) recorded in seedlings treated with 5ml.L^{-1} Tecamin Max compare to the lowest value (2.694; 1.920 %) for both season respectively recorded with untraded seedling .The results shown in Table

(2) revealed that, generally spraying seedling with benzyl adenine led to increase the N%. The highest values (3.056; 2.382%) respectively in both seasons were recorded in seedlings treated with 200 mg.L^{-1} benzyl adenine compare to the lowest values (2.436; 1.988 %) for both season respectively which recorded with untreated seedling. The interference by spraying among bio health, Tecamin max and benzyl adenine, the highest value (3.509%) in first season recorded when sweet orange seedlings treated with 12g.L^{-1} bio-health +10 ml.L^{-1} Tecamin max + 200 mg.L^{-1} benzyl adenine. while, in the second season the highest value (3.385%) was recorded in the seedlings that treated with 12g.L^{-1} bio health + 5 ml.L^{-1} Tecamin max + 100 mg.L^{-1} benzyl adenine. The lowest value (1.491%) in first season was recorded in untreated seedlings while in the second season (1.169 %) was recorded in seedlings treated with 6g.l^{-1} bio. + 0 ml.l^{-1} Tecamin + 100 mg.l^{-1} benzyl.

Table 2. effect of bio-health, Tecamin max and benzyl adenine on nitrogen content (%) in leaves of sweet orange seedlings

Treatments		2020 season				2021 season			
Bio health g.L^{-1}	Tecamin Max mg.L^{-1}	Benzyl adenine (BA)mg.L^{-1} 0	100	200	Bio × T	Benzyl adenine (BA)mg.L^{-1} 0	100	200	Bio × T
0	0	1.491 f	2.291 e	2.608 de	2.130 c	1.250 e	1.391 e	1.302 E	1.314 D
	5	1.414 f	2.756 c-e	2.898 a-e	2.356 c	1.336 e	1.379 e	1.323 E	1.346 D
	10	1.428 f	2.858 b-e	2.982 a-d	2.423 c	1.349 e	1.375 e	1.346 E	1.356 D
6	0	2.870 a-e	2.846 b-e	3.008 a-d	2.908 b	1.281 e	1.169 e	2.742 b-d	1.730 C
	5	2.578 de	2.982 a-d	3.388 a-c	2.983 b	2.505 cd	2.800 a-d	2.800 a-d	2.702 B
	10	2.898 a-e	2.870 a-e	2.996 a-d	2.921 b	2.603 cd	2.895 a-d	2.814 a-d	2.770 B
12	0	2.760 c-e	3.332 a-c	3.038 a-d	3.043 b	2.325 d	2.913 a-d	2.911 a-d	2.716 B
	5	3.168 a-d	3.010 a-d	3.080 a-d	3.086 b	2.580 cd	3.385 a	3.247 ab	3.071 A
	10	3.318 a-c	3.444 ab	3.509 a	3.424 a	2.663 b-d	2.575 cd	2.968 a-c	2.735 B
Bio health		Bio × BA			Main E of Bio.	Bio × BA			Main E of Bio.
		0	2	4		0	2	4	
0		1.444 f	2.635 e	2.829 c-e	2.303 C	1.312 e	1.382 e	1.323 E	1.339 C
6		2.782 de	2.899 b-e	3.131 a-c	2.937 B	2.129 d	2.288 cd	2.785 ab	2.401 B
12		3.082 a-d	3.262 a	3.209 Ab	3.184 A	2.523 bc	2.958 a	3.042 A	2.841 A
Tecamin		Tecamin × BA			Main E of Tecamin	Tecamin × BA			Main E of Tecamin
0		2.374 c	2.823 ab	2.885 a	2.694 b	1.619 c	1.824 c	2.318 ab	1.920 B
5		2.387 c	2.916 a	3.122 a	2.808 ab	2.140 b	2.521 a	2.457 ab	2.373 A
10		2.548 bc	3.057 a	3.162 a	2.923 a	2.205 ab	2.282 ab	2.376 ab	2.287 A
Main effect of Benzyl adenine		2.436 b	2.932 a	3.056 a		1.988 c	2.209 b	2.383 A	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Phosphorus content in leaves (%):

Data presented in Table (3) shows the application of bio health to the soil increased the P content in leaves especially in the level 12 g.L-1 that gave the highest value (0.228, 0.226) respectively in both seasons when compared with the control. Based on the results that show in Table (3) it's clear that the increasing level of Tecamin max led to increase the P content in leaves in first season, while had no significant effect of Tecamin max in the second season when spraying the sweet orange seedlings. The highest value (0.22 %) recorded when treated with 10 ml.L^{-1}

Tecamin max. According to the data in the Table (3) shows that the application of benzyl adenine in both levels and both growing seasons had no significant stimulatory effect on P %. Recording the interaction effect among the Bio health and foliar application of Tecamin max and Benzyl adenine, the highest value (0.284 and 0.268) was obtained from the interaction of high concentration of these factors in first season and from the interaction of 12g.L-1 + 10 ml.L-1 Tecamin max + 0 mg. L^{-1}Benzyl adenine in second season.

Table 3. Effect of bio-health, Tecamin max and benzyl adenine on phosphorus content (%) in leaf of sweet orange seedlings

Treatments		2020 season				2021 season			
Bio health $g.L^{-1}$	Tecamin Max $mg.L^{-1}$	Benzyl adenine (BA)$mg.L^{-1}$ 0	100	200	Bio × T	Benzyl adenine (BA)$mg.L^{-1}$ 0	100	200	Bio × T
0	0	0.107 b	0.152 ab	0.190 ab	0.150 b	0.113 g	0.114 g	0.120 G	0.115 C
	5	0.162 ab	0.161 ab	0.192 ab	0.172 b	0.121 g	0.137 fg	0.141 Fg	0.133 C
	10	0.175 ab	0.206 ab	0.228 ab	0.203 ab	0.126 g	0.130 g	0.152 e-g	0.136 C
6	0	0.146 ab	0.146 ab	0.192 ab	0.161 b	0.133 g	0.142 fg	0.149 e-g	0.141 C
	5	0.155 ab	0.164 ab	0.170 ab	0.163 b	0.136 fg	0.149 e-g	0.155 d-g	0.147 C
	10	0.187 ab	0.190 ab	0.202 ab	0.193 ab	0.164 c-g	0.182 b-g	0.212 a-f	0.186 B
12	0	0.198 ab	0.205 ab	0.219 ab	0.207 ab	0.219 a-e	0.220 a-e	0.230 a-c	0.223 Ab
	5	0.202 ab	0.206 ab	0.233 ab	0.214 ab	0.254 ab	0.222 a-e	0.228 a-d	0.234 A
	10	0.239 ab	0.271 a	0.284 a	0.265 a	0.268 a	0.185 b-g	0.210 a-f	0.221 Ab
Bio health		Bio × BA 0	2	4	Main E of Bio.	Bio × BA 0	2	4	Main E of Bio.
0		0.148 b	0.173 ab	0.203 Ab	0.175 b	0.120 d	0.127 d	0.137 Cd	0.128 C
6		0.163 b	0.167 ab	0.188 Ab	0.173 b	0.144 cd	0.158 cd	0.172 C	0.158 B
12		0.213 ab	0.227 ab	0.245 A	0.228 a	0.247 a	0.209 b	0.223 Ab	0.226 A
Tecamin		Tecamin × BA			Main E of Tecamin	Tecamin × BA			Main E of Tecamin
0		0.150 b	0.167 ab	0.200 Ab	0.173 b	0.155 a	0.158 a	0.166 A	0.160 A
5		0.173 ab	0.177 ab	0.198 Ab	0.183 ab	0.170 a	0.169 a	0.174 A	0.171 A
10		0.201 ab	0.222 ab	0.238 A	0.220 a	0.186 a	0.165 a	0.191 A	0.181 A
Main effect of Benzyl adenine		0.175 a	0.189 a	0.212 A		0.170 a	0.164 a	0.177 A	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Potassium content in leaves (%):

=Data in Table (4) shows that soil application of bio health, and foliar spraying both Tecamin max and Benzyl adenine at all concentrations led to increase in K content in the leaves of sweet orange seedlings leaves. The highest value (3.486 %) of K content in first season recorded when application of 6$g.L^{-1}$ of bio health, while in the second season the highest value (2.737) was recorded when soil application of 12 $g.L^{-1}$ bio health. But regarding of foliar application of Tecamin max and benzyl adenine, same table show the highest value (3.439, 2.416; 3.40, 2.383 %) respectively in both seasons recorded when foliar spraying with 10 $ml.L^{-1}$Tecamin max and 200 $m.g.L^{-1}$ benzyl adenine. Concerning the combinations effect of the studies factor, the highest increases in K content (4.250, 3.332 %) respectively in both seasons were resulted from the interaction among high concentrations of Bio health, Tecamin max and Benzyl adenine compared to the less results (1.700, 1.408 %) were obtained in untreated seedlings.

Table 4. Effect of bio-health, Tecamin max and benzyl adenine on potassium content (%) in leaves of sweet orange seedlings.

Treatments		2020 season				2021 season			
Bio-health	Tecamin Max	Benzyl adenine (BA)mg.L^{-1}			Bio × T	Benzyl adenine (BA)mg.L^{-1}			Bio × T
g.L^{-1}	mg.L^{-1}	0	100	200		0	100	200	
0	0	1.700 f	2.000 ef	2.525 c-f	2.075 c	1.408 i	1.589 hi	1.714 g-i	1.570 E
	5	2.150 d-f	2.750 b-f	3.050 a-e	2.650 bc	1.653 g-i	1.762 g-i	1.901 f-i	1.772 E
	10	2.800 b-f	2.850 b-f	3.500 a-d	3.050 ab	1.736 g-i	1.925 f-i	1.951 e-i	1.871 De
6	0	2.650 b-f	3.450 a-d	3.750 a-c	3.283 ab	1.627 g-i	1.820 f-i	1.850 f-i	1.766 E
	5	4.225 a	3.350 a-d	3.300 a-e	3.625 a	1.949 e-i	2.011 e-i	2.449 c-f	2.136 Cd
	10	3.500 a-d	3.550 a-c	3.600 a-c	3.550 a	2.100 d-h	2.271 c-g	2.716 b-d	2.362 Bc
12	0	2.750 b-f	3.500 a-d	3.575 a-c	3.275 ab	2.229 c-h	2.689 b-d	2.699 b-d	2.539 B
	5	3.050 a-e	3.950 ab	3.050 a-e	3.350 ab	2.464 c-f	2.672 b-d	2.832 a-c	2.656 B
	10	3.000 a-f	3.900 ab	4.250 A	3.717 a	2.583 b-e	3.133 ab	3.332 A	3.016 A
Bio-health		Bio × BA			Main E of Bio.	Bio × BA			Main E of Bio.
		0	2	4		0	2	4	
0		2.217 d	2.533 cd	3.025 Bc	2.592 b	1.599 e	1.759 de	1.855 De	1.738 C
6		3.458 ab	3.450 ab	3.550 Ab	3.486 a	1.892 de	2.034 cd	2.338 Bc	2.088 B
12		2.933 bc	3.783 a	3.625 Ab	3.447 a	2.425 b	2.831 a	2.954 A	2.737 A
Tecamin		Tecamin × BA			Main E of Tecamin	Tecamin × BA			Main E of Tecamin
0		2.367 c	2.983 bc	3.283 ab	2.878 b	1.754 e	2.032 de	2.087 c-e	1.958 C
5		3.142 ab	3.350 ab	3.133 ab	3.208 ab	2.022 de	2.148 b-d	2.394 a-c	2.188 B
10		3.100 ab	3.433 ab	3.783 a	3.439 a	2.139 b-d	2.443 ab	2.667 A	2.416 A
Main effect of Benzyl adenine		2.869 b	3.256 a	3.400 a		1.972 b	2.208 a	2.383 A	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Sodium content in leaves (%)

The results of current study referred that the untreated sweet orange seedlings gave the highest value of Na (0.0414, 0.0357 %) respectively in both seasons when compared to the seedlings treated with bio health in both concentrations (6, 12 g.L^{-1}). For the foliar application of Tecamin max, same Table shows that there is no significant effect of Tecamin max in both concentrations in first season when compared with the control, whereas in the second season foliar application of Tecamin max led to decrease Na content in leaves, the highest value (0.0316 %) recorded in un-treated seedlings. The foliar application of benzyl adenine shows negative influence in first season the highest value (0.0352 %) recorded in untreated sweet orange seedlings compare to the lowest value (0.0307 %) were resulted from spraying of benzyl adenine at high concentration. and no significant differences in second season, For the interaction each of Bio health, Tecamin max and benzyl adenine same table shows that the highest value (0.0458 %) in the first season recorded in seedlings treated with (0 g.L^{-1} bio health+ 5ml.L^{-1} Tecamin max+ 0 mg.L^{-1} benzyl adenine), and the lowest value (0.0233 %) recorded in seedlings treated with (6 g.L^{-1} bio. + 10 ml.L^{-1} Tecamin + 100 mg.L^{-1} benzyl.). In the same Table show negative effect the above factors in second season, where the highest value (0.051) recorded in the untreated seedlings, and the lowest value (0.021) recorded in seedlings treated with (12g.L-1 bio.+ 0ml.L-1 Tecamin.+ 0 m.g.L^{-1} benzyl.).

Table 5. Effect of bio-health, Tecamin max and benzyl adenine on sodium content (%) in leaves of sweet orange seedlings

Treatments		2020 season				2021 season			
Bio-health	Tecamin Max	Benzyl adenine (BA)mg. L^{-1}			Bio × T	Benzyl adenine (BA)mg. L^{-1}			Bio × T
g.L^{-1}	mg.L^{-1}	0	100	200		0	100	200	
0	0	0.0457 a	0.0378 a-e	0.0361 b-f	0.0398 a	0.0510 a	0.0360 bc	0.0360 bc	0.0410 A
	5	0.0458 a	0.0420 ab	0.0396 a-d	0.0425 a	0.0285 c-f	0.0345 b-d	0.0315 b-f	0.0315 B
	10	0.0450 a	0.0398 a-d	0.0408 a-c	0.0419 a	0.0345 b-d	0.0360 bc	0.0330 b-e	0.0345 B
6	0	0.0328 c-g	0.0258 gh	0.0243 gh	0.0276 bc	0.0390 b	0.0260 c-f	0.0240 d-f	0.0297 Bc
	5	0.0263 gh	0.0245 gh	0.0246 gh	0.0251 c	0.0240 d-f	0.0230 ef	0.0260 c-f	0.0243 C
	10	0.0278 gh	0.0233 h	0.0243 gh	0.0252 c	0.0240 d-f	0.0270 c-f	0.0240 d-f	0.0250 C
12	0	0.0319 d-h	0.0248 gh	0.0239 gh	0.0269 bc	0.0210 f	0.0240 d-f	0.0270 c-f	0.0240 C
	5	0.0310 d-h	0.0320 c-h	0.0308 d-h	0.0313 b	0.0280 c-f	0.0250 d-f	0.0250 d-f	0.0260 C
	10	0.0301 d-h	0.0292 e-h	0.0319 d-h	0.0304 b	0.0240 d-f	0.0270 c-f	0.0220 F	0.0243 C
Bio health		Bio × BA			Main E of Bio.	Bio × BA			Main E of Bio.
		0	2	4		0	2	4	
0		0.0455 a	0.0399 b	0.0388 b	0.0414 a	0.0380 a	0.0355 a	0.0335 ab	0.0357 A
6		0.0290 cd	0.0245 d	0.0244 d	0.0260 c	0.0290 bc	0.0253 c	0.0247 C	0.0263 B
12		0.0310 c	0.0287 cd	0.0288 cd	0.0295 b	0.0243 c	0.0253 c	0.0247 C	0.0248 B
Tecamin		Tecamin × BA			Main E of Tecamin	Tecamin × BA			Main E of Tecamin
0		0.0368 a	0.0295 cd	0.0281 d	0.031 a	0.0370 a	0.0287 b	0.0290 B	0.0316 A
5		0.0344 ab	0.0328 a-d	0.0317 b-d	0.033 a	0.0268 b	0.0275 b	0.0275 B	0.0273 B
10		0.0343 a-c	0.0308 b-d	0.0323 a-d	0.032 a	0.0275 b	0.0300 b	0.0263 B	0.0279 B
Main effect of Benzyl adenine		0.0352 a	0.0310 b	0.0307 b		0.0304 a	0.0287 a	0.0276 A	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Fe content in leaves (mg.kg^{-1} dry wt.):

Data in Table (6) shows that soil application of Bio health and spraying of Tecamin max and Benzyl adenine especially at high concentration (12g.L^{-1}, 10 ml.L^{-1} and 200m.g.L^{-1}) respectively in both seasons led to increase Fe content in leaves of sweet orange seedlings, where recorded it (90.327, 90.131, 91.503, 96.300, 90.714, and 96.449 mg.kg^{-1} dry wt.) respectively for both seasons when compared with the lowest value(86.488, 87.052, 85.769, 87.839, 86.711 and 87.871 mg.kg^{-1} dry wt.) for both seasons respectively were obtained in un treated seedlings. The combination effect among application of the three factors (bio health, Tecamin max and benzyl adenine) data noticed that the maximum Fe (98.345 and 109.958 mg.kg^{-1} dry we.) for both seasons respectively was obtained from the interaction of (6 g.L^{-1}bio health+ 5ml.L^{-1} Tecamin max +200 mg.L^{-1} benzyl adenine) in first season, and from the interaction (6g.L^{-1}bio health+10ml.L^{-1} Tecamin max +200mg.L^{-1} benzyl adenine) in second season. The lowest value (76.920 and 79.883 mg.kg^{-1} dry we.) recorded in both untreated seedlings in both seasons.

Table 6. Effect of bio-health, Tecamin max and benzyl adenine on iron content (mg.kg^{-1} dry wt.) in leaves of sweet orange seedlings

Treatment		2020 season				2021 season			
Bio-health	Tecamin Max	Benzyl adenine (BA)mg.L^{-1}			Bio × T	Benzyl adenine (BA)mg.L^{-1}			Bio × T
g.L^{-1}	mg.L^{-1}	0	100	200		0	100	200	
0	0	76.920 f	84.055 c-f	87.065 a-f	82.680 d	79.883 e	84.060 de	91.533 b-e	85.158 d
	5	79.370 ef	87.115 a-f	91.023 a-d	85.836 cd	81.693 e	83.790 de	93.340 b-e	86.274 cd
	10	86.640 b-f	92.725 a-d	93.480 a-d	90.948 a-c	85.428 b-e	88.143 b-e	95.603 a-e	89.724 b-d
6	0	82.628 d-f	87.364 a-f	90.420 a-e	86.804 b-d	85.248 b-e	92.668 b-e	90.775 b-e	89.563 b-d
	5	88.583 a-e	91.650 a-d	98.345 A	92.859 ab	84.705 c-e	86.165 b-e	102.448 ab	91.106 b-d
	10	93.950 a-d	88.863 a-e	84.003 c-f	88.938 a-c	100.768 a-d	92.218 b-e	109.958 a	100.981 a
12	0	87.123 a-f	87.128 a-f	89.220 a-e	87.823 b-d	87.010 b-e	92.123 b-e	87.250 b-e	88.794 cd
	5	91.865 a-d	87.095 a-f	86.645 b-f	88.535 b-d	92.348 b-e	92.805 b-e	99.853 a-d	95.002 a-c
	10	93.318 a-d	94.330 a-c	96.223 Ab	94.623 a	93.755 a-e	99.048 a-d	101.780 a-c	98.194 ab
Bio health			Bio × BA		Main E of Bio.		Bio × BA		Main E of Bio.
		0	2	4		0	2	4	
0		80.977 b	87.965 a	90.523 a	86.488 b	82.334 d	85.331 cd	93.492 a-c	87.052 b
6		88.387 a	89.292 a	90.923 a	89.534 ab	90.240 b-d	90.350 b-d	101.060 a	88.521 a
12		90.768 a	89.518 a	90.696 a	90.327 a	91.038 b-d	94.658 ab	96.294 ab	90.131 a
Tecamin			Tecamin × BA		Main E of Tecamin		Tecamin × BA		Main E of Tecamin
0		82.223 b	86.182 ab	88.902 A	85.769 b	84.047 d	89.617 b-d	89.853 b-d	87.839 b
5		86.606 ab	88.620 a	92.004 A	89.077 a	86.248 cd	87.587 cd	98.547 ab	90.794 b
10		91.303 a	91.973 a	91.235 A	91.503 a	93.317 bc	93.136 b-d	102.447 a	96.300 a
Main effect of Benzyl adenine		86.711 b	88.925 ab	90.714 A		87.871 b	90.113 b	96.949 a	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

The improve all vegetative growth characteristics of seedlings Local oranges as a result of spraying with a growth stimulator bio-health especially at a concentration of 12 g.l^{-1} could be attributed to Its content of humic acid and algae extract marine and microorganisms, as humic acids contribute to Stimulating growth through its organic compounds and amino acids and mineral elements, especially potassium Which actively participates in many physiological processes like organizing the work of stomata, as it accumulates in the guard cells, It affects the osmotic pressure, so it is with sugars As the driving force for opening and closing stomata (11), and this process directly affects the Water relations within the plant, including water absorption and nutrients from the soil. Also, humic acid increases Cell membrane permeability and nutrient uptake (17, 18), hence the increase in the content of leaves of nutrients. In addition, humic acid Contributes to the activation of chlorophyll pigment formation and synthesis sugars, amino acids and enzymes (10), activating cell division and increasing the rate Growth and development of the vegetative and root system and increase dry matter in plant tissues (19). As for the effect of microorganisms on plant growth it may be due to its ability to secrete a quantity of organizations liberated growth medium (cytokinin and Auxin and gibberellin) and that these secretions play a role in plant cell elongation due to increased plant cell division, Which is positively reflected in improving growth and increasing the process of nutrient uptake (13). The improvement of leaves nutrients content by foliar application of Tecamin max could be due to that Tecamin max as amino acid plays a major role in the mitotic cell division of apical meristems (21). This growth enhancement might be due to stimulation of growth by improving the nutrient efficiency or indirectly by their effect on the cation exchange capacity of plants (7, 28). Increasing leaves nutrients content due to

spraying with benzyl adenine could be ascribed to its role in increasing cell division and elongation and then increasing growth, which is reflected in an increase in the average number of leaves and leaf area, in addition to its physiological role by canceling the apical dominance and also has an important role in delaying the decomposition of proteins and chlorophyll and thus delaying the aging of leaves Which leads to prolonging the life of the leaf by encouraging the movement of nutrients and the continuity of protein synthesis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERNCES

1. AL-Marsoumi; F. S. H, and M E. A. AL-Hadethi. 2025. Response of local orange transplants to bio and organic fertilizers and spraying with moringa leaves extract. Iraqi Journal of Agricultural Sciences, 56(Special), 179-189. https://doi.org/10.36103/cqnr8793

2. Alalaf, A.H.E, A. T. Shayal Alalam and S. M. K. Al-Zebari. 2022. The effect of spraying amino acid fertilizer on the growth characteristics and mineral content of pomelo (*Citrus grandis*) seedlings. Iranian Society of Ichthyology -ISSN: 2383-0964.

3. Al-Janabi, A. M. I. 2018. Effect of foliar application with kinetin and urea on some growth characteristics of cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) rootstock. Journal of Research in Ecology. 6(2): 1952-1964.

4. Al-jubouri; K. D. H, I. J. Abdul Rasool, A. M.H. H. Al-Khafaji, A. J. Abdulsada, F. Y. Baktash, W. H. Hasoon, and Z. J. Al-Mousawi. 2025. Unraveling prolonged irrigation intervals and some sustainable treatments on potato starch composition, growth, and productivity in Iraq. Iraqi Journal of Agricultural Sciences, 56(1), 321-329. https://doi.org/10.36103/s9q1w418

5. Al-Mousawi, Z. J., I. J. Abdul Rasool, A. M.H. H. Al-Khafaji, F. Y. Baktash, and K. D. H. Al-jubouri, 2025. Ecofriendly treatments and drought conditions as a tool for the induction of medicinally active compounds on potatoes. Iraqi Journal of Agricultural Sciences, 56(2), 648-656. https://doi.org/10.36103/qzwfkw53

6. Al-Rawi, K. M. and A. Khalafalla. 2000. Analysis of Experimental Agriculture Design. Dar Al-Kutub Print. Pub., Univ. Mosul . pp:448.

7. Al-Taey, D.K.A., S.S.M. Al-Azawi and Al-Shareefi. 2010. Effect of spraying acetyl salicylic 250 acid on the plant tolerance for salt stress and survival percentage after transplanting of Q34 orange (*Citrus sinensis*). Babylon J. Univ. Pure Appl. Sci. 18(4):1513–1520.

8. Baqir, H. A., M.F.H. AL-Hassan, and J. W. Mahmood. 2024. Role of bio health extract on wheat growth according to Zadok's decimal scale. Res. Crop. 25 (4): 547-552 DOI: 10.31830/2348-7542.2024.ROC-1130

9. Barzegar, T., P. Moradi, J. Nikbakht and Z. Ghahremani. 2016. Physiological response of okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress. Int. J. Hortic. Sci. Technol. 3(2), 187–197. Doi: 10.22059/ijhst.2017.213448.147

10. Carota, G., M. Raffaele, M. Amenta, G. Ballistreri, S. Fabroni, P. Rapisarda, L. Vanella and V. Sorrenti .2020. In vitro effects of bioflavonoids rich lemon extract on pre-adipocyte differentiation. Natural Product Research, 0(0): 1–5. https://doi.org/10.1080/14786419.2020.1721493.

11. Castle, W. S .2010. A career perspective on citrus rootstocks, their development and commercialization. Hort. Sci. 45(1), 11-15. DOI: https://doi.org/10.21273/HORTSCI.45.1.11

12. Chen Y., M. Nobili and T. Aviad. 2004. Stimulatory effect of humic substances on plant growth. In: Magdoft F., Ray R. (eds): Soil organic matter in sustainable. Agriculture. CRC press Washington.

13. Dumas, Y., S. Smail and A. Benamara. 2004. Effect of potassium fertilization on the behavior of three processing tomato cultivars under various watering levels. Acta Hort.13. DOI: 10.17660/ActaHortic.2003.613.22

14. Ennab, H. A. 2016. Effect of organic manures, biofertilizers and NPK on vegetative growth, yield, fruit quality and soil fertility of eureka lemon trees (*Citrus limon* L. Burm).

J.Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 7(10): 767- 774.

15. Fathi, M. A., A. I. Mohamed and. A. Abd El-Bary. 2011. Effect of sitofex (CPPU) and GA3 spray on fruit set, fruit quality, yield and monetary value of "Costata" Persimmon. Nature and Science. Vol.9(8).

16. Ghaffoori, A.T. and A. F. Z. Al-Dulaimy. 2018. Effect of spray of G.gana and bio health on some growth traits of local orange *Citrus sinensis* L. saplings. Iraqi. J.Des.Stud 8 (1). ISSN:1994-7801.

17. Hartmann, H. T., D. E. Kester, F. T. Davies and R. Geneva. 2002. Plant propagation. Principles and practices. 6th. ed. Prentice-Hall, Englewood Cliffs. New Jersey.

18. Hasan, A.M., T.J. Mohamed Ali and D.K.A. Al-Taey .2019. Effects of winter foliar fertilizing and plant growth promoters on element and carbohydrate contents on the shoot of navel orange sapling. International Journal of Fruit Science, 19(1): 1-10. https://doi.org/10.1080/15538362.2019.1668331

19. Hildebrandt, T.M., A. Nunes Nesi, W.L. Arau´ jo, and H.-P. Braun .2015. Amino acid catabolism in plants. Mol. Plant. 8, 1563–1579.

20. Karmegam, M.N. and T. Daliel .2008. Effect of vermicompost and chemical fertilizer on growth and yield of hyacinth bean, lablab purpureus, Sweet dynamic plant, 2(1 and 2): 77-81.

21. Kava, M., M. Atak, K.M. Khawar, C.Y. Cifici and S. Ozean .2005. Effect of pre-sowing seed treatment with zinc and foliar spray of humic acid on yield of common bean (*Phaseolus valgaris* L.) Turkey. Int. J. Agri. Biol. 7 (6): 875-878.

22. Khaled, H. and H. A. Fawzy .2011. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil, Water Res., 6 (1): 21 – 29.

23. Medeiros, A. de S., M. M. F. de. Queiroz, R. A. Araújo Neto, P. S. Costa, A. C. Campos, R. L. S. Feraz, I. D. Magalhães, S. O Maia Júnior, L. D. F Melo and G. B. M Gonzaga. 2018. Yield of the okra submitted to nitrogen rates and wastewater in Northeast Brazilian semiarid region. Journal of Agricultural Science, 10(4), 409–416.

24. Mohamed Ali, T.J. 2018. Effect of organic remnants and zinc application on local orange saplings, (*Citrus Sinensis* L.) grafted on sours orange root stock. Plant Arch 18 (2):1306–1312.

25. Mudzunga, M. J.2000. Enhancement Of Vegetative Growth In Young Citrus Plantings. Thesis presented in partial fulfilment of the requirements for the M.Sc. Degree in Agriculture in the department of Horticultural Science, University of Stellenbosch.

26. Noori, A. M., L. M. Abdul Aziz and M. H. Muhsin. 2018. Effect of phosphorus and gibberellic acid on growth and yield of grape (*Vitis vinifera* L.). Res. on Crops. Regeneration from root explant of Gladiolus (*Gladiolus X grandiflora* Hort.). J. JPN. Soc., 19(4): 643-648.

27. Pratelli, R., and G. Pilot. 2014. Regulation of amino acid metabolic enzymes and transporters in plants. J. Exp. Bot. 65:5535–5556.

28. Quinlan, J.D. 1978. The use of growth regulators for shaping young fruit trees. Acta Hort. 80: 39-49.

29. SAS Institute. 2015. Base SAS 9.4 Procedures Guide. SAS Institute.

30. Siddiqui, Y., S. Meon, R. Ismail, M. Rahmani, and A. Ali. 2008. Bio-efficiency of compost extracts on the wet rot incidence, morphological and physiological growth of okra (*Abelmoschus esculentus* L. Moench). Sci. Hortic., 117: 9–14.

31. Van Oosten, H.J. 1978. Effect of initial tree quality on yield. Acta Hart. 65: 123- 125.

32. Youssef, M.M. A and M.F.M. Eissa .2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. E3 Journal of Biotechnology and Pharmaceutical Research. 5(1):001-006.