Response of soybean plants to exogenously applied with Ascorbic acid, Zinc Sulphate and Paclobutrazol

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Abstract: Field trials with soybean (Glycine max L var. klark) plant. were conducted at the experimental Botanical garden, Botany and Microbiology Dept., Fac. of Sci., Al- Azhar Univ., Nasr City, Cairo, Egypt, in growth seasons. The aim of this work is to study the effect of foliar application with ascorbic acid at 0, 50 and 100 ppm, zinc sulphate at 0, 50 and 100 ppm and paclobutrazole at 0, 50 and 100 ppm on growth, yield and some metabolic activities of soybean (Glycine max L var. klark) plant. Ascorbic acid was more effective than other treatments in enhancing growth parameters during stage I, while zinc sulphate was most effective during stage II. Pacloputrazole seemed to be less effective regarding growth characteristics. Different applied treatments tended to increase photosynthetic pigments, soluble carbohydrates and soluble proteins in tested 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 pacloputrazole was superior to that of ascorbic acid, zinc sulphate on increasing yield components. The highest lipid % was recorded by 50 ppm of ascorbic acid.

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1. Introduction

Soybean (*Glycine max* L.) is an important source of protein for human and animal nutrition,as well as a major source of vegetable oil. The seeds consist of approximately 40–42% protein and 20–22% oil, on a dry-matter basis (Manavalan *et al.,* 2009). Rafique *et al.* (2011) showed the best results on seedling growth, fresh and dry matter production of pumpkin seedlings due to 30 mg L^{-1} 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.

Zinc is one of the essential micronutrients required for optimum crop growth. It plays an important role in many biochemical reactions within the plants. Zinc is important in the formation of the growth hormone auxin. Auxin is produced by shoot tips, and controls cell division, leaf and shoot growth and fruit development. Zinc is also needed by leaf cells to form the green leaf pigment chlorophyll. Its regulates starch formation and proper root development (Wassel *et al*., 2007). Also, zinc play an essential role in plant physiology where it actives some of enzymes such as dehydrogenises, pretenses, peptidases and phosphohydrolases. Zinc is a micronutrient needed in small amounts by crop plants, but its important in crop production has increased in recent years (Fageria, 2009). 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.

Therefore in the light of such findings, the present study was undertaken to investigate the effect of foliar application of ascorbic acid, zinc sulphate and paclobutrazol on growth, yield and some biochemical constituents of soybean plants grown under field conditions.

2. Materials and Methods

Seeds of soybean (*Glycine max* var. klark) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Uniform 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 (4 m width and 15 m. length) containing 7 ridges representing the following treatments: Distilled water (as controls), AA (50 ppm), AA (100 ppm), Zn (50 ppm), Zn (100 ppm), PBZ (50ppm) and PBZ (100 ppm).

The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated whenever 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 30 days, while the second treatment was made when the age of plants was 60 days. The plant samples were collected for analysis when the plants were 40 (Stage I) and 70 (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 (1987). 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). Lipases activities were determined by method of Elwan *et al*. (1976).

Total lipids determined by using a soxhlet apparatus according to Guenther (1972).

All statistical calculations were done using computer programs.Microsoft excel version 10 and spss (statistica package for the social science version 20.00) statistical program. at 0.05, 0.01 and 0.001 level of probability (Snedecor and Cochran, 1982) the One-way ANOVA and Post hoc-LSD tests (the least significant difference) was presented using mean ± Error.

3. Results and Discussion

3.1. Growth parameters

Results in tables (1 & 2) revealed that application of ascorbic acid or zinc sulphate at 50 and 100 ppm created significant stimulative effects on growth parameters of soybean 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 zinc sulphate 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. Khudsar *et al.* (2004) studied the effect of Zn (50, 100, 200, 300 and 400 μg/g soil dry mass) on *Artemisia annua* plants. They found that, total leaf area, length and dry mass of shoots and roots were increased with the age of the plant. El-Sallami and Gad (2005) found that, Zn sprays at 100 ppm increased the vegetative growth measurements plant height, number of leaves as well as the fresh and dry weights of aster plants. Amira (2013) working on almond (*Prunus amygdalus*) and found that foliar application of ascorbic acid at 500 mg/L^{-1} increase significantly primary root length, increasing ascorbic acid to1000 mg/L^{-1} increased plant length, while the foliar application of Zn on 15 and 30 mg/L^{-1} increased significantly chlorophyll %.

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 paclobutrazolat 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.

3.2. Photosynthetic pigments

The contents of chlorophyll a; b; total chlorophyll (a + b) and carotenoids of soybean plants (Figs. 1, 2, 3 & 4) -showed, in most cases, consistent and gradual increases in response to various treatments applied. Ascorbic acid at 100 ppm was more effective followed by paclobutrazolat 50 ppm than other treatments in enhancing chlorophyll contents, while Zn at the two applied levels was more effective than other treatments in enhancing carotenoid content. The obtained results are in consistent with those observed by a number of investigators (Hamza *et al*., 2007; 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.

Rashed and Ahmed (1997) on broad bean plants. They found that, chlorophyll contents were increased by using foliar application with Zn (50 ppm). Hassanein *et al*. (2000) found that, spraying cow pea (*Vigna sinensis*) plants with 10, 50 and 250 mg/L-1 of B or Zn caused high significant increases in the contents of chlorophyll (a) and chlorophyll b. Also, Tobbal (2006) showed that, spraying *Celosia* plants with Zn (400 mg/L^{-1}) increased contents of chlorophyll a, b and total (a + b). The same author reported that, the increase in chlorophyll a, b and total chlorophyll (a + b) could be ascribed to the effect of this element on increasing the biosynthesis of photosynthetic pigments and/or retarding their degradation.

Table 1. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on shoot length, root length and number of leaves of soybean plants. Values given are means of ten replicates

Treatment (ppm)	Shoot length (cm)		Root length (cm)		Number of leaves	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	40.53±0.45	53.63±1.40	21.73±0.58	20.20±0.26	22.67±0.32	35.53±0.47
Asc 50	45.33±1.53	56.90±0.36	24.40±0.43	24.23±0.21	25.30±0.36	37.53±0.47
Asc 100	48.27±1.42	54.32±0.85	26.80±0.10	27.00±0.20	27.50±0.50	39.27±0.25
Zn **50**	42.73±1.62	58.79±0.67	23.57±0.42	25.87±0.25	25.23±0.32	40.00±0.20
Zn **100**	44.33±1.50	57.45±1.20	27.64±0.30	28.57±0.52	28.30±0.30	38.47±0.45
Pac 50	36.54±1.44	47.90±0.56	17.33±0.42	16.50±0.44	18.37±0.47	31.53±0.47
Pac 100	30.53±1.30	42.95±1.49	16.20±0.26	14.20±0.30	19.57±0.51	28.63±0.55
F ratio	57.363	95.511	388.160	844.313	261.613	299.115
P value	***	***	***	***	***	***

****= highly significant

Table 2. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on fresh weight and dry weight of shoots and roots of soybean plants. Values given are means of ten replicates

Treatment (ppm)	F.wt. of shoots (g.)		D.wt. of shoots (g.)		F.wt. of roots (g.)		D.wt. of roots (g.)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	12.06±0.16	33.60±0.79	3.06±0.05	9.31±0.27	2.20±0.15	5.50±0.45	1.25±0.14	1.44±0.02
Asc 50	25.46±0.45	36.30±0.36	5.37±0.47	15.20±0.17	4.20±0.13	7.56±0.46	1.46±0.03	2.05±0.05
Asc 100	22.28±0.75	48.97±0.25	6.45±0.45	11.39±0.34	3.10±0.43	6.70±0.47	1.53±0.24	2.15±0.13
Zn **50**	25.23±0.21	40.27±0.30	6.41±0.44	12.35±0.46	4.30±0.46	7.40±0.21	1.40±0.06	3.88±0.53
Zn **100**	22.16±0.23	51.43±0.51	4.65±0.14	7.45±0.45	2.70±0.47	8.80±0.52	1.43±0.01	4.58±0.13
Pac 50	10.34±0.49	22.46±0.45	2.47±0.41	8.53±0.72	3.70±0.35	6.50±0.45	0.60±0.03	0.78±0.02
Pac 100	11.33±0.49	29.43±0.51	3.26±0.25	12.04	6.80±0.52	7.50±0.26	0.95±0.02	1.95±0.02
F ratio	712.167	1469.539	63.245	136.064	36.28	17.587	21.70	105.143
p value	***	***	***	***	***	***	***	***

****= highly significant

3.3. Soluble carbohydrates

Results of the present work (Figs. 5 & 6) revealed that total soluble carbohydrates contents of soybean plants were tended to increase, with some exceptions, in response to the treatment with either ascorbic acid at 50 ppm or zinc at both 50 and 100 ppm. Application of paclobutrazol at 50 ppm markedly increased total soluble carbohydrates contents in shoot at stage II and fruits whereas, paclobutrazol at 100 ppm significantly increased these contents in roots and shoots only at stage I as compared with the control plants. The highest increment in carbohydrates contents was observed in the fruits as a consequence of applying 50 ppm of either paclobutrazol or zinc.

On the other hand, ascorbic acid at 100 ppm tended to decrease total soluble carbohydrates contents in roots and shoots of the tested plants. This was the case throughout the two stages of growth. Also, treatment with zinc and paclobutrazol (at both doses) significantly decreased these contents in roots during stage II. Concerning the fruits, data indicated that 100 ppm of zinc 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*., 2009; Farahat *et al*., 2007; Eid *et al*., 2010), benzyl adenine (Youssef, 2004) or paclobutrazol (Amin, 2007; Hamza *et al*., 2007) on carbohydrate contents. Tobbal (2006) revealed that, contents of total soluble carbohydrates of both *Celosia* and *Zinnia* shoots were significantly increased in response to the treatment with Zn.

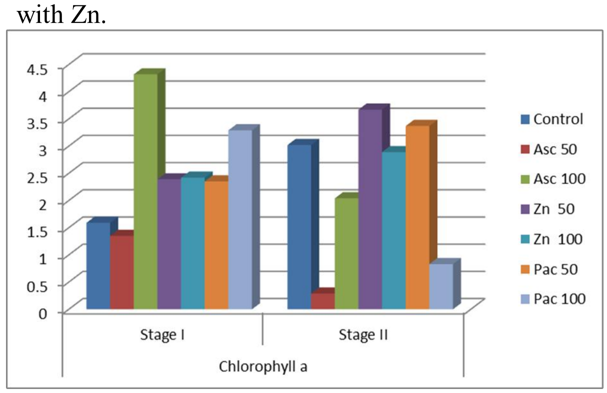

Figure 1. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on chlorophyll a content (mg/g. F. wt) of soybean plants. Values given are means of three replicates

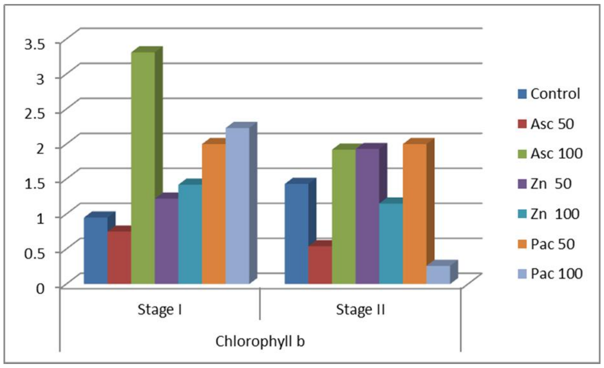

Figure 2. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on chlorophyll b content (mg/g. F. wt) of soybean plants. Values given are means of three replicates

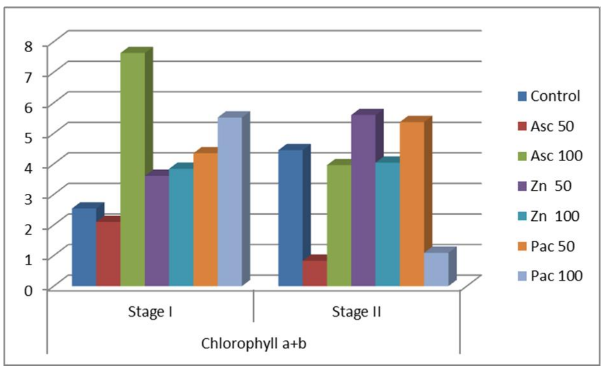

Figure 3. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on chlorophyll a + b content (mg/g. F. wt) of soybean plants. Values given are means of three replicates

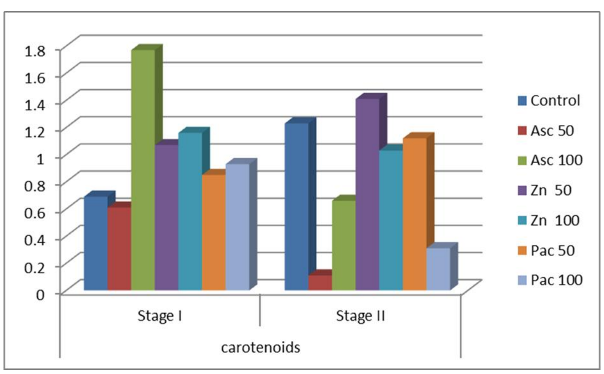

Figure 4. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on carotenoids content (mg/g. F. wt) of soybean plants. Values given are means of three replicates

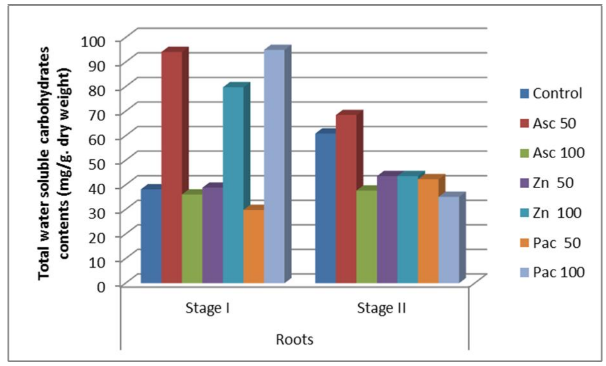

Figure 5. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on total water soluble carbohydrates contents (mg/g. dry weight) in roots of soybean plants

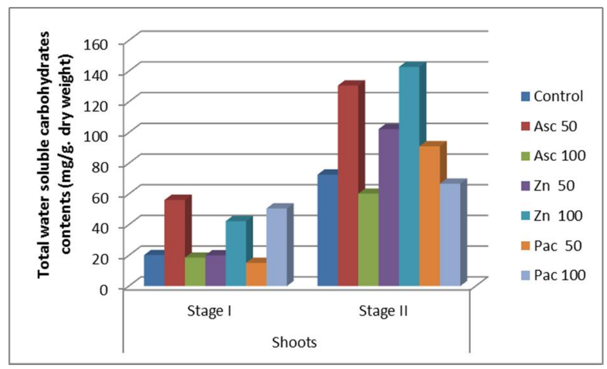

Figure 6. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on total water soluble carbohydrates contents (mg/g. dry weight) in shoots of soybean plants

3.4. Soluble proteins

In the present study, it was found that protein contents in shoots; roots as well as in the fruits of soybean plants, mostly, were significantly increased in response to all doses applied of ascorbic acid benzyl adenine or paclobutrazol (Figs. 7 & 8).

These obtained results are in harmony with those reported by Abdel-Halim (1995) observed that ascorbic acid increased protein content of wheat grains; Gamal El-Din (2005) reported that, Zn treatments (100 and 200 mg/L^{-1}) increased protein contents of fenugreek seeds. Also, Tobbal (1999) found that, the contents of soluble proteins in shoots, roots and yielded seeds of fenugreek and chickpea plants were increased in response to the treatment with Zn (100 ppm) as foliar spraying. 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.

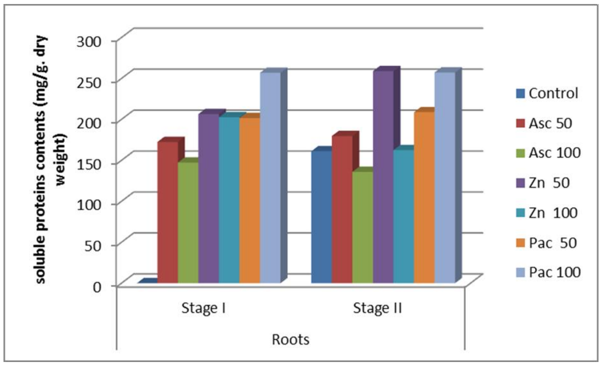

Figure 7. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on soluble proteins contents (mg/g. dry weight) in roots of soybean plants

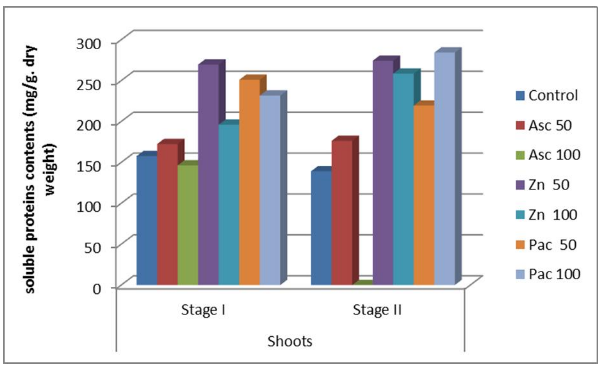

Figure 8. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on soluble proteins contents (mg/g. dry weight) in shoots of soybean plants

3.4. Enzymes activities

The obtained data (Fig. 9) indicated that both doses applied of ascorbic acid (Asc), Zinc (Zn) and paclobutrazol (Pac), with two exaptions, significantly increased 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 Zn at 100 ppm and Asc at 50 ppm, respectively. The most proteases activities was recorded by Zn 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 15 mg/L^{-1} Asc.

Regarding the activities of amylases, results in figure (10) indicated that foliar application of Asc, Zn 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.).

It was also observed that (figure 11) either 100 ppm or Asc and also 50 ppm of Pac at both stages (I & II) of growth and 100 ppm of Zn or 50 ppm of Pac at stage I significantly increased the lipolitic activities of soybean plants. In the contrary, Asc at 50 ppm (stages I & II); Zn at 50 ppm (stage I) or Pac at 50 ppm (stage II) caused significant decreases of these activities. The potent effects of Zn on stimulating the activities of certain enzymes were recorded by other investigators. In this respect, Tobbal (2006) summarized that, treating *Celosia* plants with Zn resulted in insignificant increases in the activities of amylases, proteases, catalases, peroxidases and polyphenoloxidases. El-Mashad (1998) reported that, application of Zn caused highly significant increases in peroxidases activities of shoots and roots of *Vigna sinensis* plants. Contrary to the stimulatory effects of Zn as regards enzymes activities, Tobbal (1999) revealed that, activities of proteases in fenugreek and chickpea plants were significantly decreased in response to treating the plants with Zn 0.1%. Sharaf *et al.* (2009) revealed also that, application of Zn caused variable responses as regards the activities of the detected enzymes, in broad bean plants, activities of amylases were markedly increased, activities of proteases were significantly decreased, and insignificant changes were recorded as regards the activities of catalases and peroxidases. In lupin plants, treatment with Zn caused significant increases in the activities of amylases and peroxidases, activities of proteases were significantly decreased while activities of catalases were insignificantly affected.

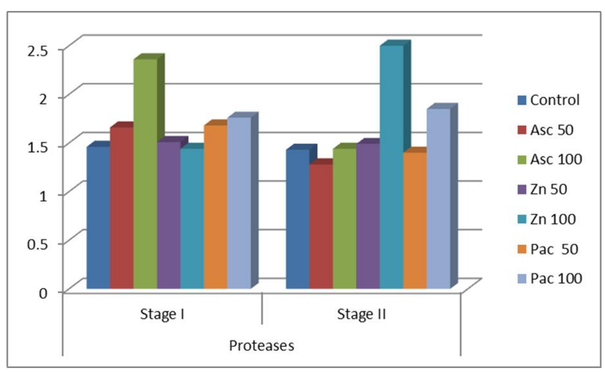

Figure 9. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on activities of proteases enzymes (mg/g. dry weight equivalent) of soybean plants

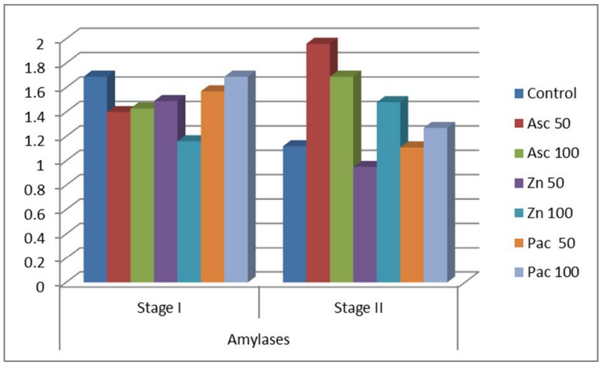

Figure 10. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on activities of amylases enzymes (mg/g. dry weight equivalent) of soybean plants

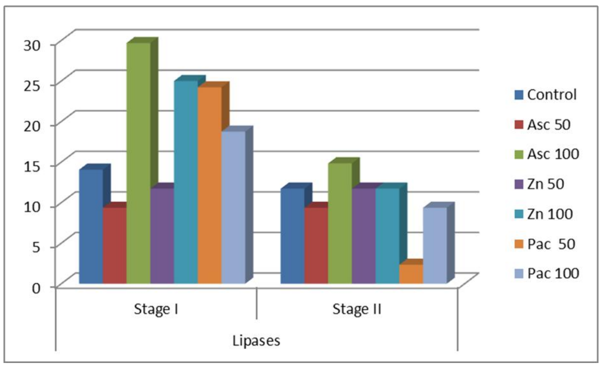

Figure 11. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on activities of lipases enzymes (mg/g. dry weight equivalent) of soybean plants

3.5. Yield components and nutritional value of the yielded seeds

Results recorded in figure (12) indicated that foliar application of ascorbic acid (Asc), Zinc (Zn) and paclobutrazol (Pac) significantly increased yield components of soybean plants. The highest value of all yield, mostly, were obtained with plants treated with Pac. The sequence of increase in weight of 100 seeds was as follows Pac > Zn > Asc. The increments of weight of 100 seeds estimated by 39.46%, 33.76% and 26.80% in response to treating with Pac, Zn 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 connection, Abdul-Jaleel *et al.* (2007) reported that the application of PBZ. 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.

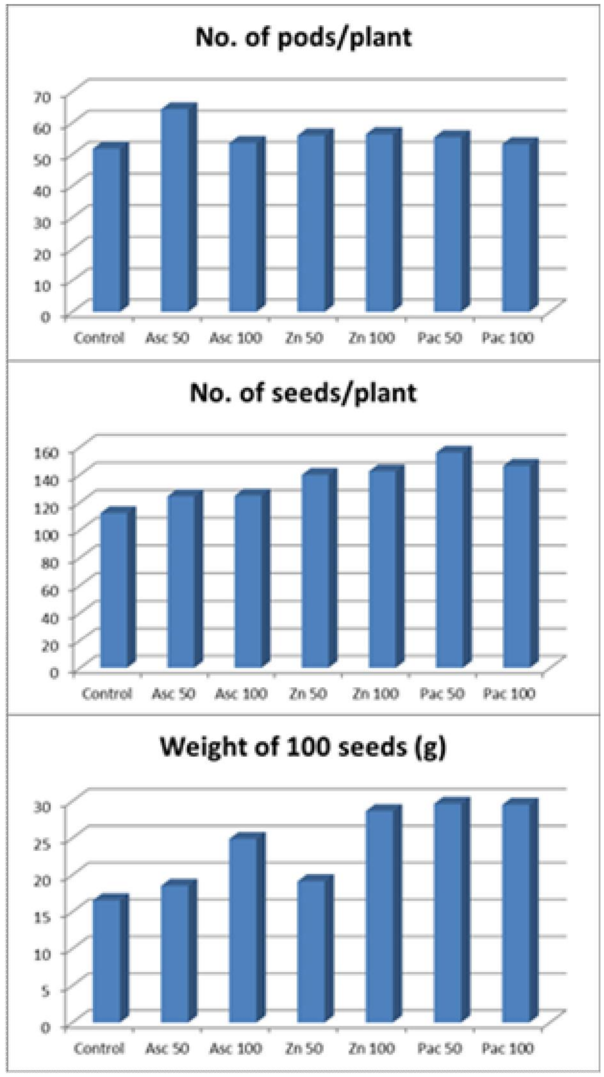

Figure 12. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on yield components of soybean plants

The obtained results (Fig. 13) showed that carbohydrates contents were found to be significantly increased only in response to applying Zn at 100 ppm, while treatment the plants with either Zn at 50 ppm or Pac at 50 ppm caused significant decrease in these contants of the seed yield.

It was also observed (Figs. 14 & 15) that both doses applied of Asc, Zn and Pac tended to significant increase both proteins contents and total lipids of the yielded seeds. The increases in the contents of carbohydrates and proteins of the seeds yield were shown to be the following order: Zn > Pac > Asc. On the other hand, the sequence of total lipid % was according to the following order: Asc > Pac > Zn. In this regard, Vasudevan *et al*. (1996) reported that spraying three sunflower cultivars with cytokinin 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 Zn application, especially at 40 mg/L. 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 outcome of the obtained results in the present investigation, it can be suggested that treatment of ascorbic acid, zinc and paclobutrazol had a beneficial effect on growth and chemical constituents as well as yield quality of soybean plants.

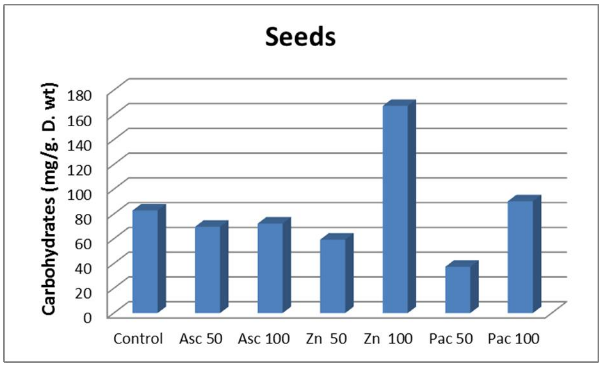

Figure 13. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on soluble carbohydrates of the seeds yield of soybean plants

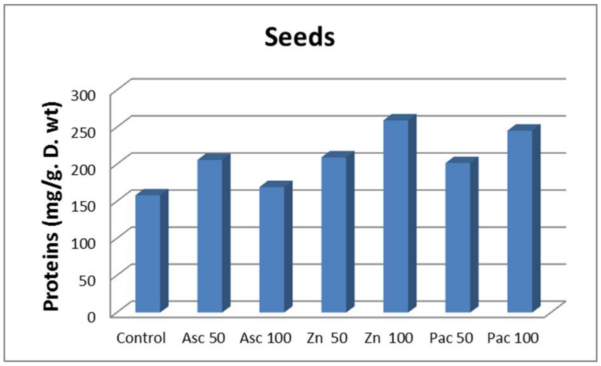

Figure 14. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on proteins of the seeds yield of soybean plants

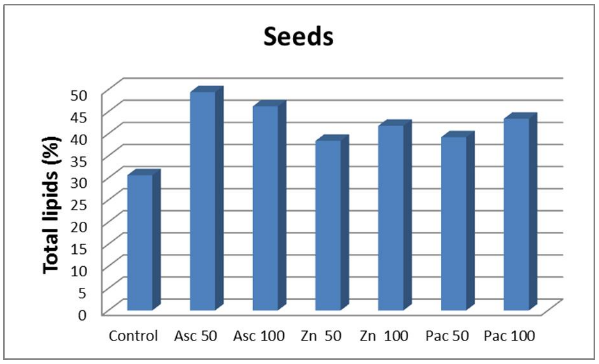

Figure 15. Effect of ascorbic acid (Asc), Zn and paclobutrazol (Pac) on total lipids of the seeds yield of soybean plants

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