Effect of salicylic Acid and Boron on Growth, Fruit Productivity and Its Quality of Peppers (*Capsicum annuum L.* cv.Twingo F1).
1. Fruit Productivity and Antioxidant Contents

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Abstract: The present investigation aimed to study the effect of foliar spraying with different concentrations of SA, B, and their combinations on pepper plants during two summer seasons of 2006 and 2007. Dry weight/plant and fruit yield/plant as well as some antioxidant compounds including, free phenols, carotenoids, Vitamin C and peroxidase activity in the leaves and fruits in addition to cuticle thickness of pericarip were studied. The results showed that SA (10^{-6} M) with B (5ppm) produced greatest amount of dry weight, fruit yield as well as the measured antioxidant compounds in the fruits and enhanced the cuticle thickness of pericarip as well. The accumulation of these different compounds in fruits may be improved the nutritional values of pepper fruits. Therefore, for producing a pepper fruits with high-quality should use combination of SA and B at low concentrations as foliar spray on the pepper plant.

Keywords: pepper, Salicylic acid, Boron, and antioxidants.

INTRODUCTION

Pepper *Capsicum annuum* L. ; Solanaceae is an important vegetable crop, not only because of its economic importance, but also for the nutritional value of its fruits, which an excellent source of natural colors and antioxidant compounds, like phenolics, vitamin C and carotenoids (Howard *et al,* 2000 ; Navarro *et al.,* 2006). The intake of these compounds in food is an important health-protecting factor. They have been recognized as being beneficial for prevention of widespread human diseases including cancer. Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers (Namiki 1990), cardiovascular diseases, when taken daily in adequate amounts (Bramley, 2000 and Sies, 1991), and consequently, are essential antioxidants that protect against propagation of the oxidative chain. It is also known that vitamin C play an important role in pepper fruits as, chelates heavy metal ions (Namiki, 1990, Rietjens *et al.,* 2002), reacts with singlet oxygen and other free radicals, and suppresses peroxidation (Bielski *et al.,* 1975), reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Harris,1996). Carotenoids play an important role in fruit coloring and act as antioxidants, reacting with free radicals, mainly peroxide radicals and singlet molecular oxygen (Namiki, 1990 & Russo and Howard, 2002).

Several studies strongly suggest that SA and other salicy- lates as a simple ubiquitous plant phenolic and it is considered as hormone-like substances play an important role in many biological responses in plants. SA has been reported to regulate a number of processes including plant growth and development, seed germination, fruit yield and quality, glycolysis, flowering, disease resistance, ethylene production, salinity tolerance and heat production in thermogenic plants (Raskin, 1992; Klessig and Malamy, 1994 & Hayat and Ahmad 2007). Ion uptake and transport (Harper and Balke, 1981), photosynthetic rate, stomatal conductance and transpiration (Khan *et al.,* 2003) could also be affected by SA application.

The effect of SA on plant growth, fruit yield and its quality has been discussed by several researchers (Kord and Hathout 1992, Zhang *et al,* 2003). It is reported that SA positively affected the antioxidants, for example, exogenous treatment with SA in low concentration enlarged activity of some antioxidant enzymes (peroxidase and superoxid dismutase) in barley leaves exposed to herbicide stress (Ananieva *et al.,* 2004) and carrot exposed to salinity and boron toxicity (Eraslan *et al.,* 2007). In addition to, 1 mM SA induced catalase activity and H_2O_2 in tobacco leaves during 3 hours after treatment demonstrated his role in plant defense mechanisms (Chen and Klessing, 1991).

Micronutrients can affect the plant biochemistry and physiology, which can influence the resistance or tolerance mechanisms of plants to pathogens. Boron (B) is an essential micronutrient for plant growth and development, pollen germination and growth of pollen tubes as well as it is involved in a number of metabolic pathways and functions such as cell wall synthesis and structure, lignification, carbohydrate metabolism, phenol metabolism, plasma membrane integrity, salt tolerance and plant disease (Marschner, 1995, Dordas and Brown, 2005, Stavrianakou *et al.,* 2006, Simoglou and Dordas, 2006, López-Gómez *et al.,* 2007 & Singh *et al.,* 2007).

In view of some afore-mentioned reports which indicated that SA or B could alleviated stress factors by stimulation of the antioxidants content (Eraslan *et al.,* 2007 & López-Gómez *et al.,* 2007), to further the present study examined the effects of exogenous application of the SA, B or their interaction effect through foliar application which could improve pepper fruit yield for food safety and antioxidants content for prevention of widespread human diseases.

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MATERIALS AND METHODS

Plant material, treatments and growth conditions:

The experiment was carry out under the screenhouse conditions at the experimental station of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt during the summer seasons of 2006 and 2007. Seeds of pepper *Capsicum annum* L. Solanaceae cv. Twingo F1; provided from the Egyptian Vegetable Research Center, Ministry of Agriculture, Egypt were germinated in styrophom trays under the screenhouse conditions. The trays were filled with a soil mixture (modified cornell peat-lite mixes in 1:1 ratio, Hartmann *et al.,* 1990). After emerging, seedlings were watered with a commercial nutrient solution (19-19-19 N-P-K with micronutrient) at a dilution of 1:200. The seedlings were maintained under 58-85% humidity and with a day/night temperature ranged from 20-35 °C for six weeks. Uniform seedlings (45 days old) were transplanted in hills 0.25 m apart. Four plots, each 40 m^2 (2 ridges, each 20 m in length and 1.0 m in width) were prepared at the beginning of June in both seasons. Plots were distributed using complete randomized block design. Soil texture was sandy (91% sand, 3.% clay and 6% silt), pH 8.27, EC 3.48 dSm^{-1}, Ca 20.87 meq^{-1}, Mg 9.78 meq^{-1}, K 0.884 meq^{-1}, Na 3.25 meq^{-1}, HCO_3 6 meq^{-1}, Cl 7.28 meq^{-1} and SO_4 21.52 meq^{-1}.

In the screenhouse, temperature, relative humidity and light density were fluctuated between 25-40 °C, 58-85% and 25-30 klux respectively, during the experiment. Plants 75 days old (at initiation of flowering stage) were sprayed with different concentrations of salicylic acid (0.0, 10^{-6} and 10^{-4} M) and / or Boron (0.0, 5.0 and 10.0 ppm) as well as their interactions. Borax $Na_2B_4O_7.10H_2O$ was used as a source of Boron (10.6% B). Solution of boron, salicylic acid and their interaction with a surfactant triton 0.1% were sprayed three times with two weeks intervals on the whole plant shoot. The volume of sprayed solution was approximately 50 ml per plant each time. The same amount of dionized water plus triton 0.1% was used as a control. All sprays were done in the morning (8-9 a.m.). Plants were received irrigation and fertilization as recommended by Ministry of Agriculture.

Measurements and Observations:

All plant samples were taken after 150 days from sowing for the following measurements:

Plant biomass:

Shoot dry weight of 10 plants from each replicate were determined using gravimetric method. Dry weight was determined after fixing plant material at 90 °C and drying (at 70 °C) up to a constant weight.

Yield and fruit quality:

Marketable green-yellowish fruits were harvested at 105 days from transplanting then fruit weight /plant was recorded.

Antioxidants analysis:

All Chemicals analysis were estimated in the 3rd leaf from the plant tip and first fruit formed in the plant (at main stem).

a) Ascorbic acid concentration (mg/g F.W.) in the fruits was estimated according to (Pearson 1970).

b) Carotenoids were extracted from the fresh leaves and fruit pericarp by acetone 85% and determined spectrophotometricaly according to Lichenthaler and Wellburn (1983), and then calculated as mg/g DW.

c) Ethanolic extract (96% ETOH) of pepper leaves and fruit pericarps were prepared according to Abdel-Rahman *et al.,* (1975), then the free phenols determined spectrophotometricaly at 650 nm with folin- ciocalteu reagent according to (William *et al.,* 1965).

Peoxidase activity determination

Preparation of peroxidase extract:

0.5 g fresh leaves and fruit pericarp were homogenized in a mortar and pestle with 0.1 M phosphate buffer (pH 6.5) at 4 °C and stirred for 20 min (Urbanek *et al.,* 1991). The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18000g for 15 min, 4 °C. The supernatant was used for determination of enzyme activity.

Peroxidase [E.C. 1.11.1.7] assay:

The reaction mixture consisted of 3.5ml of 0.1 M phosphate buffer (pH 6.5), 0.3 ml of 0.1 % o-dianisidine solution, 0.2 ml of enzyme extract and 0.2 ml of 0.2 M hydrogen peroxide solution (Urbanek *et al.,* 1991). The reaction mixture was incubated at 30 °C for 10 min and the oxidation of O-dianisidine measured by changes in optical density at 430 nm (Beckman DK-2 Spectrophotometer). Corrections were done for the oxidation rate of O-dianisidine in the absence of H_2O_2 in the reaction mixture. Soluble peroxidase activity was expressed as optical density per milligram of protein per minute. One unit of peroxidase activity (AU) was taken as the change of 1.0 unit of optical density per minute.

Anatomical studies

Fruit pericarp specimens from second year were killed and fixed in F.A.A, then dehydrated in ethyl alcohol series, embedded in Paraffin wax, sectioned to thickness of 15μ, double stained with Safranin and Light green, cleared in Xylene and mounted in Canada balsam according to Willey (1971).Cuticle measurements were calculated by eyepiece micrometer.

Data analysis:

Data were statistically analyzed using ANOVA/MANOVA of Statistica 6 software (Statsoft, 2001) with mean values compared using Duncan's multiple range test with a significance level of at least $p \leq 0.05$.

RESULTS

Effect of SA, B and their interactions on some antioxidants contents in leaves and fruits of pepper plants:

The data (Figure 1) proved that no treatment with either of SA or B and their interaction combination enhanced carotenoids and free phenols content over the control treatment. In most cases the control treatment had high carotenoids and free phenols content in the leaves. However, in fruit high vitamin C, carotenoids and free phenols were occurred under low concentration of SA in combination with low concentration of B (5 ppm) in both seasons. Fruit antioxidants were increased by 48% and 289%, 51% and 75% in addition to 48% and 81% respectively in both seasons. In most cases, either of SA or B at their tested concentrations did not improve the content of carotenoids and free phenols in pepper fruits, except only SA at 10^{-6} which increased fruit carotenoids content in the first season and B at 10 ppm increased fruit carotenoids content in the second season. The same trend also was true for peroxidase activity in leaves and fruits which were significantly increased when the pepper plants sprayed with the combination of SA and B at low concentration compared with control, where peroxidase activity increased by 82% in leaves and 67.4% in fruits. However, peroxidase activity in leaves and fruits did not significantly affect when pepper plants sprayed with SA or B alone (Figure 1).

Fruit antioxidant traits positively correlated to each other (Table 2), especially vitamin C and carotenoids content. Such results appear to support that these traits are associated for their response to the treatments with SA, B and their interactions.

Effect of SA, B and their interactions on growth, fruit yield and cuticle thickness of pericarp:

High values of shoot dry weight appeared in plants which foliar sprayed either with SA at low concentration alone or in combination with boron at 5 ppm than those treated with boron alone or control plants in both seasons. The positive effect of low level of SA on shoot dry weight amplified when SA combined with low level of boron (5 ppm) in the second season. The combination of SA (10^{-6}) and boron (5 ppm) enlarged shoot dry weight by 15.9% and 90.4% in both seasons, respectively. The lowest fruit yield (483.35 and 283.35 g/plant) was obtained in plants under control, and the maximum values (780 and 610.9 g/plant) in plants sprayed with SA + B at low concentration, where the increasing percentage was 61.6% and 115.6% in fruit weight per plant over the control treatment in both years, respectively (Table 1). Also, the significant highest values of cuticle thickness of fruits pericarp were recorded with the treatments of SA at low concentration alone (125μm) or in combination with B at low concentration (127μm). However the lowest values were observed in control plants and treated plants with SA at high concentration (Table 1 and Figure 2).

Correlation coefficient Table (2) indicated that the fruit yield is highly correlated with the shoot dry weight (r =0.59*), also, the yield significant positively correlated with vitamin C and carotenoids (r = 0.57* and 0.36*, respectively).

Table (1): Effect of SA, B and their interactions on shoot dry weight, fruit weight per plant and pericarp thickness of pepper fruits.

Treatments		Shoot Dry Weight (g/plant)		Fruit Characters		
				Fruit Weight (g/plant)		Cuticle thickness(μm)
B	SA	2006	2007	2006	2007	2007
0.0	0.0	26.14 bc	18.41 c	483.4 ef	283.4 d	62.5 d
	10^{-6}	33.51 a	31.73 ab	700.0 ab	508.4 b	125.0 a
	10^{-4}	30.00 ab	22.37 c	566.5 de	408.3 c	64.7 d
5.0	0.0	27.65 bc	19.74 c	405.0 f	449.2 c	111.3 b
	10^{-6}	30.30 ab	35.05 a	780.0 a	610.9 a	127.0 a
	10^{-4}	27.45 bc	22.98 c	592.5 cd	450.0 bc	95.1 c
10.0	0.0	22.65 c	25.30 bc	575.0 d	600.0 a	112.2 b
	10^{-6}	30.73 ab	25.15 bc	758.0 a	608.4 a	93.4 c
	10^{-4}	23.35 c	23.57 c	665.0 bc	408.35 c	93.5 c

Values are the means of three replicates. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test

Table (2): Correlation coefficients between traits of pepper plants (cv. Twingo F1) treated with SA, B and their interactions.

	Antioxidants							Shoot Dry Weight
	Leaves			Fruits				
	Carotenoids	Free Phenols	Peroxidase activity	Vitamin C	Carotenoids	Free Phenols	Peroxidase activity	
Leaf Free Phenols	0.05							
Leaf Peroxidase	-0.43*	-0.03						
Fruit Vitamin C	-0.26	0.10	0.32					
Fruit Carotenoid	-0.33*	0.17	0.27	0.68*				
Fruit Free Phenols	-0.20	0.38*	0.55*	0.22	0.45*			
Fruit Peroxidase	-0.11	0.09	0.39*	0.45*	0.32	0.33		
Shoot Dry Weight	-0.13	0.14	0.18	0.66*	0.44*	0.23	0.10	
Fruit Weight	-0.30	0.23	0.27	0.57*	0.36*	0.11	0.14	0.59*

*significant at 0.05 probability levels

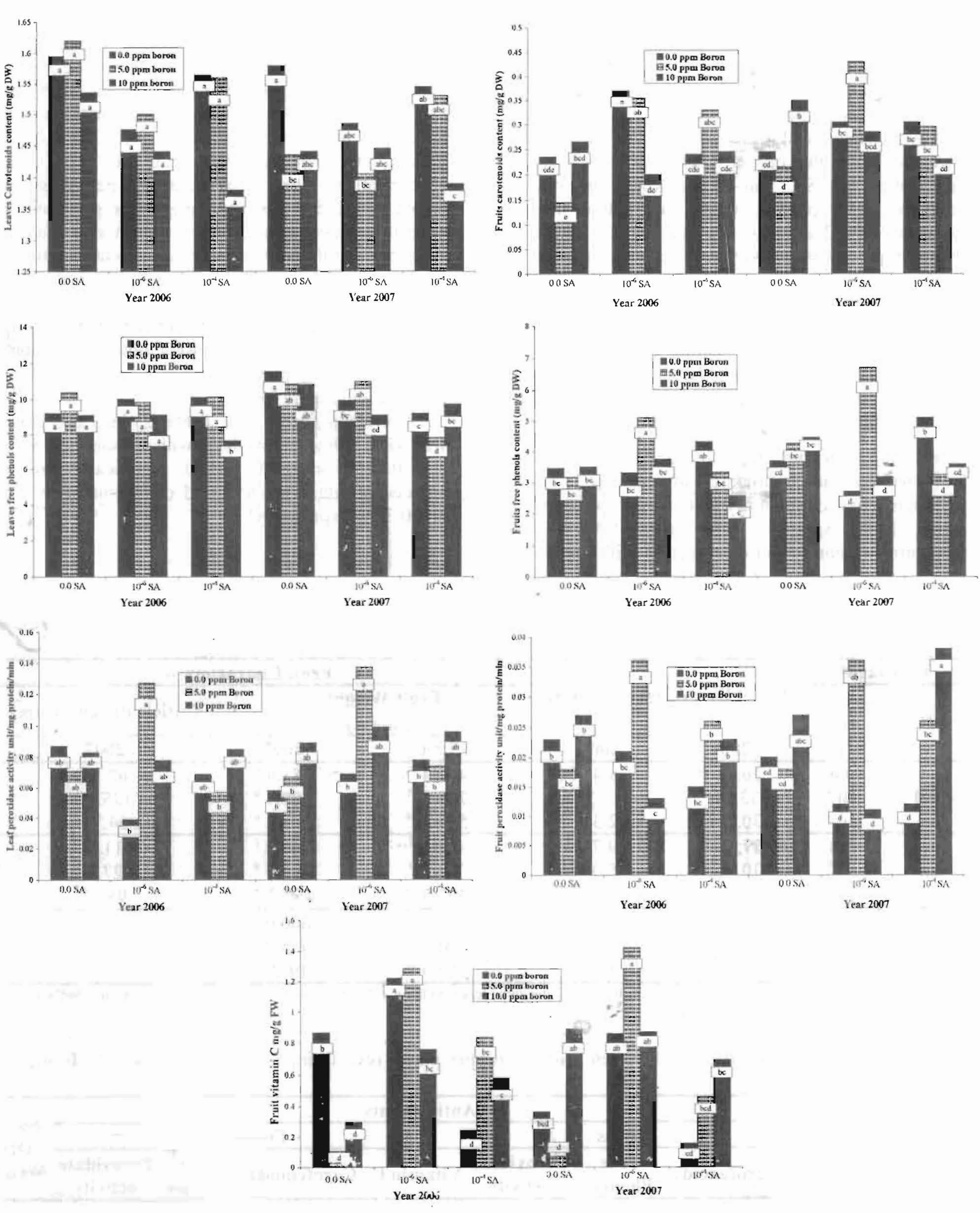
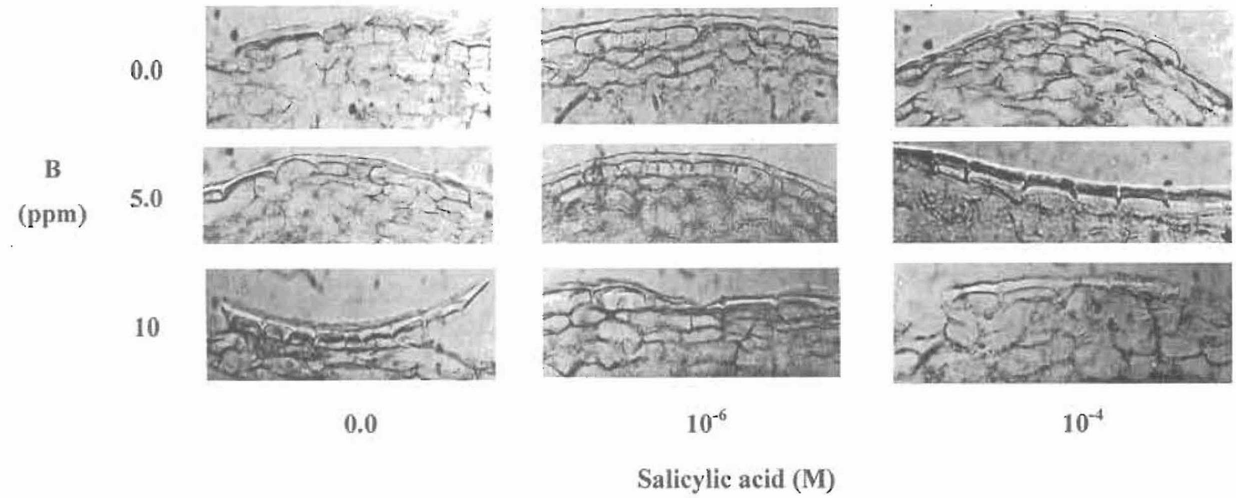


Figure (1): Effect of SA, B and their interactions on some antioxidants content in leaves and fruits of pepper plants.



DISCUSSION

Effect of SA, B and their interactions on some antioxidants contents in leaves and fruits of pepper plants.

The results of this investigation proved that SA or B alone generally did not affect antioxidants concentration in pepper leaves and fruits. However, the combination of SA and B at low level enhanced the concentration of antioxidants (carotenoids, vitamin C, free phenols and peroxidase), significantly. These results partially are in agreement with those of Eraslan *et al.* (2007) in carrot, who found that carotenoids, anthocyanin content and the total antioxidant activity were positively affected by the application of SA. Similarly, Fernandes *et al.* (2006) found that treatment of the primary leaves of cowpea with 10 mM salicylic acid increased the total peroxidase activity contributed by the anionic isoform. The results indicated that the best treatment increased the content of enzymatic and non-enzymatic antioxidants in fruits and only enzymatic in leaves. This finding may be support that the plant fruits possess more protection means than the plant leaves.

Effect of SA, B and their interactions on growth, fruit yield and cuticle thickness of pericarp.

Bioproductivity of plants has been one of the main topics of agricultural sciences and different experts (geneticists, plant breeders, biotechnologists, plant nutritionists, plant physiologists, etc) have been trying to describe it.

Plants have enzymatic and non-enzymatic antioxidant systems, to remove the reactive oxygen species, which produced as secondary metabolites under normal or stress conditions (Ashraf and Harris, 2004; Navarro *et al.*, 2006) for better growth and produce high fruit yield. The pervious statements supported our results which indicated that high values of shoot dry weight and fruit yield as well as fruit cuticle thickness were correlated with the treatment contained SA and B at low concentration. Its clear that, SA and B markedly improved fruit yield by inducing of antioxidants which are involved in the regulation of both cell division and expansion as reported by Franceschi and Tarlyn, (2002), Pignocchi *et al.* (2003), Chen and Gallie, (2006) and Hayat and Ahmad (2007) and cell wall lignification which was protected the fruits from any stress. This is consistent with findings of Dordas and Brown, (2005), Stavrianakou *et al.* (2006), Simoglou and Dordas, (2006), López-Gómez *et al.* (2007) and Singh *et al.* (2007). Also, peroxidases has a function in lignification, auxin regulation, wounding and defense against pathogen infection (Hiraga *et al.*, 2001; Kawano, 2003), which supported the present results.

The positive correlation between fruit weight and vegetative growth positively indicates that the processes of photo-assimilate translocation from the leaves and/or compartmentation within the fruits, as induced by SA and B, may induce the sink demand and thereby the fruit yield production. Also, high correlation between fruit yield and antioxidants content, one may speculate that, the higher antioxidant within the fruits has provided suitable metabolic conditions for the processes involved in fruit weight and fruit number to be more rate efficient.

In general, plants sprayed with low concentration of SA and boron (10^{-6} M & 5 ppm, respectively) showed the highest values in most investigated parameters in both seasons compared with all treatments as well as control. Whereas, SA has an inhibition effect with indirect manner on plant when used in high concentration (Taiz and Zeiger, 2006), therefore pepper plants responded better to SA at low concentration. In the same way, B as a micronutrient must used in low concentration, due to avoid their toxic effect on plants (Xu *et al.*, 2007).

It could be concluded that spraying pepper plants with SA in 10^{-6} M with B at concentration of 5 ppm increased the amount of some antioxidant compounds like vitamin C (ascorbic acid), carotenoids and free phenols as well as peroxidase activity in pepper fruits, which resulted to produce a healthy plants with high yield. These results led to accumulate of different antioxidant compounds in fruits which improved the nutritional value of pepper fruits which very important for human health.

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تأثير الرش بحامض السلسيلك والبورون على النمو والإنتاجية وكذلك جودة الثمار في الفلفل (صنف توينجو)
١- المحصول المنتج ومحتوى مضادات الأكسدة

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كان هدف هذا البحث هو دراسة تأثير رش نباتات الفلفل بالتركيزات المختلفة من حامض السلسيلك والبورون وكذلك اتحادهم معا خلال موسمي الزراعة ٢٠٠٦ و ٢٠٠٧. حيث تم دراسة تأثير المعاملات بحامض السلسيلك والبورون على كلا من الوزن الجاف للنباتات والمحصول الثمرى بالإضافة إلى بعض مضادات الأكسدة مثل الفينولات الحرة و الكاروتينات و فيتامين ج بالإضافة إلى نشاط إنزيم البيروكسيديز في أوراق وثمار الفلفل وكذلك تم دراسة سمك طبقة الكيوتكيل في منطقة البيريكارب في ثمار الفلفل. أظهرت النتائج أن أعلى قيم للوزن الجاف للنباتات ومحصول الثمار لكل نبات تم الحصول عليها عندما تم رش نباتات الفلفل بحامض السلسيلك ($١٠^{-٦}$) متحدا مع البورون (٥ جزء في المليون). نفس المعاملة أعطت أعلى قيم للمركبات المضادة للأكسدة التي تم تقديرها مثل الفينولات الحرة و الكاروتينات و فيتامين ج بالإضافة إلى نشاط إنزيم البيروكسيديز كما أدت نفس المعاملة الى زيادة سمك طبقة الكيوتكيل لثمار الفلفل مقارنة بمعاملة الكنترول. وعلى ذلك فأن تراكم هذه المركبات في الثمار حسنت من قيمتها الغذائية وبناءا على ما تقدم من النتائج المتحصل عليها فأن إنتاج محصول ثمري عالي من الفلفل ذو جودة عالية يستلزم رش نباتات الفلفل بمخلوط حامض السلسيلك والبورون بتركيزات منخفضة.