EFFECT OF ASCORBIC ACID, BENZYL ADENINE AND PACLOBUTRAZOL ON GROWTH, YIELD AND SOME METABOLIC CONSTITUENTS OF SUNFLOWER PLANTS

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ABSTRACT

A field experiment was conducted to study the effect of foliar spray of ascorbic acid (50 and 100 ppm), benzyl adenine (50 and 100 ppm) and paclobutrazol (25 and 50 ppm) on growth, yield and some physiological parameters of (*Helianthus annuus* var. Sakha 53). Ascorbic acid was more effective than other treatments in enhancing growth parameters during stage I, while benzyl adenine was most effective during stage II. Paclobutrazol seemed to be less effective regarding growth characteristics. All doses applied tended to increase photosynthetic pigments, total soluble carbohydrates and soluble proteins of sunflower plants. The changes in proteolytic, amylolytic and lipolytic activities were also recorded. This was associated by improving yield quality and the nutritional value of the seeds. The effect of paclobutrazol was superior to that of ascorbic acid, benzyl adenine on increasing yield components. The highest lipid % was recorded by 50 ppm of ascorbic acid, whereas the highest carbohydrates and proteins of the yielded seeds were observed with plants treated with 100 ppm benzyl adenine.

INTRODUCTION

Sunflower (Helianthus annuus L.) is an important crop and ornamental plant in the world. It is used for animal feed and also it is the second most important crop producing edible oil after soybean (Shehata and El-Khawas, 2003; Fairless, 2007). Growth regulators and vitamins are known to affect plant growth through primary and secondary metabolism (Ewais et al., 2003; Reda et al., 2007). Rafique, et al. (2011) showed the best results on seedling growth, fresh and dry matter production of pumpkin seedlings due to 30 mg L⁻ ascorbic acid treatments. Seedlings fresh weight, protein contents, protease and nitrate reductase activities were significantly affected by 30 mg L⁻¹ ascorbic acid. Moreover, Mazher et al. (2011) found stimulatory effect of ascorbic acid (100 and 200 ppm) on all growth parameters (plant height, number of branches, number of leaves, stem diameter, root length as well as fresh and dry weights of all plant organs) of *Codiaeum variegatum* L. Several researchers, mentioned that benzyl adenine (BA) improve vegetative growth and yield quality, such as Mazrou (1992) on datura, Menesi et al. (1994) on Tagetes erecta, Zinna elegans and Celusia argentia, Farahat et al. (2002) on fennels, Vijay and Laxmi (2001) on mungbean, El-Abagy et al. (2003) on faba bean. El-Maadawy et al. (2006a) indicated that treating pot marigold plants with BA at 100 ppm gave the highest number of inflorescences, which was significantly higher than the control in both seasons. Different benzyl adenine concentrations significantly increased inflorescence diameter, compared to control. Spraying plants with BA had also generally favourable effect on fresh and dry weight of inflorescences as compared to unsprayed plants. Zedan (2000) on Coriander and Zhang et al. (2006) on soybean observed that paclobutrazol treatments caused significant reductions in plant height, internodes length, leaf length and leaf area/plant, while dry weight per plant was increased. Zhang et al. (2006) on soybean observed that paclobutrazol treatments caused significant reductions in plant height, internodes length, leaf length and leaf area/plant, while dry weight per plant was increased. Therefore in the light of such findings, the present study was undertaken to investigate the effect of foliar application of

ascorbic acid, benzyl adenine and paclobutrazol on growth, yield and biochemical constituents of sunflower plants grown under field conditions.

MATERIALS AND METHODS

Seeds of sunflower "Helianthus annuus" (Sakha 53) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Uniform sunflower seeds were planted in natural loamy soil conditions in Botanical garden, Botany and Microbiology Dept., Fac. of Sci., Al- Azhar Univ., Nasr City, Cairo, Egypt, in a plot (4m width and 15m length) containing 7 ridges representing the following treatments: distilled water (as controls), Asc (50, 100 ppm), BA (50, 100 ppm) and Pac (25, 50 ppm). The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated when ever required. Concentrations of the used plant growth regulators were chosen according to a preliminary experiment in which they caused a maximum germination percentage. The plants were sprayed twice with the above mentioned treatments. The first treatment was made when the age of plants was 33 days, while the second treatment was made when the age of plants was 65 days. The plant samples were collected for analysis when the plants were 40 (Stage I) and 72 (Stage II) days old. At the end of the growth season, analysis of the seeds yielded from the different treatments and the control were done. Contents of chlorophylls were estimated using the method of Vernon and Selly (1966). Contents of carotenoids were carried according to Lichtentahler (1981). Contents of soluble carbohydrates were measured according to the method of Umbriet et al. (1969). Contents of soluble proteins were estimated according to the methods of lowery et al. (1951). Activities of amylases were determined using the method of Afifi et al. (1986). Proteases activities were estimated using the method of Ong and Guacher (1972). Lipase activities were determined by method of Elwan et al. (1976). total lipids determined by using a soxhlet apparatus according to Guenther (1972). Statistical analysis of the obtained results was done using (L.S.D.) according to Snedecor and Cochran (1982).

RESLTS AND DISCUSSION

1- Growth parameters:

The obtained results (Tables 1 & 2) revealed that application of ascorbic acid or benzyl adenine at 50 and 100 ppm created significant stimulative effects on growth parameters of sunflower plants. These effects were clear with the resulted induced increases in shoots and roots lengths; number of leaves/plant, fresh and dry weight of shoots and roots. Ascorbic acid was more effective than other treatments in enhancing growth parameters during stage I, while benzyl adenine was most effective during stage II. These findings are in accordance with Ewais (2003) reported that application of ascorbic acid improved growth and yield characteristics of broad bean plants. Recently, Rafique et al. (2011) found that the best results on seedling growth, fresh and dry matter production of pumpkin seedlings by 30 mg L^{-1} ascorbic acid treatments. With respect to benzyl adenine, many investigators obtained similar positive effects on the growth of other plants such as mungbean (Vijay and Laxmi; 2001); faba bean (El-Abagy et al. 2003) marigold (El-Maadawy et al. 2006). Also, Ibrahim et al. (2010) found that foliar application of benzyl adenine (BA) at (50, 100 and 150 ppm) were significantly affected on croton plant height, number of branches and leaves/plant, root length, leaf area and fresh and dry weights of stem, leaves and roots. On the contrary, results shown in tables 1 & 2 revealed that growth characteristics were significantly lowered by paclobutrazol treatments. This was the case, with two exceptions, throughout the two stages of plant growth. The exceptional cases were represented by significant increases in shoot dry weight at stage I & II in responses to paclobutrazol at 50 ppm. These results are in agreement with those observed by Zedan (2000) on coriander and Zhang et al. (2006) on soybean. They reported that paclobutrazol treatments caused significant reductions in plant height, internodes length, leaf length and leaf area/plant, while dry weight per plant was increased.

Treatment (ppm)	Shoot length (cm)		Root leng	th (cm)	Number of leaves	
rieauneni (ppin)	Shoot leng	ui (ciii)	Koot leng		Number of leaves	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	86.50	152.40	21.70	29.50	22.40	25.40
Asc 50	96.90	171.20	24.60	34.00	25.20	27.70
Asc 100	101.50	162.30	26.90	37.20	27.00	29.30
BA 50	92.30	187.70	23.80	35.60	25.10	30.20
BA 100	91.40	180.70	27.40	39.00	28.00	28.50
Pac 25	74.60	142.90	17.80	26.70	18.60	21.70
Pac 50	65.70	134.40	16.50	23.90	19.70	19.10
LSD at 0.05	1.55	4.61	0.71	0.87	0.68	0.58

Table (1): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on
shoot length, root length and number of leaves of sunflower *Helianthus annuus*
(var. Sakha 53) plants. Values given are means of ten replicates.

Table (2): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on fresh weight and dry weight of shoots and roots of sunflower plants. Values given are means of ten replicates.

Treatmen	F.wt. of	shoots (g.)	D.wt. of s	shoots (g.)	F.wt. of roots (g.)		D.wt. of roots (g.)	
t (ppm)	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	309.30	633.30	30.08	62.43	43.20	54.50	6.25	7.44
Asc 50	340.30	681.00	34.20	98.29	42.20	133.56	6.46	29.05
Asc 100	345.66	693.20	39.47	71.52	41.10	71.70	6.53	23.15
BA 50	345.40	671.20	31.36	86.19	42.30	187.40	6.40	41.88
BA 100	331.80	784.10	32.67	100.54	35.70	207.80	6.43	52.58
Pac 25	176.50	333.50	17.73	51.70	23.70	61.50	2.60	10.78
Pac 50	305.20	545.30	32.20	88.04	45.80	114.50	5.95	5.95
LSD at	11.82	24.50	1.52	3.88	2.01	8.71	0.46	3.37
0.05								

2- Photosynthetic Pigments:

The contents of chlorophyll a; b; total chlorophyll (a+ b) and carotenoids of sunflower plants (Table 3) showed, in most cases, consistent and gradual increases in response to various treatments.

Table (3): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on

Treatment	Chlorop	hyll a	Chloro	phyll b	Chlorop	hyll a+b	Carot	enoids
(ppm)	Stage I	Stage II						
Control	1.59	3.02	0.95	1.43	2.54	3.41	0.69	1.23
Asc 50	1.34	0.29	0.75	0.04	2.10	1.43	0.61	0.11
Asc 100	7.32	2.04	3.31	0.94	10.63	7.37	1.77	0.66
BA 50	2.39	3.67	1.22	1.93	3.61	4.41	1.07	1.41
BA 100	2.42	2.89	1.42	1.15	3.84	4.10	1.16	1.03
Pac 25	2.35	3.37	2.00	2.00	4.35	4.95	0.85	1.12
Pac 50	3.29	0.83	2.23	0.26	5.52	3.94	0.93	0.31
LSD at 0.05	0.10	0.12	0.06	0.13	0.06	0.12	0.05	0.06

chlorophyll and carotenoids contents (mg/g. F. wt) of sunflower plants. Values given are means of three replicates.

applied. Ascorbic acid at 100 ppm was more effective followed by paclobutrazol at 50 ppm than other treatments in enhancing chlorophyll contents, while benzyl adenine (at both doses) was more effective than other treatments in enhancing cartenoid content. The obtained results agree with those observed by a number of investigators (Hamza *et al.* 2007; El-Maadawy *et al.* 2006 and Abdel Aziz *et al.* 2009). Hamza *et al.* (2007) recorded that treating plants of *Pelargonium zonale* with paclobutrazol and cycocel significantly increased the total chlorophyll content when compared with the control treatment. El-Maadawy *et al.* (2006a) working on *Calendula afficinalis* L. plants, observed that BA (100) had a favorable effect on chlorophyll synthesis and accumulation in the leaves. Abdel Aziz *et al.* (2009) found that ascorbic acid at 100 ppm significantly increased chlorophyll a, b, a+b and carotenoids of gladiolus plants.

3- Soluble Carbohydrates:

Results of the present work (Table 4) revealed that total soluble carbohydrates contents of sunflower plants were tended to increase, with some exceptions, in response to the treatment with either ascorbic acid at 50 ppm or benzyl adenine at 100 ppm. Application of paclobutrazol at 25 ppm markedly increased total soluble carbohydrates contents in shoot at stage II and in fruits whereas, paclobutrazol at 50 ppm significantly increased these contents in roots and shoots only at stage I as compared with the control. The highest increment in carbohydrates contents was observed in fruits as a consequence of applying 25 ppm of paclobutrazol and benzyl adenine at 50 ppm. On the other hand, ascorbic acid at 100 ppm tended to decrease total soluble carbohydrates contents in roots and shoots of sunflower plants. This was the case throughout the two stages of growth. Also, application of benzyl adenine and paclobutrazol (at both doses) significantly decreased these contents in roots during stage II. Concerning the fruits, data indicated that 100 ppm of benzyl adenine or 50 ppm of paclobutrazol induced significant decreases in carbohydrates contents. In agreement with these results a number of investigators observed stimulating effect regarding the effect of ascorbic acid (Abdel Aziz et al. 2006; Farahat et al. 2007; Eid et al. 2010), benzyl adenine (Youssef, 2004) or paclobutrazol (Amin, 2007; Hamza et al. 2007) on carbohydrate contents. On the other hand, Elgayar (2004) revealed that treatment of soybean with benzyl adenine (25 and 50 ppm) resulted in slight effects on carbohydrate. On the contrary, El-Abagy et al. (2003) found that spraying faba bean plants with benzyl adenine (25 and 50 ppm) decreased carbohydrate percentage content.

Table (4): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol(Pac) on total water soluble carbohydrates contents (mg/g. dry weight) of sunflower plants. Values given are means of three replicates.

Treatment	Roots		S	Fruits	
(ppm)	Stage I	Stage II	Stage I	Stage II	
Control	37.20	60.96	20.23	72.57	161.53
Asc 50	103.14	68.47	56.08	130.61	165.98
Asc 100	34.10	37.70	18.54	60.15	170.23
BA 50	36.90	43.60	20.06	102.26	220.88
BA 100	77.78	43.64	42.29	142.76	135.94
Pac 25	27.85	42.30	15.15	91.07	322.64
Pac 50	92.99	35.13	50.56	66.67	54.18
LSD at 0.05	19.28	8.91	6.46	16.19	12.29

4- Soluble Proteins:

In the present study, it was found (Table 5) that protein contents in shoots; roots as well as in the fruits of sunflower plants, mostly, were significantly increased in response to all applied doses of Asc, BA or Pac. The obtained results are in harmony with those reported by **Abdel-Halim (1995)** who observed that ascorbic acid increased protein content of wheat grains; **El-Abagy** *et al.* (2003) found that spraying faba bean plants with benzyl adenine significantly increased crude protein content and **Wanas (2007)** indicated that application of paclobutrazol considerably increased the levels of crude protein in leaves of treated faba bean plants compared with those of untreated ones.

Table (5): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on soluble proteins contents (mg/g. dry weight) of sunflower plants. Values given are means of three replicates.

Treatment	Roots		Sh	Fruits	
(ppm)	Stage I	Stage II	Stage I	Stage II	
Control	12297	161.19	157.60	139.18	317.76
Asc 50	172.52	179.76	172.52	176.33	323.29
Asc 100	147.29	135.96	146.27	182.23	372.19
BA 50	206.60	258.94	269.67	274.29	347.02
BA 100	202.58	162.53	196.08	258.51	307.09
Pac 25	258.54	208.80	250.99	219.43	373.91
Pac 50	256.90	257.27	231.62	284.26	283.19
LSD at 0.05	24.48	23.29	15.12	12.70	14.036

5 Enzymes Activities:

The obtained data (Table 6) indicated that both doses applied of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac), with two exaptions, caused significant increase in the activities of proteases especially at stage I of growth. The exceptional cases were represented by significant decreases in proteases at stage I & II in responses to BA at 100 ppm and Asc at 50 ppm, respectively. The most proteases activities was recorded by BA at 100 ppm (stage II), followed by Asc 100 ppm (stage I). The stimulating effect of ascorbic acid on protease activity, obtained in the present study, are harmony with those observed by Rafique et al. (2011) who found that protease activity was higher in pumpkin seedling from seeds treated with 15mgL^{-1} Asc. Concerning the activities of amylases, results in table (6) indicated that foliar application of Asc, BA and Pac resulted, mostly, in either significantly reduced (at stage I) or significantly increased (at stage II). In this regard, Prusakova et al. (2004) reported a similar view that the growth retarding activity of triazole compounds such as paclobutraz appears in the inhibition of amylase activity in barley (Hordium vulgare L.). **Bialecka and Kepczynski (2003)** found that BA at 10^{-5} M had no effect on α -amylase activity in Amaranthus caudatus seeds. It was also observed (Table 6) that 100 ppm of Asc or 25 ppm of Pac at both stageses (I & II) of growth and 100 ppm of BA or 50 ppm of Pac at stage I significantly increased the lipolitic activities of sunflower plants. In the contrary, Asc at 50 ppm (stages I & II); BA at 50 ppm (stage I) or Pac at 50 ppm (stage II) caused a significant decreases of these activities.

equiva	equivalent) of sunflower plants. Values given are means of three replicates.							
Treatment	Prot	eases	Am	ylases	Lipases			
(ppm)	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II		
Control	1.46	1.43	1.69	1.12	14.06	11.72		
Asc 50	1.66	1.28	1.40	1.96	9.37	9.37		
Asc 100	2.36	1.44	1.43	1.69	29.68	14.84		
BA 50	1.51	1.49	1.49	0.95	11.72	11.72		
BA 100	1.44	2.50	1.16	1.48	25.00	11.72		
Pac 25	1.68	1.40	1.57	1.11	24.21	2.34		
Pac 50	1.76	1.85	1.69	1.27	18.75	9.37		
LSD at 0.05	0.063	0.031	0.018	0.006	1.89	2.28		

Table (6): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on activities of proteases, amylases and lipases enzymes (mg/g. dry weight equivalent) of sunflower plants. Values given are means of three replicates.

6- Yield components and nutritional value of the yielded seeds:

Results recorded in table (7) indicated that foliar application of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) significantly increased yield components of sunflower plants. The highest value of all yield, mostly, were obtained with plants treated with Pac. The sequence of increase in diameter of head; weight of head; weigh of seeds/plant and weight of 100 seeds was as follows Pac > BA > Asc. The increments of weight of 100 seeds estimated by 39.46%, 33.76% and 26.80% in response to treating with Pac, BA and Asc, respectively compared with the control plants. The positive effect of Pac on yield components followed the negative trend obtained previously on vegetative growth. Thus, it could be stated that treatments of Pac had a beneficial effect on yield components. In this respect, Abdul Jaleel et al. (2007) reported that the application of Pac on Catharanthus roseus had significant effects on photosynthetic and anatomical responses thus can be used for improving productivity in medicinal plants. Moreover, Lolaei et al. (2012) reported that the highest leaf number, leaf area, petiole length, and total soluble solid percent were observed in control plants, while highest fruit number, fruit weight fruit, fruit set, flower number and yield of strawberry were obtained in plants treated with 90 mg L⁻¹ Pac. They also mentioned that foliar application of Pac prior to flowering is recommended to increase the yield of strawberry. The obtained results (Table 8) showed that carbohydrates contents were found to be significantly increased only in response to applying BA at 100 ppm, while treatment the plants with either BA at 50 ppm or Pac at 25 ppm caused significant decrease in these contents of the seed yield. It was also observed (Table 8) that both doses applied of Asc, BA and Pac tended to significant increase both proteins contents and total lipids of the vielded seeds. The increases in the contents of carbohydrates and proteins of the see ds yield were shown to be the following order: BA > Pac > Asc. On the other hand, the sequence of total lipid % was according to the following order: Asc > Pac > BA. In this regard, Vasudevan et al. (1996) reported that spraying three sunflower cultivars with cytokinins produced the highest seed oil content. Talaat and Youssef (1998) showed that oil in seeds of rosella plants were significantly increased as a result of BA application, especially at 40 mg/L. Abed (2001) observed that BA significantly increased oil and protein % in seeds of cotton plants. Ibrahim et al. (2001) found that treatment of sunflower plants with kinetin 50 ppm gave a significant increase in crude fat %, respectively. Yousif et al. (2012) observed that 0.5 g l^{-1} sucrose + 150 g l^{-1} ascorbic acid increased fresh and dry weight, and total carbohydrates percentage in snapdragon cut spike flowers.

From the bulk of data obtained in the present investigation, it can be suggested that treatment of ascorbic acid, benzyl adenine and paclobutrazol had a beneficial effect on growth and chemical constituents as well as yield quality of sunflower plants.

Treatment (ppm)	Diameter of	Weight of head	Weigh of	Weight of 100
	head (cm)	(g.)	seeds/plant (g.)	seeds (g.)
Control	12.07	72.14	47.15	4.15
Asc 50	17.67	114.38	58.98	5.43
Asc 100	16.30	104.84	64.89	5.91
BA 50	18.71	132.8	87.14	6.91
BA 100	16.38	126.43	95.66	5.62
Pac 25	18.43	145.90	112.48	6.47
Pac 50	18.22	146.38	100.74	7.24
LSD at	0.96	19.90	9.60	0.44
0.05				

Table (7): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on yield components of sunflower plants. Values given are means of ten replicates.

Table (8): Effect of ascorbic acid (Asc), benzyl adenine (BA) and paclobutrazol (Pac) on soluble carbohydrates, proteins and total lipids of the seed yield of sunflower plants. Values given are means of three replicates.

Treatment	Carbohydrates	Proteins	Total lipids (%)
(ppm)	(mg/g. D. wt)	(mg/g. D. wt)	
Control	82.87	158.13	30.6
Asc 50	69.72	205.80	49.4
Asc 100	72.26	169.19	46.2
BA 50	59.33	209.23	38.4
BA 100	167.33	259.23	41.8
Pac 25	37.35	201.56	39.2
Pac 50	90.33	245.30	43.4
LSD at 0.05	13.39	18.13	3.72

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تأثير حمض الاسكوربيك والبنزيل أودنين والباكلوبوترازول على نمو وايض وانتاجية نبات دوار الشمس

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أجريت تجربة حقلية لدراسة تأثير الرش الورقي من حامض الاسكوربيك (٥٠, ١٠ جزء في المليون)، البنزيل أدنين (١٠, ١٠ جزء في المليون) والباكلوبوتر ازول (٢٥, ٥٠ جزء في المليون) على النمو والمحصول وبعض العمليات الفسيولوجية لدوار الشمس. وكان حمض الاسكوربيك أكثر فعالية من المعاملات الأخرى في تنشيط خصائص النمو خلال المرحلة الأولى، في حين كان البنزيل أدنين الأكثر فعالية خلال المرحلة الثانية من النمو. وجد ان الباكلوبوتر ازول أقل فعالية فيما يتعلق بخصائص النمو. كل المعاملات أدت إلى زيادة أصباغ التمثيل الضوئي، و الكربو هيدرات والبروتينات الذائبة. وسجلت أيضا التغيرات في أنشطة الانزيمات المحالله للبروتينات والنشويات والدهون. وارتبطت هذه التغيرات مع على زيادة المحصول والقيمة الغذائية للبذور. وكان تأثير الباكلوبوتر ازول أعلى من حامض الأسكوربيك والبنزيل أدنين على زيادة المحصول. أعلى نسبة زيوت تم الحصول عليها من معاملة النباتات بحامض الأسكوربيك (١٠٠ ، بينما سجلت المعاملات والبروتينات المحلله للبروتينات والنشويات والدهون. وارتبطت هذه التغيرات مع على زيادة المحصول. أعلى نسبة زيوت تم الحصول عليها من معاملة النباتات بحامض الأسكوربيك (١٠٠ جزء في المليون) ، بينما سجلت المعامين المحاربة في أنشطة الانزيمات المحلله للبروتينات والنشويات والدون. تحسين جودة المحصول والقيمة الغذائية للبذور. وكان تأثير الباكلوبوتر ازول أعلى من حامض الأسكوربيك والبنزيل أدنين المليون).