

Physiological role of bio-regulators in improving growth and productivity of chickpea (*Cicer arietinum* L.) plant

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ABSTRACT

Two field experiments were carried out during the two successive season (2014/2015 and 2015/2016) at the Research and Production of National Research Centre, Al Nubaria District El-Behaira Governorate, Egypt. The response of Chickpea (*Cicer arietinum* L.) Cv. Giza 531 and Giza 195) to foliar application of plant growth regulators (Benzoic acid, Ascorbic acid, Fulvic acid, Humic acid) at vegetative and reproductive stages of the plant under field conditions was studied in a factorial experiment with four factors based on randomized complete block design. Spraying of Benzoic acid 100 gm/L, Ascorbic acid 200 mg/L, Fulvic acid 6 g/L and Humic acid 10 ml/L. Significantly increased the plant height, number and dry weight of branches, leaves and pods/plant leaf area/plant, leaf area index and SLW at the two growth stages on Giza 531 compared with Giza 195. Foliar application of Benzoic acid, Ascorbic acid, fulvic acid and Humic acid on chickpea Cv. 195, lead to overall better performance of the plant and increase the growth and yield, as well as, its components compared with untreated plant (control treatment). The most favorable treatments for growth parameters, yield and its components and photosynthetic pigments content per leaves were foliar spraying with Benzoic acid 100 mg/L, followed by ascorbic acid 200 mg/L, fulvic acid 6 g/L and humic acid 10 ml/L on chickpea Cv. Giza 531 compared with Giza 195. Foliar application of bioregulators significantly increased the nitrogen, phosphorus, potassium, total carbohydrate and protein in chickpea seeds Giza 531 compared with Giza 195. It could be conclude that spraying bioregulators on chickpea plant have promotion effect on the seed yield criteria which have promising potential as sources of low-cost protein and minerals for possible use as food/feed supplements.

Keywords: Chickpea (*Cicer arietinum* L.), bio- regulators, growth characteristics, yield, biochemical constituents

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most cultivated and consumed legume crop in the world. It is the second most important pulse crop in the world (used in human and animal feed). Chickpea is one of the most important grain legumes grown in semi-arid regions. Chickpea is a good source of protein and carbohydrate and its protein quality is better than other legumes such as pigeon pea, black gram and green gram (Kaur and Singh, 2005). There are several biotic and abiotic stresses, which limit chickpea cultivation (Zarel *et al.*, 2011).

In Egypt, because the continuous increase in population, animal protein become not sufficient for several millions of malnourished people. Egypt governorate made many efforts to decrease the gap between production and consumption in animal protein by improving and increasing legume crops area and productivity in Egypt, the cultivated area reached to (9276 fad.) in 2009 year, which produced (7581 tons, seeds (FAO Statistics, 2010) and its concentrated in the governorates of Assiut, Qena and El-Behira, plant growth regulators are representing one the controlling factors that regulate growth, biosynthesis of chemical constituents, yield and may be improve adaptation of plants to environment. Ascorbic acid and Benzoic acid are regarded as two of the most effective antioxidants against various stresses in plants (Conklin, 2001; Khan *et al.*, 2011). It has a great effect on physiological processes such as cell division, plant, plant growth and the biosynthesis of cell wall, metabolites and phytohormones. Moreover, ascorbic acid and Benzoic acid plays a vital role in

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renovation of chloroplast and mitochondrion membranes (Pavel *et al.*, 2005; Barth *et al.*, 2006). High endogenous ascorbic acid and Benzoic acid in plants is necessary to counteract oxidative stress in addition to regulating other metabolic processes of plant (Athar *et al.*, 2008). Ascorbic acid and Benzoic acid level in plant organs can be elevated by its exogenous application methods such as foliar spray, seed priming or rooting medium (Chen and Galile, 2004). In an experiment, Farjam *et al.*, (2014) demonstrated that the exogenous application of ascorbic acid and salicylic acid on chickpea plants increased the plant biomass significantly compared with control plants and cell membrane damage in chickpea leaves was decreased through foliar application of ascorbic acid and salicylic acid. However, Benzoic acid is a biosynthetic precursor of salicylic acid and has been tested in different crops (Raskin, 1992). Benzoic acid is potentially known to provide abiotic stress tolerance (Senaratan *et al.*, 2003). Benzoic acid is naturally synthesized by plant and classified in the group of carboxylic acid.

Humic acid fulvic acids are commonly used in organic crop production. Application of human materials to plant affects cell membranes, leading to enhanced transport of minerals, improved protein synthesis, plant hormone-like activity, promoted photosynthesis, modified enzyme activities, solubilization of micro and macro elements, reduction of active levels of toxic minerals, and increased microbial population (Seyed-Bagheri, 2010). Humic compounds such as humic acid and fulvic acid have been shown to stimulate plant growth in terms of increasing plant height and dry or fresh weight as well as enhancing nutrient uptake. Gad El-Hak *et al.*, (2012) conclude the salicylic acid and humic acid are very beneficial to the growth yield and some yield components of pea. They reported that foliar application of salicylic acid at 200 ppm and humic acid at 1 g/L are useful to obtain high quantity/quality peas.

Huda *et al.*, (2003), Ozer *et al.*, (2010) findings were shown that humic substances have a positive effect on plant growth, physiologic events increases in root and shoot lengths, stimulate the germination and increase in grain yield of chickpea (*Cicer arietinum* L.) Rasaei *et al.*, (2012) with regard to the physiological effects of application of humic acid on green peas indicated that use of humic acid showed significant effect on value of chlorophyll a and b and content of relative water of leaf as well as value of solution sugars in leaf. Khraman. (2017) showed that use of Humic acid was significant on weight of 100 seeds in 5% level.

Therefore, effect of spraying chickpea plant with different concentrations of Ascorbic acid, Benzoic acid, fulvic acid and humic acid on vegetative growth, yield components and change in some metabolic constituents of two chickpea (Giza 531 and Giza 195) of the seeds were assessed in the work.

Materials and Methods

Two field experiments were carried out in the production and research station of the National Research centre, at Al-Nubaria Region El-Beharia Governorate, Egypt, during two winter growing seasons (2014/2015 and 2015/2016) to study the effect of foliar application of different concentration of bio-regulators (i.e., ascorbic acid, benzoic acid, fulvic acid, humic acid) on some growth criteria, photosynthetic pigments and yield as well as some metabolic constituents of chickpea seeds (*Cicer arietinum* L. Variety Giza 531 and Giza 195). The experimental design treatments were arranged in complete block design with six replications. The plot area was 10.5m² (3.5 x 3.0 m) and consisted of five rows 70 cm apart and the distance between hills along the row 25cm apart and top water as control treatments seeds of chickpea (*Cicer arietinum* L.) Cv. Giza 531 and Giza 195) were obtained from Agricultural Research Centre, Giza, Egypt and Sown at the last week of October, in both seasons.

The normal cultural treatments of growing chickpea in the location were followed pre-sowing (50 kg/fed). Calcium super-phosphate (15.5% P₂O₅) was applied to the soil. While, nitrogen fertilizer 100 kg/ N/fed.) as ammonium nitrate (33.5%) were applied in two equal doses before the first and the second irrigation, respectively. In both seasons, a foliar spray was applied twice to chickpea plant during vegetative growth at 45 and 60 days after sowing with ascorbic acid, benzoic acid, fulvic acid, humic acid concentration solutions were sprayed over the entire-surface of plant including the adaxial and abaxial surfaces of leaves. No additives or surfactants such as tween were added. In addition, untreated plants (control) were sprayed only with tap water.

The plant growth characters were measured at 75 and 105 days after sowing, i.e., green yield as plant height, number of branches, leaves, and pods/plant, dry weight of branches, leaves and pods/plant and leaf area (cm²/plant) following the suggestions of Bremner and Taha (1966) photosynthetic pigment (Chlorophylls a,b, carotenoids as well as total pigments) in the fresh leaves was determined at two growth stages (75 and 105 days after sowing) according to Saric *et al.*, (1967). Leaf area index (LAI) was determined according to Watson (1952) and specific leaf weight (SLW mg/cm²) was calculated as Pearce *et al.*, (1969).

At harvest time, ten guarded plants were taken out randomly from the middle two ridges of each plot to determine the mean values of yield and its related parameters, i.e., plant height, number of branches/plant, number of pods and seeds/plant weight of pods/plant, seed and straw yield per plant and per ton/fed. Plant samples were taken from each plot and dried at 70°C for 48h until constant dry weight. Representative dry samples of seeds were taken from each plot to determine total carbohydrate content the methods of (Dubois *et al.*, 1956), Crude protein percentage was obtained by determine total nitrogen in seeds according to A.O.A.C. (1990). Phosphorus was determined according to watanab and Olsen (1965) and potassium by Jackson (1965).

Date for both growing seasons was carried out according to Snedecor and Cochran (1990). L.S.D. test at 5% level used for comparison between means.

Results and Discussion

Growth parameters:

Data presented in Tables (1, 2 and 3) show that spraying chickpea plant (Giza 531) with Benzoic acid 100 mg/L, Ascorbic acid 200 mg/L, Fulvic acid 6g/L and Humic acid 10 ml/L caused significant stimulative effect on plant height, number of branches, leaves and pods/plant dry weight of brnaches, leaves and pods, leaf area/plant, LAI and SLW at 75 and 105 days after sowing compared with Giza 195 and control. In addition, data indicate that the most effective treatment to harvest the greatest mean values from growth and its attributes was foliar spraying with Benzic acid 100 mg/L, Ascorbic acid 200 mg/L, fuluvic acid 6 g/L and Humic acid 10 g/L respectively, compared with control. Hartwigsen and Evans (2000) Ulukan *et al.*, (2012).

Data illustrated in Table (2) observed that foliar application with benzoic acid 100 mg/L on chickpea plants resulted significant increases in the previous growth measurements at 75 and 105 days after sowing in comparison with control treatment (untreated plants). Moreover, increasing the concentration of ascorbic acid up to 200 mg/L. Caused stimulative increases in growth and its attributed under study compares with control. Furthermore, the superiority of fuluvic acid 6 g/L than 10 ml/L Humic acid in its effects on growth parameters were significant at 75 and 105 days age except the differences in plant height. Number of branches, leaves and pods/plant, dry weight/plant. It was noted that fuluvic acid and Humic acid treatments were more effective than benzoic acid and ascorbic acid treatments in increasing vegetative growth of chickpea plant at the two different growth stages (Table 1, 2). Serenella *et al.*, (2002), Rasaan *et al.*, (2013).

The increment in growth characters studied was more pronounced with spraying fuluvic acid at 6g/L and Humic acid at 10m/L An increase of 54, 43, 40 and 31% over control occurred in dry weight of branches, leaves, and pods at 75 days after sawing with the application of benzoic acid 100 mg/L and ascorbic acid at 200 mg/L on chickpea plants, respectively. While, the Benzoic acid, ascorbic acid showed corresponding increments 50, 41, 38 and 22%, respectively (Table 1, 2). From our results in (Table 1, 2), data indicate that foliar application with fuluvic acid 6 g/L and Humic acid 10 ml/L gave the greatest mean values from growth characters (Giza 531, Giza 195) compared with control, Benzoic acid and ascorbic acid treatments. It may be due to that ascorbic acid foliar nutrient, benzoic acid, fuluvic acid and Humic acid had a promotion effect on growth and active constituent compounds on various plant and also synergistic effect on plant.

Finally, our obtained results regarding the effect of bioregulators on growth attributes of chickpea plants are confirmed with those obtained Sadaket *et al.*, (2013), Amin *et al.*, (2013) and Youssef *et al.*, (2015) Ahmed *et al.*, (2016).

Table 1: Effect of Ascorbic acid, Benzoic acid, Fulvic acid and Humic acid on growth characters of chickpea plant (Average of two seasons).

Bio-regulators Concentration		Plant height (cm)		No. of Branches/Plant		No. of Leaves/Plant		No. of Pods/Plant	
		A	B	A	B	A	B	A	B
Giza 531	Control	74.60	79.01	10.67	12.49	126.75	119.84	39.71	50.21
	Benzoic acid 100 mg/L	80.29	91.90	11.48	13.38	137.66	128.90	42.90	59.49
	Ascorbic acid 200 mg/L	84.33	95.81	12.39	15.62	142.50	131.89	49.40	60.34
	Fulvic acid 6 g/L	86.44	97.70	14.47	17.59	153.74	141.78	51.54	70.49
	Humic acid 10 mL/L	88.50	98.68	15.50	19.86	161.63	149.67	55.62	77.60
L.S.D. at 5%		2.04	2.52	1.03	1.97	3.85	5.89	2.92	3.01
Giza 195	Control	72.78	78.55	9.65	11.74	122.70	117.55	34.74	49.62
	Benzoic acid 100 mg/L	80.69	88.4	11.39	12.68	130.82	124.64	40.82	59.54
	Ascorbic acid 200 mg/L	83.70	90.39	12.43	13.59	138.93	129.39	43.90	62.46
	Fulvic acid 6 g/L	85.81	93.23	14.69	15.40	142.24	133.62	49.27	69.38
	Humic acid 10 mL/L	87.90	95.61	14.70	16.52	159.48	140.71	52.64	74.50
L.S.D. at 5%		1.91	2.3	0.59	1.12	2.11	3.31	2.08	2.96

A: 75 day after sowing B: 105 day after sowing

Table 2: Effect of Ascorbic acid, Benzoic acid, Fulvic acid and Humic acid on growth characters of chickpea plant (Average of two seasons)

Bio-regulators Concentration		Dry weight of branches/Plant (g)		Dry weight of Leaves/Plant (g)		Dry weight of Pods/Plant (g)		Leaf area (cm ² /Plant)	
		A	B	A	B	A	B	A	B
Giza 531	Control	4.09	3.89	3.88	3.56	6.33	8.81	1089.23	990.41
	Benzoic acid 100 mg/L	4.92	4.56	4.51	3.87	6.59	10.69	1187.9	1049.55
	Ascorbic acid 200 mg/L	5.33	4.9	4.69	4.09	7.14	11.78	1259.84	1164.39
	Fulvic acid 6 g/L	6.19	5.87	5.66	5.3	9.67	12.95	1291.71	1177.26
	Humic acid 10 mL/L	6.56	5.98	5.71	5.41	10.27	14.05	1309.67	1221.60
L.S.D. at 5%		0.39	0.67	0.27	0.11	0.56	1.21	8.89	10.46
Giza 195	Control	0.39	3.49	3.76	3.43	5.29	7.68	1078.53	987.80
	Benzoic acid 100 mg/L	4.43	3.88	3.82	3.49	6.13	9.89	1129.46	1034.29
	Ascorbic acid 200 mg/L	4.87	4.39	4.58	4.27	7.43	10.96	1199.39	1097.43
	Fulvic acid 6 g/L	5.82	4.96	5.39	4.03	8.12	12.9	1287.2	1171.50
	Humic acid 10 mL/L	5.97	5.67	5.52	5.21	8.39	13.55	1289.76	1167.82
L.S.D. at 5%		0.18	0.41	0.13	0.1	0.49	1.07	7.81	9.49

A: 75 day after sowing B: 105 day after sowing

Table 3: Growth characters of chickpea plant as effected by bioregulators (Average of two seasons).

Bio-regulators Concentration		LAI		SLW mg/Cm ²	
		A	B	A	B
Giza 531	Control	2.66	2.46	2.89	3.12
	Benzoic acid 100 mg/L	2.86	2.64	3.47	3.43
	Ascorbic acid 200 mg/L	3.01	2.78	3.55	3.49
	Fulvic acid 6 g/L	3.32	3.00	4.01	3.60
	Humic acid 10 mL/L	3.43	3.02	4.12	3.78
L.S.D. at 5%		0.11	0.14	0.04	0.06
Giza 195	Control	2.63	2.43	2.92	3.18
	Benzoic acid 100 mg/L	2.78	2.68	3.59	3.50
	Ascorbic acid 200 mg/L	2.89	2.72	3.69	3.58
	Fulvic acid 6 g/L	2.96	2.83	3.75	3.61
	Humic acid 10 mL/L	3.02	2.88	3.78	3.68
L.S.D. at 5%		0.05	0.07	0.03	0.05

A: 75 day after sowing B: 105 day after sowing

Yield and its Components:

Table (4) shows clearly that chickpea plants sprayed with fulvic acid 6 g/L and Humic acid 10 mL/L caused a significant increases in plant height, number of branches, pods, and seeds/plant, weight of pods, seed and straw/plant and seed and straw (yield/ton/fed.) as well as total carbohydrate %, crude protein %, nitrogen %, phosphorus %, potassium % Giza 531 and Giza 195. Compared with control treatment (Untreated plants) at 75 and 105 days after sowing.

Regarding, the effect of foliar spraying with concentrations of Benzoic acid and ascorbic acid on yield and its attributes of chickpea plant, data recorded in table (4) observed that there were significantly marked positive effect on plant height, number of branches and pods and seed/plant, seed and straw yield/plant and seed and straw yield (ton/fed) as well as total carbohydrate, and crude protein per dry seed under foliar with Benzoic acid 100 mg/L and ascorbic acid 200 mg/L in comparison with untreated plants (control treatment). Furthermore, increasing concentration of fulvic acid 6 g/L and Humic acid 10 mL/L caused an increment in yield and its attributes compared with control or Benzoic acid and ascorbic acid in Giza 531 and Giza 195 Nardi *et al.*, (2002), Zarghommejad *et al.*, (2014).

Table 4: Yield and its components of chickpea plants as effected by bioregulators (Average of two seasons)

Bio-regulators Concentration	Yield and its Component	Plant height (cm)	No. of Branches/Plant	No. of Pods/Plant	wt. of Pods/Plant	No. of seeds/Plant	Seed yield plant / (g)	Straw yield plant/ (g)	Seed yield ton/fed	Straw yield ton/fed
	Giza 531	Control	80.20	10.32	52.60	49.51	78.8	27.78	34.29	0.889
Benzoic acid 100 mg/L		91.34	12.61	56.82	52.34	89.59	31.69	39.17	0.978	1.32
Ascorbic acid 200 mg/L		94.49	14.09	61.46	54.12	113.46	32.5	41.21	1.029	1.41
Fulvic acid 6 g/L		97.54	16.69	72.54	55.76	118.29	54.41	44.01	1.069	1.44
Humic acid 10 mL/L		99.68	17.38	77.23	58.48	123.63	37.39	46.21	1.093	1.51
L.S.D. at 5%		2.05	0.87	4.08	1.76	2.83	1.92	1.88	0.04	0.09
Giza 195	Control	79.50	9.74	50.01	47.80	76.40	25.60	33.67	0.870	1.20
	Benzoic acid 100 mg/L	89.49	11.6	54.8	50.67	87.59	30.55	37.6	0.922	1.28
	Ascorbic acid 200 mg/L	93.35	13.55	63.56	54.01	105.64	31.34	38.93	0.969	1.31
	Fulvic acid 6 g/L	95.29	15.43	69.44	55.5	109.53	33.63	42.16	1.016	1.37
	Humic acid 10 mL/L	97.10	16.39	75.28	56.92	115.72	34.42	43.91	1.025	1.41
L.S.D. at 5%		1.82	0.69	2.79	1.43	1.39	1.26	1.33	0.02	0.06

Chemical constituents:

Data presented in Table (5) shows that application either Benzoic acid, ascorbic acid and fulvic acid, Humic acid at any concentration increased nitrogen, phosphorus, potassium, total carbohydrate and crude protein in the dry seed chickpea compared with their control. However, the four concentration of the four bioregulators caused a significant increase in the values of chemical constituents compared to untreated plants. Furthermore, the results in Table (5) indicated that among the treatments Benzoic acid 100 mg/L and ascorbic acid 200 mg/L followed by fulvic acid 6 g/L and Humic acid 10 mL/L and the combination between the four bioregulators recorded the highest level of N, P, K, cured protein and total carbohydrate in Giza 531 and Giza 195 at 75 and 105 days after sowing Amin *et al.*, (2013), El-Awadi *et al.*, (2014), Ahmed *et al.*, (2016), Canellas *et al.*, (2015).

Photosynthetic pigments:

Data presented in Table (6) shows that Chla, b, Carotenoids of chickpea Cv. Giza 531 and Giza 195 cultivars growing in Sandy soil, the four acid, i.e Benzoic acid, ascorbic acid, fulvic acid and Humic acid were sprayed at different concentration (100 mg/L 200 mg/L 6 g/L and 10 mL/L).

Application of fulvic acid and Humic acid increased Chl a, b and carotenoids increased compared with control treatment in Giza 531, and Giza 195. It is worthy to mention that fulvic acid and Humic acid treatments were more effective in increasing the photosynthetic pigments in Giza 531 or Giza 195 than Benzoic acid and ascorbic acid treatment. Fulvic acid and Humic acid treatment had much higher photosynthetic pigment than Benzoic acid and ascorbic acid at 75 and 105 days

after sowing as compared to controls. It is worthy that our results are in great harmony with those obtained by Malik and Ashraf (2012), Sadak *et al.*, (2013) and Aim *et al* (2013).

Table 5: Yield and its components of chickpea plants as effected by bioregulators on seeds (Average of two seasons).

Yield and its Component		Total Carbohydrate	Crude Protein (%)	N (%)	P (%)	K (%)
Bio-regulators Concentration						
Giza 531	Control	59.43	21.39	3.10	0.32	1.86
	Benzoic acid 100 mg/L	61.52	22.58	3.24	0.34	1.97
	Ascorbic acid 200 mg/L	62.78	23.7	3.72	0.38	2.39
	Fulvic acid 6 g/L	63.86	23.9	3.90	0.40	2.54
	Humic acid 10 mL/L	64.94	25.87	4.01	0.44	2.76
L.S.D. at 5%		1.02	1.19	0.18	0.02	0.15
Giza 195	Control	58.9	20.87	3.01	0.31	1.71
	Benzoic acid 100 mg/L	60.81	21.65	3.12	0.33	1.88
	Ascorbic acid 200 mg/L	61.69	22.84	3.33	0.35	1.99
	Fulvic acid 6 g/L	61.74	22.63	3.40	0.37	2.31
	Humic acid 10 mL/L	62.92	23.72	3.52	0.39	2.47
L.S.D. at 5%		0.81	1.05	0.12	0.01	0.11

Table 6: Photosynthesis pigments content of chickpea plants as effected by bioregulators 75 and 105 days after sowing (Average of two seasons).

Treatments		At 75 days after sowing			At 105 days after sowing		
		Chl.a	Chl.b	Carotenods	Chl.a	Chl.b	Carotenods
Giza 531	Control	1.76	0.69	1.52	1.83	0.71	1.9
	Benzoic acid 100 mg/L	1.86	1.42	1.69	1.88	1.47	1.97
	Ascorbic acid 200 mg/L	2.28	1.67	2.13	2.49	1.78	2.56
	Fulvic acid 6 g/L	2.69	1.82	2.49	2.76	1.94	2.81
	Humic acid 10 mL/L	3.53	2.08	2.52	3.8	2.38	2.98
L.S.D. at 5%		0.07	0.09	0.03	0.08	0.04	0.06
Giza 195	Control	1.59	0.35	1.49	1.69	0.65	1.78
	Benzoic acid 100 mg/L	1.76	0.44	1.56	1.82	0.79	1.89
	Ascorbic acid 200 mg/L	1.87	1.51	1.64	1.89	1.81	1.94
	Fulvic acid 6 g/L	2.75	1.62	2.33	2.83	1.9	2.88
	Humic acid 10 mL/L	2.82	1.78	2.47	2.9	2.51	2.96
L.S.D. at 5%		0.04	0.06	0.03	0.05	0.03	0.02

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