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Effect of Gibberellic Acid and Indole 3-acetic Acid on Improving Growth and Accumulation of Phytochemical Composition in *Balanites aegyptiaca* Plants

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ABSTRACT

Seeds of *Balanites aegyptiaca* were soaked in Gibberellic acid solutions (0, 50,100, 150, ppm) and Indol 3-acetic acid solutions (IAA) (1000, 2000, 3000 ppm) for 14 h to study their effects on the germination percentage, plant height, number of branches and leaves, total chlorophyll content, dry weight and protein, carbohydrates, alkaloids, tannins, saponins and phenol contents. The study resulted in significant increases in most of these parameters mentioned previously. In contrast, a decrease in phenols content was found using all concentrations of both GA_3 and IAA. The concentrations of 50 ppm of GA_3 and 2000 ppm of IAA gave the best results by increasing the growth and phytochemical composition.

Key words: *Balanites aegyptiaca*, alkaloid, carbohydrates, chlorophyll, phenols, protein, saponin, tannins

INTRODUCTION

Balanites aegyptiaca (L.) Delile belongs to the family Balanitaceae, popularly known as the desert date, soapberry tree and thorn tree and easily can be propagated by seeds. It is a evergreen woody tree (10 m) with a stem diameter of about 30 cm and snarled mass of barbed branches. Thorns are up to 8 cm, soft at first and then woody. The leaves are distinctive pairs of grey-green colour and ovate shape; flowers are fragrant in yellow-green clusters. Fruits are oblong, 5 cm with both ends round, yellow when ripe with a hard seed inside (El-Sadat-Neveen, 2005; Mostafa, 2009). It is a slow-growing tree with a capacity of coppicing. It is found throughout the arid region of the world, grows in the dry, sunny areas of Africa, the Middle East, India and Burma. The tree is an important species for dry areas.

Plant Growth Regulators (PGRs) are the chemicals which influence the plant growth when they are applied in very little quantities. It is known that the developmental processes in plants are regulated by the action and balance of the different groups of growth regulators, which may act as promoters or inhibitors of these processes. However, there is little available information concerning the possible involvement of these compounds in the secondary metabolism of plant: terpenes, phenols and alkaloids (Ortuno *et al*., 1999). There are many reports which indicate that application of growth regulators enhance plant growth and crop yield (Hernandez, 1997).

Schelin *et al.* (2003) concluded that GA_3 did not result in significantly higher germination than the control plants of *Balanites aegyptiaca*. This result disagree with the finding of Zarad *et al.* (1998) who found that the germination percentage and rate of *Balanites aegyptiaca* were highest with GA_3 (500 ppm) treatment (>70% germination). The vegetative growth characteristics of Gladiolus plants were improved as a result of using GA_3 application (Dataram *et al.*, 2001; Kirad *et al.*, 2001; Prasad *et al.*, 2002). GA_3 levels used enhanced the vegetative growth of *Zantideschia aethiopica* plants (Attia, 2004; Brooking and Cohen, 2002).

Youssef (2004) found that spraying *Sterlizia reginae* plants with GA_3 at 100 or 200 ppm improved the vegetative growth parameters. In addition, Abou El-Elela (2007) showed that spraying *Acanthus mollis* plant with GA_3 enhanced vegetative growth measurements.GA_3 also increased mobilization of starch in cotyledons by increasing amylase activity. It was thought that enhanced germination and seedling growth by PGRs might be mediated through changes in the activities of enzymes of carbohydrate metabolism (Kaur *et al.*, 2000). Plant growth regulators (ex. IAA, GA_3 and cytokinins) induced a marked accumulation of protein content and carbohydrates content (Abdel-Latef, 2003; Abou Al-Hamd, 2007). Indol 3-acetic acid also increased growth and yield of black seeds as found by Hussein *et al.* (2003).

The fruits of *Balanites aegyptiaca* can be eaten and they have been used in the treatment of liver and spleen disease, as they were recorded to kill the snails which carry schistosomaiasis and bilharzias flukes. The roots are used for abdominal pains, a purgative gum from the wood is mixed with maize meal porridge to treat chest complaints. Edible fruit has 30-40% of edible oil. Seed kernels yield the sapogenin and yanogenin. Seeds are given in cough and colic bark, unripe fruit and leaves are pungent, bitter, purgative and anthelmintic for cattle (El-Sadat-Neveen, 2005). The usefulness of these plants materials medicinally is due to the presence of bioactive constituents such as alkaloids, saponines, tannins and phenols. Saponins are the active component which prevent disease invasion of plants by parasitic fungi, hence have some antifungal properties (Osuagwu *et al.*, 2007). Saponins are useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effects in the food industry. It is also used in the manufacture of shampoos, insecticides various drug preparations and synthesis of steroidal hormones (Sodipo *et al.*, 2000).

Alkaloids play some metabolic role and control development in living system (Edeoga and Eriata, 2001). They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids (Stevens *et al.*, 1992). Alkaloids are known to exhibit marked physiological activity when administered to animals (Okwu, 2004). Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic antispasmodic and bactericidal effects (Stray, 1998).

Tannins are known to inhibit pathogenic fungi; the presence of phenolic compounds in the leaves of the plants may act as antimicrobial agents. Phenolic compounds may be the reason for the therapeutic, antiseptic, antifungal or bactericidal properties of the plants (Osuagwu *et al.*, 2007). Yousef and Gomaa (2008) investigated the effect of GA_3 treatments (0, 100, 200 and 300 ppm) on Dahlia applied by three methods i.e., tubers soaking before planting, foliage spraying and tubers soaking+foliage spraying. They found that all tested treatments of GA_3 succeeded in improving the studied vegetative traits as well as leaf chemical composition determinations. The investigation was carried out to study the effect of Gibberellic Acid (GA_3) and Indol 3-acetic acid (IAA) on the growth and phytochemical composition of *Balanites aegyptiaca*.

MATERIALS AND METHODS

The investigation was carried out during the two successive seasons of 2008 and 2009 at the Nursery of Ornamental Plants, Faculty of Agriculture, South Valley University, Qena, Egypt.

Seeds were soaked in Gibberellic acid solutions (0, 50,100, 150, ppm) and Indol 3-acetic acid (IAA) (0, 1000, 2000, 3000 ppm) for 14 h on June 23 and April, 3 for first and second seasons, respectively. After soaking, The seeds were washed with tap water and sown in 20 cm plastic pots containing a soil mixture of clay and sand (1:1 v/v) ninety seeds were sown for each treatment. The seeds were sown in three replications; each replication contained three pots (ten seeds in each pot). After 45 days from sowing seedlings were transplanted into a 20 cm plastic pots containing the soil mixture of clay and sand (3:1 v/v). Plants were irrigated using the available water with Electro Conductivity (EC.) Value of 3.70 and 5.81 dSm. in the first and second seasons, respectively.

Experimental layout and statistical analysis: The experimental layout was a randomized complete block design containing three replications (Steel and Torrie, 1982). Each replication contained seven treatments and every treatment consisted of 10 plants (7 treat. X 3 repl. X 10 plant = 210 plant).

Plant growth and phytochemical parameters:

Seed germination: Mean seed germination percentages were calculated after twenty one days from sowing. Data were subjected to angular transformation prior to statistical analysis. All the followed data were recorded after five months, plant height (cm), number of branches, numbers of leaves were recorded.

Chlorophyll estimation: Total chlorophyll (SPAD units) was determined in the leaves at the end of experiment with SPAD apparatus as described by Yadava (1986). Carbohydrates content in the leaves was carried out after the anthrone sulphoric acid method of Herbert *et al.* (1971). Protein content in the leaves was determined according to Lowry *et al.* (1951). Saponins and alkaloid determination in the leaves was carried out according to Osuagwu *et al.* (2007). Tannins were determined using Washington (1970) method. Phenols determination was carried out using Cheng and Hanning (1955) method.

RESULTS AND DISCUSSION

Table 1 showed that the growth criteria (germination percentage, plant height, number of branches and leaves, total chlorophyll and dry weight) significantly increased when soaking of *Balanites aegyptiaca* seeds in the different concentrations of Indole Acetic Acid (IAA) as compared with the control. Except plants treated with 1000 and 3000 ppm which having lowest No. of branches in the first season and total chlorophyll in both seasons, respectively.

With respect to GA_3, all concentrations were increased significantly seed germination, plant height, No. of leaves, dry weight of vegetative growth and total chlorophyll, except for 150 ppm which decreased plant height, no of leaves, dry weight in the first season. No significant differences were found between plants treated with 100 and 150 ppm compared to control for No. of leaves, 100 ppm for dry weight, 150 ppm for plant height in the first season and total chlorophyll in the second season. The concentration of 150 ppm decreased dry weight in the first season only.

The different concentrations of IAA and GA_3 were increased protein content (soluble, insoluble and total) and carbohydrates content (soluble, insoluble and total) when compared with untreated plants in both seasons (Table 2). While, treating seeds with 3000 ppm IAA were decreased insoluble

Table 1: Effect of GA_3 and IAA on seed germination percentage, plant height (cm), number of branches per plant, number of leaves per plant, dry weight (g/plant) and total chlorophyll content (SPAD unit)

	Seed germination percentage		Plant height (cm)		No. of branches per plant		No. of leaves		Dry weight of vegetative growth (g)		Total chlorophyll content (SPAD unit)	
Treatment	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
GA_3												
Cont.	67.5e	60.0e	53.6c	26.6c	3.0b	1.0b	55.3de	35.0c	3.5bc	0.90g	65.1bc	58.3ab
50 ppm	75.9d	68.6d	61.0b	38.6b	3.0b	1.3b	84.3a	71.3a	3.5bc	1.54a	73.3a	49.7b
100 ppm	99.2a	81.7c	62.3ab	37.6b	2.0b	1.3b	54.3de	43.3bc	2.9cd	1.20d	69.0ab	63.2a
150 ppm	97.0b	99.8a	53.0c	50.6a	2.1b	2.3a	46.0e	49.3b	2.3d	1.42c	69.3ab	55.5ab
IAA												
1000 ppm	96.4b	88.3b	58.6bc	45.3ab	2.5b	2.5a	59.3cd	37.3c	3.5bc	1.01f	70.6ab	63.0a
2000 ppm	96.5b	99.9a	61.3b	47.3a	3.0b	2.5a	68.3bc	38.3c	4.6a	1.50b	70.0ab	57.0ab
3000 ppm	88.7c	99.9a	68.3a	28.3c	5.0a	2.6a	76.3ab	35.1c	4.0ab	1.08e	63.3c	50.4b

*and** = significant at p = 0.05 and 0.01, respectively. Values in the same column not followed by the same letter are significantly different at the 5 % level of probability

Table 2: Effect of GA_3 and IAA on the contents of protein and carbohydrate (soluble, insoluble and total) [mg g^{-1} dry weight]

	Soluble protein		Insoluble protein		Total protein		Soluble carbohydrates		Insoluble carbohydrates		Total carbohydrates	
Treatment	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
GA_3												
Cont.	157.5f	160.2d	37.8e	27d	195.3f	187.2d	294.4d	160.6f	146.6c	87.3e	381.7f	307.2e
50 ppm	170.1c	162.9b	63.9a	45.9a	234.0b	208.8ab	327.2a	261.8a	209.4a	113.2c	440.4b	471.2a
100 ppm	169.8c	162.0c	35.1f	44.1ab	196.2ef	206.1bc	315.3b	223.2b	205.9a	126.0b	429.6cd	429.1b
150 ppm	161.1e	158.4e	27.9g	33.3cd	197.7e	191.7d	303.6c	184.6d	128.4d	106.2d	421.5e	313.0e
IAA												
1000 ppm	166.5d	162.9b	39.4d	37.8bc	205.9d	200.7c	297.8cd	187.2d	176b	136.2a	434.0bc	363.2d
2000 ppm	187.2a	163.8a	49.6b	49.5a	236.8a	213.3a	325.1a	200.4c	178.3b	135.8a	460.9a	378.7c
3000 ppm	171.6b	162	45.0c	47.7a	216.6c	209.7ab	318.2b	168.3e	124.6d	105.4d	423.6de	292.9f
LSD	1.0**	0.49**	1.0**	8.0**	2.0**	6.2**	6.4**	4.0**	5.0**	6.7**	7.1**	11.9**

*and** = significant at p = 0.05 and 0.01, respectively. Values in the same column not followed by the same letter are significantly different at the 5% level of probability

carbohydrates in the first season and total carbohydrates in the second season. Also the concentration of 100 GA_3 was decreased soluble protein in the second season, insoluble protein and insoluble carbohydrates in the first season. The concentration of 100 ppm GA_3 decreased insoluble protein in the first season.

Alkaloids, saponins and tannins contents in the leaves of *Balanites aegyptiaca* plants were increased significantly by using all concentrations of IAA as compared with the control in both seasons (Table 3). Also alkaloids and tannins contents were increased with all concentrations of GA_3. While saponins content increased only by using 50 and 100 ppm GA_3 in the first and second seasons, respectively. It can be seen that treating seeds of *Balanites aegyptiaca* with IAA were more effective than GA_3 treatments on accumulation of phytochemical composition. The phenolic

Table 3: Effect of GA_3 and IAA on the content of alkaloids, spooning, tannins and phenols (mg g^{-1} dry weight)

	Alkaloids content		Saponins content		Tannins content		Phenols content	
Treatment	First season	Second season	First season	Second season	First season	Second season	First season	Second season
GA_3								
Cont.	0.17c	0.32e	0.73c	0.71b	2.11d	1.86d	6.20a	8.99a
50 ppm	0.27ab	0.36d	0.8b	0.71b	2.83ab	2.92a	5.99a	7.83b
100 ppm	0.33a	0.49b	0.7c	0.8a	2.28cd	2.43bc	5.39b	6.48c
150 ppm	0.29ab	0.36d	0.71c	0.69b	2.6bcd	2.15cd	5.19bc	6.45c
IAA								
1000 ppm	0.27ab	0.38c	0.95a	0.8a	3.24a	2.17cd	4.96bcd	6.36c
2000 ppm	0.33a	0.54a	0.81b	0.84a	2.95ab	2.8ab	4.85cd	5.95d
3000 ppm	0.23bc	0.39c	0.81b	0.8a	2.66bc	2.35c	4.65d	5.62e
LSD	0.08*	0.02**	0.04**	0.05**	0.55*	0.44**	0.47**	0.22**

*and** = significant at p = 0.05 and 0.01, respectively. Values in the same column not followed by the same letter are significantly different at the 5% level of probability

compounds markedly decreased in plants treated with the different levels of GA_3 and IAA compared with the untreated plants (Table 3). The results found in Table 1-3 concluded that the concentrations of 50 ppm of GA_3 and 2000 ppm of IAA were the best concentrations to increase the growth and phytochemical composition of *Balanites aegyptiaca*.

Plant hormones exert far reaching effects on plant growth, the precise action depending on the concentrations of the substances present and the sensitivity of the organ concerned. Applied GA_3 and IAA showed increase in growth criteria (germination percentage, plant height, number of branches and leaves, total chlorophyll content, dry weight). Similar results were observed by Naeem *et al.* (2004) where they found that application of GA_3 showed remarkable increase in the number of compound leaves and the length of shoot and the application of IAA resulted in an increase in the number of compound leaves. GA_3 and IAA had regulatory effect to enhance the plant height, number of branches, number of leaves as compared to other plant growth regulators and control (Sarkar *et al.*, 2002). Ashraf *et al.* (2006) concluded that IAA is successful in enhancing the plant growth and yield of barley cultivars and alleviated the adverse effect of water stress.

GA_3 and IAA treated plants exhibited higher values of dry weight and chlorophylls content than did the control (Abdel-Latef, 2003; Afroz *et al.*, 2005; Abou Al-Hamd, 2007). Enhanced germination and seedling growth by plant growth regulators may be mediated through changes in the activities of carbohydrate metabolism enzymes (Kaur *et al.*, 2000). The increase in the dry matter due to soaking in GA_3 and IAA solution might be attributed to rapid increase in cell division, cell enlargement and accumulation of building units that accompanied by greater saccharides content than those of untreated plants (Abdel-Latef, 2003; Abdel-Latef *et al.*, 2009).

Our results revealed that GA_3 and IAA induced a marked accumulation of protein content and carbohydrates content. This accumulation of carbohydrates due to GA_3 and IAA treatment might be linked with the efficiency of photosynthetic apparatus, which leads to increase in plant productivity and dry matter production (Azooz *et al.*, 2004). Increasing protein content by plant growth regulators may be due to increase the formation of rough endoplasmic reticulum that provides the appropriate medium for increasing polyribosome and mRNA (Kaber, 1987).

GA_3 and IAA stimulated a considerable increase in alkaloids, saponins and tannins contents of *Balanites aegyptiaca* plants, but markedly decreased phenolic compounds. The increasing of seed

germination percentage as a result of the exogenous application of both growth regulators was positively correlated with the decrease in total phenols. This result agrees with those of Baskin and Baskin (1998) and Araby *et al.* (2006).

Many contributions support these results, although some others suggest that this phytoregulators might modulate the expression of secondary compounds of a terpenic (Ortuno *et al.*, 1993), phenolic (Garcia Puig *et al.*, 1995) and alkaloid (Cho *et al.*, 1988) nature.

A decrease in the plant growth were found, while alkaloid content in the leaves were increased in the second season with comparable to first season, this may be due to the increase in water salinity in the second season, where plants were irrigated using the available water with Electro Conductivity (EC) Value of 3.70 and 5.81 dSm in the first and second seasons, respectively. This result confirmed with the finding of Abdul-Jaleel *et al.* (2008) on *Catharanthus roseus*. It can be seen that this decrease in plants treated with GA_3 or IAA in most cases were lower than that of the control. This may be due to that, the exogenous application of GA_3 or IAA alleviated the adverse effects caused by salt stress as found by Radi *et al.* (2001), Gaber (2003) and Ashraf *et al.* (2006) by harmonizing the ionic stress of the plants and reduced the net accumulation of Na^+ and Cl^- as well as by modulating the endogenous level of abscisic acid.

In conclusion, phytohormones like GA_3 and IAA had a good potential for improving plant growth and accumulation of some phytochemical composition. Treating seeds of *Balanites aegyptiaca* with IAA were more effective than GA_3 treatments. The concentration of 2000 ppm of IAA gave the best results.

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