

EFFECT OF FOLIAR SPRAY OF SALICYLIC ACID AND SOME MICRONUTRIENTS ON THE LEAFY YIELD, QUALITY AND CHEMICAL COMPOSITION OF LETTUCE.

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ABSTRACT

Two field trials were conducted at Baramoon Experimental Farm, Dakahlia Governorate, Egypt, during the winter seasons of 2004/2005 and 2005/2006 to study the effect of foliar spray of salicylic acid (SA) at rates of 0, 50 and 100 ppm and mixtures of some micronutrients (Fe + Mn + Zn), *i.e.*, 120 ppm Fe + 60 ppm Mn + 60 ppm Zn, 240 ppm Fe + 120 ppm Mn + 120 ppm Zn and untreated plants as well as their interaction on leafy yield, quality and chemical composition of lettuce cv. Balady. Lettuce plants were sprayed two times at 30 and 45 days after transplanting. The sub plots were devoted to the mixtures of some micronutrients. The obtained results indicated that, spraying SA increased all studied traits, except NO₃ accumulation that was significantly decreased. The best results were obtained at 100 ppm SA treatment in both seasons. For micronutrients treatments, foliar application of 240 ppm Fe + 120 ppm Mn + 120 ppm Zn was the most favorable treatment for yield, quality and chemical composition of lettuce. The interaction between the experimental factors had significant effects on all studied traits in both seasons. Foliar treatment of 100 ppm SA with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn was optimal to lettuce leafy yield and quality, especially, reducing nitrate accumulation in leaves, consequently, minimizing the harmful effects on human health.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important green leafy vegetable crop for fresh consumption in Egypt. It offers a cheap but rich source of a number of micronutrients and vitamins (Tarwadi and Agte, 2003). However, it is one of the highest free NO₃⁻ accumulators (El-Awamry, 2002). Nitrate content is an important quality characteristic of leafy vegetables. Nitrate itself is relatively non-toxic but its metabolites may produce a number of health effects (Santamaria, 2006). There are numerous factors affecting the productivity and quality of lettuce plants. Growers may apply huge amounts of mineral nitrogen fertilizers aiming to increase only the quantity of yield without any care by its risk (nitrate accumulation in plant tissues). Even with the application of the recommended dose of nitrogen fertilizer, the high potential of lettuce yield could not be realized and the high NO₃ accumulation could be occurred due to the inadequacy of micronutrients (Hanafy Ahmed *et al.*, 1997; El-Agrodi *et al.*, 2001). Accordingly, to improve lettuce yield and quality and avoid environmental pollution it is necessary to optimize plant nutrition. Finding environmentally safe methods for achieving these aims became an important task that might be affected through specific treatments. Such treatments include the foliar application of organic acids such as salicylic acid (SA) (Hanafy Ahmed *et al.*, 2002) and some micronutrients (Kheir *et al.*, 1991).

Salicylic acid is an endogenous plant substance that can also be applied externally. It is a common plant-produced phenolic compound.

Compounds in this group can function as plant growth regulators. It has an effect on physiological process in plant at low concentration (Raskin, 1992; Arteca, 1996). In this respect, Hanafy Ahmed *et al.* (2002) found that foliar application of SA was effective in increase rocket yield and N, P, K, Fe, Mn, and Zn concentrations in leaves. In addition, SA spray showed its distinct role in increasing nitrate reductase activity and chlorophyll content of tomato plants (Kalarani *et al.*, 2002).

Although micronutrients are needed relatively in very small quantities, their deficiency or excess induce great disorders in the physiological processes of plants (Marschner, 1995; Srivastava and Gupta, 1996). In recent years, micronutrients deficiency problems have been increasing in vegetable crops in Egypt. Some reasons are high pH in soil that reduce the availability of micronutrients especially iron, manganese and zinc, decreased use of farmyard manure on agricultural soil, higher crop yields which increase plant nutrient demands, and use of high analyses NPK fertilizers containing lower quantities of micronutrients contaminants (El-Fouly, 1983). Therefore, the practices of supplying micronutrients to plants through their leaves have been recommended to overcome such problem.

El-Shewy (1981) found that treating lettuce plants with Fe as foliar application enhanced plant growth, increased total yield and more accumulation of N, P, K, and Fe in plant leaves. Kheir *et al.* (1991) on spinach, lettuce, rocket and jew's mallow, found that foliar application of micronutrients solution (1% Fe, 0.5% Mn, 0.25% Zn and 0.1% Cu) reduced the nitrate concentration. In addition, it had a pronounced effect on the contents of N, P, K, Fe, Mn, and Zn and fesh yield. Mahmoud (1993) stated that spraying lettuce plants with Fe at 100 ppm increased significantly leaves contents of N, P, and K compared with control untreated plants. Hanafy Ahmed *et al.* (1997) reported that foliar application of micronutrients had a negative relationship with nitrate accumulation in jew's mallow and radish plants. El-Agrodi *et al.* (2001) found that foliar application of (300 ppm Fe + 100 ppm Mo) increased the fresh weight per lettuce plant, total fresh yield, and N, Fe and Mo contents in the leaves, moreover, it decreased the values of nitrate content. In addition, Sarma *et al.* (2005) found that foliar application of micronutrients increased cabbage yield, quantity of protein and chlorophyll content.

Thus, the present work aimed to study the effects of foliar application of salicylic acid and different doses of some micronutrients (Fe, Mn and Zn) on leafy yield, quality and chemical composition of lettuce.

MATERIALS AND METHODS

Tow field experiments were carried out at Baramoon Experimental Farm, Dakahlia Governorate, Egypt, during the winter seasons of 2004/2005 and 2005/2006, to achieve the study objectives. The experimental soil was clay loam in texture with pH 7.8 and EC 0.8 dSm⁻¹. The available content of Fe, Mn, and Zn were 27, 12, and 1.1 ppm, respectively. The characteristics of the soil were determined using the procedures recommended by Page (1982) and Klute (1986). Lettuce seedling cv. Balady was transplanted when they were five weeks old on 24 and 26 October in both seasons, respectively.

A split plot in randomized complete blocks design with three replications was used. The main plots were assigned to three levels of salicylic acid (SA) (0, 50 and 100 ppm). The sub plots were devoted to the mixture of some micronutrients treatments, *i.e.*, (120 ppm Fe + 60 ppm Mn + 60 ppm Zn), (240 ppm Fe + 120 ppm Mn + 120 ppm Zn), and control (untreated plants). Each sub plot included 5 ridges and 5 m long, occupying an area of 15 m², transplants were set on the two sides of the ridges with a space of 20 cm between each transplant. Each treatment was separated by two guard ridges.

The foliar application of SA and micronutrients were applied at 30 and 45 days after transplanting. The plants were sprayed until dropping by using a hand-sprayer. Bio-film as a wetting agent was applied at 0.5 ml/liter of solution. Micronutrients (Fe, Mn, and Zn) in the form of FeSO₄.7H₂O (20% Fe), MnSO₄.H₂O (32.5% Mn), and ZnSO₄.7H₂O (22% Zn) were used.

Plants were fertilized with ammonium sulfate (20.6% N) at a rate of 300kg/fed., calcium superphosphate (15.5% P₂O₅) at a rate of 250 kg/fed., and potassium sulfate (48% K₂O) at a rate of 100 kg/fed.. These fertilizers were applied at two equal doses; the first was added after 3 weeks and the second after 5 weeks from seedling transplanting. Other agricultural practices were carried out as recommended for the commercial production fields.

At the harvesting time (85 days after transplanting), sample of nine plants were taken at random from the three central rows of each sub plot and the following traits were measured:

- 1- Plant fresh weight.
- 2- Plant height.
- 3- Leaves weight/ plant.
- 4- Accumulation of N, P, K and NO₃ in leaves, which was estimated based on leaves fresh weight and dry matter and element percentage in leaves. Total nitrogen was determined with micro-kjeldahl method according to Chapman and Pratt (1961). Phosphorus was colorimetrically determined followed by Jackson (1973). Potassium was determined using a flame photometer as described by Jackson (1973). NO₃⁻ was extracted using 2% acetic acid and determined according to Singh (1988).
- 5- Contents of iron, manganese, and zinc in leaves, which were measured using atomic absorption spectrophotometer
- 6- Protein percentage: It was calculated based on multiplying N percentage by the conversion factor 6.25.

In addition, plants of plots were weighted in kg/plot to determine yield, then it was converted to estimate yield in ton/fed.

The data were statistically analyzed as split plot design according to Snedecor and Cochran (1982). Comparisons among means of treatments were tested using LSD values at 5% level.

RESULTS AND DISCUSSION

1- Yield and its components:

1.1- Effect of salicylic acid (SA) levels:

Data in Table 1 reveal that, increasing SA levels caused significant increases in plant fresh weight, plant height, leaves weight/plant, and yield/fed. in both seasons. These results are in agreement with those reported by Hanafy Ahmed *et al.* (2002).

1.2- Effect of micronutrients (Fe, Mn, and Zn) mixtures:

Data in Table 1 show that the effect of micronutrients mixtures on plant fresh weight, plant height, leaves weight/ plant, and total yield/fed. was significant in both seasons. The highest values of these traits were obtained from 240 ppm Fe + 120 ppm Mn + 120 ppm Zn treatment followed by 120 ppm Fe + 60 ppm Mn + 60 ppm Zn treatment then untreated plants.

Table 1: Means of lettuce yield and its components as affected by salicylic acid (SA) levels, micronutrients (Fe, Mn, and Zn) mixtures and their interaction during 2004/2005 and 2005/2006 seasons.

Treatments		Plant fresh weight (g)		Plant height (cm)		Leaves weight/plant (g)		Total yield (ton/fed.)	
SA Levels (µM)	Micro-nutrients Mixtures (M)	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006
		0		615	615	33.9	35.2	540	561
50		677	663	35.7	36.7	590	605	46.10	46.07
100		724	707	37.7	38.6	618	630	49.06	48.88
LSD (5%)		22	30	0.8	0.9	15	17	2.07	2.12
	M ₁	565	571	33.4	34.3	475	491	37.20	38.01
	M ₂	673	661	35.9	37.1	590	608	45.85	45.72
	M ₃	778	778	38.1	39.1	684	696	53.85	53.82
LSD (5%)		47	48	1.1	0.9	28	32	3.16	3.36
0	M ₁	511	519	31.7	33.0	427	446	33.53	34.45
	M ₂	619	614	34.0	35.3	543	571	42.20	42.55
	M ₃	715	735	36.0	37.3	651	665	49.48	50.79
50	M ₁	373	578	33.3	34.0	486	504	37.74	38.57
	M ₂	681	668	35.7	36.9	600	613	46.43	46.23
	M ₃	778	767	38.1	39.3	685	697	54.15	53.42
100	M ₁	612	616	35.1	35.9	512	524	40.33	41.02
	M ₂	718	700	37.9	39.2	627	640	48.92	48.37
	M ₃	842	831	40.7	40.7	715	725	57.92	57.26
LSD (5%)		82	83	1.8	1.6	49	55	4.64	5.14

M₁: untreated plants.

M₂: 120 ppm Fe + 60 ppm Mn + 60 ppm Zn.

M₃: 240 ppm Fe + 120 ppm Mn + 120 ppm Zn.

The enhancing effect of micronutrients on yield and its components may be attributed to the important role of these elements in many biochemical and physiological processes as the synthesis of chlorophyll and protein and mineral uptake as well as activity of enzyme systems (Marschner, 1995; Srivastava and Gupta, 1996). These results are in correspondence with those obtained by El-Shewy (1981); Kheir *et al.* (1991); El-Agrodi *et al.* (2001) and Sarma *et al.* (2005).

1.3- Effect of the interaction between SA levels and micronutrients (Fe, Mn, and Zn) mixtures:

The same data in Table 1 indicate that, the interaction had significant effects on yield and its components in both seasons. The highest means of these traits were obtained from the treatment of 100 ppm SA with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn, while the lowest values were obtained from untreated plants in comparison with other treatments.

These pronounced positive effects may be attributed to the direct favorable effect of SA and micronutrients (Fe, Mn, and Zn) on mineral content (N, P, K, Fe, Mn, and Zn) of lettuce leaves (Tables 2 and 3). Those which known to be closely associated with the main internal physiological activities and biosynthesis of chlorophyll, enzymes, amino acids, sugars, ATP, nucleic acids and others (Marschner, 1995; Srivastava and Gupta, 1996). On the other hand, the high values of yield components at these treatments interpret the increase in their total yield/fed. compared with other treatments.

2- Accumulation of N, P, K, and NO₃ in leaves:

2.1- Effect of salicylic acid (SA) levels:

Data in Table 2 indicate that spraying SA increased N, P, and K accumulation and decreased NO₃ accumulation in leaves in both seasons. The plants sprayed with SA at 100 ppm gave the highest values of N, P, and K accumulation with the lowest values of NO₃ accumulation in leaves in comparison with untreated plants, but there are insignificant differences between 50 and 100 ppm SA in N, P, and K accumulation in leaves in both seasons. These results are in line with those obtained by Hanafy Ahmed *et al.* (2002) on tomato plants.

For NO₃ accumulation, this result can be explained on the base that SA increase nitrate reductase activity which may be inhibit NO₃ accumulation in plant as it was reported by Kalarani *et al.* (2002).

2.2- Effect of micronutrients (Fe, Mn, and Zn) mixtures:

From Table 2, it could be seen that the effect of micronutrients mixtures on N, P, K, and NO₃ accumulation in leaves was significant in both seasons. The plants sprayed with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn gave the highest values of N, P, and K accumulation and the lowest values of NO₃ accumulation in leaves in comparison with untreated plants.

These results are in agreement with those obtained by El-Shewy (1981); Kheir *et al.* (1991); Mahmoud (1993) and El-Agrodi *et al.* (2001).

2.3- Effect of the interaction between SA levels and micronutrients (Fe, Mn, and Zn) mixtures:

Data presented in Table 2 indicate that the interaction between SA levels and micronutrients (Fe, Mn, and Zn) mixtures had significant effects on N, P, K, and NO₃ accumulation in leaves in both seasons. Also, it can be noticed that, plants sprayed with 100 ppm SA with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn had the highest values for N, P, and K accumulation and the lowest values for NO₃ accumulation in leaves in comparison with other treatments.

The increases in N, P, and K accumulation may be resulted from increased leaves weight/plant parameter of the same treatment (Table 1). For decreasing NO₃ accumulation, such result may be due to that, foliar application of micronutrients had a negative relationship with nitrate accumulation in lettuce leaves (Kheir *et al.*, 1991 and Hanafy Ahmed *et al.*, 1997). At the same time, SA will allow an increase in the nitrate reductase activity that decreases NO₃ accumulation.

Table 2: Means of N, P, K, and NO₃ accumulation in leaves as affected by salicylic acid (SA) levels, micronutrients (Fe, Mn, and Zn) mixtures and their interaction during 2004/2005 and 2005/2006 seasons.

Treatments		N accumulation (mg/plant leaves)		P accumulation (mg/plant leaves)		K accumulation (mg/plant leaves)		NO ₃ accumulation (mg/kg dry wt)	
SA Levels (ppm)	Micro-nutrients Mixtures (M)	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006
		0		1240	1334	151	165	1370	1441
50		1315	1423	172	184	1453	1525	255	254
100		1370	1447	182	192	1488	1554	236	232
LSD (5%)		72	86	11	11	112	85	9	9
	M ₁	1105	1190	135	147	1187	1248	309	314
	M ₂	1347	1441	138	180	1464	1544	257	254
	M ₃	1474	1574	202	213	1660	1727	224	223
LSD (5%)		120	114	18	17	96	114	12	13
	M ₁	998	1088	116	130	1068	1135	346	358
0	M ₂	1260	1354	147	162	1383	1449	288	293
	M ₃	1463	1563	191	202	1658	1738	262	261
	M ₁	1131	1211	139	150	1219	1275	299	306
50	M ₂	1343	1486	176	185	1481	1561	250	245
	M ₃	1472	1572	202	216	1660	1738	215	212
	M ₁	1185	1271	150	161	1275	1336	281	276
100	M ₂	1438	1483	182	193	1527	1621	231	224
	M ₃	1487	1586	213	221	1661	1705	194	196
LSD (5%)		208	197	30	29	167	198	20	22

M₁: untreated plants.

M₂: 120 ppm Fe + 60 ppm Mn + 60 ppm Zn.

M₃: 240 ppm Fe + 120 ppm Mn + 120 ppm Zn.

3- Contents of Fe, Mn, and Zn and protein percentage in leaves:

3.1- Effect of salicylic acid (SA) levels:

Data in Table 3 show that spraying SA caused significant increases in Fe, Mn, and Zn content and protein percentage in both seasons. The maximum values in this respect were obtained from 100 ppm SA treatments in both seasons, but there are insignificant differences between 50 and 100 ppm SA in Fe, Mn, and Zn content in both seasons.

The positive effect of SA on Fe, Mn, and Zn content was summarized by Hanafy Ahmed *et al.* (2002). In regard to the pronounced positive effect on protein content due to SA may be attributed to the role of SA in increasing the uptake of N and other elements by plants (Tables 2 and 3), consequently, the biosynthesis of protein.

3.2- Effect of micronutrients (Fe, Mn, and Zn) mixtures:

It is evident from the same data in Table 3 that, Fe, Mn, and Zn content and protein percentage in leaves of lettuce plants were significantly enhanced by micronutrients treatments compared with untreated plants in both seasons. The highest values of these traits were resulted from the treatment of 240 ppm Fe + 120 ppm Mn + 120 ppm Zn.

These findings are supported by the results of El-Shewy (1981); Kheir *et al.* (1991); and El-Agrodi *et al.* (2001) and Sarma *et al.* (2005).

Table 3: Means of Fe, Mn, and Zn content and protein percentage in lettuce leaves as affected by salicylic acid (SA) levels, micronutrients (Fe, Mn, and Zn) mixtures and their interaction during 2004/2005 and 2005/2006 seasons.

Treatments		Fe content (mg/g dry wt)		Mn content (mg/g dry wt)		Zn content (mg/g dry wt)		Protein (%)	
SA Levels (ppm)	Micro-nutrients Mixtures (M)	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006	2004/2005	2005/2006
		0		0.94	0.91	0.15	0.14	0.16	0.15
50		1.13	1.07	0.18	0.17	0.19	0.18	25.7	26.5
100		1.20	1.15	0.20	0.19	0.21	0.20	26.5	27.4
LSD (5%)		0.11	0.10	0.017	0.016	0.018	0.018	0.4	0.3
	M ₁	0.82	0.78	0.14	0.13	0.15	0.14	24.7	25.6
	M ₂	1.13	1.07	0.18	0.17	0.19	0.18	24.9	26.6
	M ₃	1.32	1.28	0.21	0.20	0.22	0.21	26.0	27.4
LSD (5%)		0.14	0.14	0.020	0.019	0.021	0.021	0.6	0.5
	M ₁	0.64	0.63	0.11	0.10	0.12	0.11	23.7	24.7
	M ₂	0.99	0.94	0.15	0.14	0.16	0.15	24.9	25.8
	M ₃	1.20	1.15	0.18	0.17	0.20	0.18	26.0	26.8
0	M ₁	0.85	0.80	0.15	0.13	0.15	0.15	24.5	25.6
	M ₂	1.19	1.10	0.18	0.17	0.19	0.18	26.0	26.6
	M ₃	1.34	1.31	0.21	0.20	0.22	0.21	26.6	27.3
50	M ₁	0.96	0.90	0.17	0.16	0.17	0.17	25.7	26.6
	M ₂	1.20	1.18	0.20	0.19	0.21	0.20	26.5	27.4
	M ₃	1.43	1.37	0.23	0.22	0.24	0.23	27.4	28.1
LSD (5%)		0.19	0.18	0.024	0.023	0.024	0.024	1.1	0.9

M₁: untreated plants.

M₂: 120 ppm Fe + 60 ppm Mn + 60 ppm Zn.

M₃: 240 ppm Fe + 120 ppm Mn + 120 ppm Zn.

3.3- Effect of the interaction between SA levels and micronutrients (Fe, Mn, and Zn) mixtures:

The interaction between the two studied factors had significant effect on Fe, Mn, and Zn content and protein percentage in leaves in both seasons. It is clear from Table 3 that the combined treatment of 100 ppm SA with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn produced the highest values for studied traits, whereas the lowest values were obtained from untreated plants in comparison with other treatments.

Such results could be explained on the basis of the fact that, foliar application of micronutrients can be efficiently use as a good management tool to overcome the soil constraints that can reduced micronutrients availability to plants such as high soil-pH, therefore, efficient usage of micronutrients was increased, particularly, at the higher SA level that helps to increase micronutrients uptake (Hanafy Ahmed *et al.*, 2002), consequently, the biosynthesis of protein.

Conclusion

It could be concluded that, spraying lettuce plants at the age of 30 and 45 days after transplanting by 100 ppm salicylic acid with 240 ppm Fe + 120 ppm Mn + 120 ppm Zn was the most favorable treatment for leafy yield, quality and chemical composition under the experimental conditions.

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تأثير الرش الورقي بحامض الساليسليك و بعض العناصر الصغرى على المحصول ائورقي و الجودة و التركيب الكيمائي للخس.

إيهاب عوض الله إبراهيم، محمأ السعيد أبو النصر، سهير السيد عبده الجندي.
قسم بحوث الخضر - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة - مصر.

أجريت تجربتان حقليتان في المزرعة البحثية بالبرامون، بمحافظة الدقيلية خلال الموسمين ٢٠٠٤/٢٠٠٥ و ٢٠٠٦/٢٠٠٥ لدراسة تأثير الرش الورقي بحامض الساليسليك بمعدل صفر، ٥٠، ١٠٠ جزء في المليون وبخليط من العناصر الصغرى (الحديد+ المنجنيز + الزنك) بتركيز ١٢٠ + ٦٠ + ٦٠، و ٢٤٠ + ١٢٠ + ١٢٠ جزء في المليون على الترتيب، وبدون رش، بالإضافة إلى التفاعل بينهما على المحصول الورقي ومكوناته (الوزن الطازج للنبات، ارتفاع النبات، وزن الأوراق للنبات) والمحتويات الكيميائية في الورقة (تراكم النتروجين و الفسفور و البوتاسيوم و النترات، و محتواها من الحديد، المنجنيز، الزنك، و النسبة المئوية للبروتين) لنباتات الخس البلدي، و قد تم الرش مرتين بعد ٣٠، ٤٥ يوم من الشتل. أوضحت النتائج أن الرش بحامض الساليسليك أدى إلى حدوث زيادة معنوية في جميع الصفات المدروسة فيما عدا تراكم النترات في الأوراق التي انخفضت بإضافته، و قد حققت معاملة الرش بحامض الساليسليك بتركيز ١٠٠ جزء في المليون أفضل النتائج. و بالنسبة للرش بالعناصر الصغرى كانت أفضل معاملة هي الرش بخليط الحديد، المنجنيز، الزنك بتركيز ٢٤٠، ١٢٠، ١٢٠ جزء في المليون على الترتيب، فقد أدت هذه المعاملة إلى زيادة المحصول و الجودة لنباتات الخس في كلا الموسمين. كذلك أشر التفاعل بين عاملي الدراسة معنويا على جميع الصفات المدروسة في كلا الموسمين، فقد حققت معاملة الرش بحامض الساليسليك بتركيز ١٠٠ جزء في المليون مع خليط الحديد و المنجنيز و الزنك بتركيز ٢٤٠، ١٢٠، ١٢٠ جزء في المليون على الترتيب أفضل النتائج بالنسبة للمحصول الورقي و الجودة لنباتات الخس، وخاصة خفض تراكم النترات في الأوراق، و بالتالي تقليل التأثير الضار للنترات على صحة الإنسان.