Stimulation effect of some bioregulators on flowering, chemical constituents, essential oil and phytohormones of tuberose (*Polianthes tuberos L.)*.

[(1)] Lobna, S. Taha and [(2)] Rawia, A.Eid

Department of Ornamental Plant and Woody Trees.National Research Centre,Dokki, Cairo, Egypt
* Corresponding author, emil, lobnasalah82@yahoo.com

Abstract: Bulbs of tuberose plants were soaked (24h) or sprayed with solutions of spermidine and ATP at 50, 75 or 100ppm for each. Both bioregulators (especially at 100 ppm) augmented plants bulblets and flowering characteristics (No. of bulblets, fresh and dry weights of bulblets, no of days to flowering, No of florets/spike, spike length, length of rachis and fresh and dry weights of spike) Spermidine was more effective than ATP for bulblets parameters but ATP was preferred for flowering parameters, photosynthetic pigments (Chl a, b and carotenoides), chemical constituents of plants (Indoles, phenols and total carotenoides). Essential oil content of flowers was significantly improved by soaking or spraying of ATP at 50, 75 or 100ppm. The highest amount of endogenous GA3 produced with ATP at 100ppm. However, spermdine (100ppm) caused the highest amount of cytokinins. Using ATP as soaking or spraying treatment at 75 or 100ppm resulted in the highest amount of ABA.

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Key words: Spermidine, ATP, flowering, chemical constituents, essential oil, phytohromones, tuberose.

1-INTRODUCTION

Polianthes tuberose linn (Family, Amaryllidareae) is a night blooming plant that distributed in hotter parts, mainly south America or Mexico. Besides its popularity as an ornamental garden plants with beautiful blossoms, flowers are used in perfume industry and also diuretic and emetic activity. Bulbs are used for curing raches in infant (Rammamurthy *et al.,* 2010)[(1)].

Spermidine is one of the major polyamine forms in plants. Polyamines are low-molecular weight polycations constitute a part of the overall nitrogen metabolism and the nitrogen source in the nutrient solution determines the polyamine synthesis and accumulation in plant (Altman and Levin, 1993)[(2)] . It was suggested that polyamines are able to bind with several negatively charged molecules, such as DNA (Pohjanpelta and Holtta, 1996)[(3)], protein (Apelbaum *et al.,* 1988)[(4)], membrane phospholipids and proteins (Tassoni *et al.,* 1996)[(5)] and pectic polysaccharides (D'oraci and Bangi, 1987)[(6)]. Polyamines were localized in the vacuoles, mitochondria and chloroplasts (Abd El-Wahed and Krima, 2004)[(7)]. They modulate several growth and developmental processes viz., cell division, differentiation, flowering fruit ripening, embryogenesis, senescence and rhizogenesis (Kakkar *et al.,* 2000)[(8)].

Phosphorus plays an important role in many enzyme reactions depending on phosphorylation and energy conservation and transfer for a wide range of biochemical processes (Walker, 1980[(9)]; Stevenson, 1986[(10)]; and Marshner and Cakmak, 1986[(11)]).

Phosphorus nutrition is doubly critical because the total supply of phosphorus in most soils is low and is not readily available for the plant use. Most of the basically adenosine- tri- phosphate (ATP) generating pathway, i.e. photophophorylation, glycolysis, TCC-Cycle, and oxidative phosphorylation are restricted [Lyons and Breidenbach (1990)[(12)] and Ortiz (1991)[(13)]]. In addition, synthesis of bioconstituents, minerals uptake, translocation and retention processes are dependent on the adenosine tri-phosphate (ATP) supply (Mengel and Kirkly, 1982). Besides the involvement of ATP in the system of gene expression and function, it is also directly involved in gene (DNA) structure (Dashek, 1997)[(15)]. The AMP (the hydrolytic derivative of ATP) is the main precursor of cytokinins (Jameson, 1994)[(16)].

Phytohormones regulate the protective responses of plants against both biotic and abiotic stresses by means of synergistic or antagonistic actions (Schemlz *et al.* 2003)[(17)].

The major objective of this investigation was to determine the effects of spermidine as polyamine and adenosine-tri-phosphote (ATP) on the promotion of plant growth flowering, chemical constituents, essential oil content and endogenous phytohormons of *Polianthes tuberosa* L.

2-Materials and Methods

The field experiments were carried out in Oseim district, 6 of October governorate during the two successive seasons 2008 and 2009. The aim of study was to investigate the effect of spermidine and ATP soaking and foliar application on the flowers, essential

oil content and some chemical constituents of *Polianthes tuberosa L.* plants.

Experimental procedures: Bulbs of tuberose were obtained from ornamental plant research Dept., Ministry of Agric, Egypt for cultivation. The soil is clay loam in texture (sand 37, silt 28 and clay 35%), tented to alkalinity in reaction (pH 7.91). It had low content of calcium carbonate (2.04%); organic matter (1.40%) and E.C. (0.45ds/m). High in available phosphorus, potassium (3.2 and 78mg/100g soil), and low in available Fe, Mn, Zn, Cu (9.3, 3.24, 0.84, 0.89 mg/100g soil), respectively.

On April, 2008 and 2009 bulbs of tuberose plants were soaked in prepared solution of spermidine and ATP reach at 50, 75 and 100ppm for 24h. after that planted in rows, at spacing of 30 cm between bulbs within each row, and 60cm between rows. The plants were fertilized with 80: 40: 60 g/m^2 from NPK, calcium superphosphate (15.5% P_2O_5) was added before planting while the plant were fertilized with ammonium nitrate (33.5%) and potassium sulphate (48% K_2O) after 30 days from planting at two side dressings. Plants were sprayed three times with spermdine and ATP each at (50, 75 and 100ppm). The control plants were sprayed with water. The experiments were set up in a completely randomized block design with three replications. After the flowering period of each season, the following data were recorded, number of days to flowering, spike length (cm), spike diameter (cm), length of the rachis (cm), number of flowers/spike, fresh and dry weight of spike (g), No.of bullblets /plants, fresh and dry weight of bulblets (g). Treatments were arranged in a complete block design with three replicates. The data were statistically analyzed using analysis of variance according to Snedecor and Cochran (1980)[18].

Chemical Analysis : Soil surface samples (0-30 cm depth) were taken before planting from the experimental site. Soil was air-dried and sieved through 2mm sieve. Physical and chemical characteristics, were evaluated according to Ankerman and Large (1974)[18]. Total carbohydrates were determined in the ground line dry powder of tuberose plants using the colorimetric method described by Herbert et al. (1971)[20], photosynthetic pigments including chlorophyll (a and b) as well as carotenoids content were determined in fresh branchlets as mg/gm fresh weight, according to the producer achieved by Saric *et al.* (1967)[21].

Total phenols : 1gm of the fresh leaves were macerated in 5-10 ml 80% ethanol for at least 24 hr at 0°C, the alcohol was clarified the remained residue was re-extracted with 5-10ml 80% ethanol 3times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of folin-Denis as described by Daniel and George (1972)[22].

Endogenous phytohormones :

The shoot of the plants of this group were frozen in liquid nitrogen and stored in deep freezer at -20°C until used for estimation of endogenous hormones.

Endogenous phytohormones were determined by using HPLC method

The frozen plant material were homogenized with absolute ice clod methanol in wavering blander and stored at 3°C for 24h. The alcohol was filtered and two subsequent extraction were carried out using 80% ice cold methanol and the alcoholic extracts were evaporated to aqueous phase as described by (Badr *et al.,* 1971)[22]. The aqueous phase was adjusted to pH 8.2 and partitioned three times against equal volumes of petroleum ether according to (Van Bragt, Laba, 1969)[24].

Separation of gibberellins, auxins and cytokinins were carried out according to Hiraja *et al.,* (1972)[25]. Endogenous hormones IAA, GA3 and were determined by their absorption at 269 nm. The peak area of the samples were compared to the corresponding peak areas of a standard solution containing known concentration analyzed using a waters 54100 high performance liquid chromatography (HPLC).

3-Results and Discussion

Bulblets and flowering characteristics

Data given in Table 1 show that soaking treatment of spermidine at 100ppm was a companied by an increase in the number of bulbets, fresh and dry weights of bulblets as well as number of florets/ spike length and length of rachis. The increment were 55.5%, 86.1% and 56.6%, 29.61, 20.3% and 4.2%, respectively as compared with control plants in the two seasons. While, the application of spermidine at 100ppm as spraying treatment caused the highest fresh and dry weights of spike (100.31 and 27.71, respectively).

Relations between polyamine and flowering processes have been observed by many investigators. In cherry flower buds, polyamines that increase rapidly with the onset of active metabolism were dected in all stages of bud development (Wang et al., 1985)[28]. Conjugated polyamines are known to be associated with the physiology of flowering metabolite synthesis (Slocum and Galston, 1985)[27]. Spraying *Calendula* officinalis plants with brassinosteroid significantly promoted their flowering characteristics (Shalaby and Talaat, 1998)[28]. Nahed *et al.* (2009)[28] found that application of putrescine at 200 ppm had a promative effect on cormlets and florets caracters of gladiolus plants.

Regarding the effect of ATP, it was found that (Table 1) the best results in bulblets and florets

parameters (No of day to flowering, No of florets/spike, spike length, length of rachis, fresh and dry weight of spikes were obtained by using soaking treatment of ATP at 100 ppm.

Rawia *et al.,* (2009)[30] on *Matthiola incana* L. indicated that ATP application was considered the most effective treatment for increasing growth characters i.e No of flowers and dry weight of flowers. This may be attributed to the major role of ATP in plant metabolism according mengel and Kirkly (1982)[14].

Table (1): Effect of spermidine and ATP on tuberose bulblets and flowering characteristics (mean of the two seasons).

Treatment	Concentration (ppm)	No. of bulblets	F.w of bulblets (g)	D.W of bulblets (g)	No of days to flowering	No of florets /spike	Spike length (cm)	Length of rachis (cm)	F.W of spikes (g)	D.W of spike (g)
Soaking										
	Control	12.23	17.80	2.45	91.2	20.11	68.21	2.81	80.23	18.00
	50	20.53	71.07	3.81	89.30	22.62	73.25	3.00	85.63	21.42
Sperimidine	75	24.11	83.50	4.51	86.10	25.14	83.11	3.62	90.21	24.11
	100	27.51	127.90	5.65	86.40	28.60	85.62	4.90	97.41	26.71
	50	18.71	64.50	3.15	84.53	30.40	77.42	4.11	100.25	28.41
ATP	75	22.82	81.20	4.18	84.53	32.11	88.32	4.71	105.3	30.18
	100	26.32	100.30	4.74	82.10	36.21	91.25	5.32	109.2	33.47
Spraying										
	50	17.21	86.11	2.87	89.3	22.21	70.23	2.85	77.61	16.31
Sperimidine	75	23.00	80.21	3.97	86.3	23.11	76.21	4.22	81.14	17.94
	100	25.21	111.41	5.00	86.00	26.71	82.74	4.28	100.31	27.71
	50	16.80	61.35	1.83	84.62	26.22	72.12	3.82	78.53	17.89
ATP	75	21.41	78.92	3.21	84.65	28.52	78.07	4.42	85.21	23.41
	100	23.58	88.31	3.97	84.69	32.22	83.51	4.58	103.43	29.84
LSD (0.05)		3.52	0.81	0.32	1.38	0.31	1.81	0.04	2.52	1.11

Photosynthetic pigments:

The data recorded in table 2 show that soaking treatment of ATP at 100 ppm significantly increased chlorophyll a, b and a+b to highest amounts (0.57, 0.25 and 0.82 mg /g. F.W, respectively) followed by soaking treatment of spermidine at 100ppm. Whereas, application of spermidine or ATP as spraying treatment (100 ppm) increased carotenoides content to highest values (0.69 and 0.67 mg/g F.W, respectively) in tuberose plants as compared with control (Table 2). These results indicated that tuberose plant content of pigments responds to spermidine and ATP applicaton positively. This could be due to the spermidine capability (as polyamine) to stabiles protoplast and prevent both loss of chlorophyll during senescence in protoplast and leaves (Kaur-Sawhney, 1980)[31]. Ma *et al.*, (1996)[32] suggested that he effect of polyamines in inhibiting chlorophyll degradation may be related to the inhibition of peroxidase activity. Increasing carotenoids content may due to convert these substances to pyruvic acid that led to enhance bioynthesis of leaf carotenoids (Martin – Tanguy, 2001)[33].

Regarding the effect of ATP, the above mentioned results are in harmony with those obtained by Rawia *et al.,* (2009)[30] who observed that the highest values of chlorophyll a and b in *Matthoila incana* plant was obtained from ATP application.

Table (2). Effect of spermidine and ATP on photosynthetic pigments (mg/g F.w)of *Polianthes tuberose* L (mean of two seasons).

Treatment	concentration (ppm)	Chl a	Chl b	a+b	Carotenides
Soaking					
	Control	0.21	0.09	0.30	0.23
	50	0.25	0.16	0.41	0.28
Spermedine	75	0.29	0.18	0.49	0.34
	100	0.43	0.22	0.65	0.37
	50	0.52	0.20	0.72	0.50
ATP	75	0.56	0.23	0.79	0.57
	100	0.57	0.25	0.82	0.64
Spraying					
	50	0.20	0.14	0.34	0.52
Spermedine	75	0.27	0.17	0.44	0.60
	100	0.34	0.22	0.56	0.69
	50	0.47	0.15	0.62	0.61
ATP	75	0.49	0.17	0.66	0.65
	100	0.53	0.22	0.75	0.67
LSD (0.05)		0.002	0.004	0.001	0.004

Chemical constituents:

Total indoles :

Data presented in table 3 show that spermindine and ATP significantly affected total indoles content of *Polianthes tuberose* plants. This was shown by the increased indoles content in plants with soaking or spraying spermidine or ATP at 100 ppm which were significantly variable compared to the effect of the control. This showed that spermidine

treatments enhanced phytohormons which can play an important role as regulator of growth and development of plant endogenous polyamine (Shunquan *et al.*, 2001)[34]. In the same time, role of ATP in activating most processes in plant metabolism was mentioned by Mengel and Kirkly (1982)[14].

Total soluble phenols :

Data presented in Table 3 show significant differences in total soluble phenols content of *Polianthes tuberose* plants as a result of spermidine or ATP application. Spraying spermidine treatment at 100ppm increased the phenolic content to the highest value (19.2mg/g F.w) followed by ATP at 100 ppm as soaking or spraying treatment (15.2 and 15.6, respectively). Spermidine treatment at 100ppm (soaking or spraying) was more effective than the ATP one. It could be due to the convertion of spermidine into another substance in the plant. It could derive from sugars, free amino acids, phenolic compounds and essential oil (Abd El-Wahed *et al.*, 2004)[7]. Spermidine converts to diaminopropane that can be converted into β-alanine, which in turn deaminates the production of cinnamic and para coumaric acids, respectively, from which the more complex phenolics are converted (Herrman, 1976)[35].

Total carbohydrate content :

Data in table 3 show the total carbohydrate % in tuberose plants. It can be emphasized that application of ATP at 100 ppm (soaking or spraying) resulted in the highest values of total carbohydrates (25.3% and 23.21%, respectively) followed by soaking treatment of ATP at 75ppm and spermidine at 100ppm (22.5 and 22.2, resepectively) as compared with control. These results were confirmed by those of Dessouky (2002)[35] on *Borago officinalis* and Rawia (2004)[30] on *Mattiolia incana.*

Essential oil content (%) :

Data presented in Table 3 indicate that application of spermidine or ATP as soaking or spraying treatment significantly affect tuberose essential oil percent. It could be deduced from the present results that essential oil percent increased to highest percentage by application of ATP as soaking or spraying treatment at 50, 75 or 100 ppm followed by similar treatments of spermidine as compared with control. These results are in close agreement with those reported by Baljieet *et al.*, (1996)[36] on *Guizatia abyssinica*, Shalan (2001)[38] on *Lagenaria siceraria*, Dessouky (2002)[36] on *Borago officinalis* and Abd El-Wahed *et al.* (2004)[7] on *Chamomilla recutita.*

Table 3: Effect of spermidine and ATP on chemical constituents and oil content (%) of *Polianthes tuberose L.* (mean of two seasons).

Treatment / concentration (ppm)		Indoles mg/g F.w	Phenoles mg/g F.W	Carbohydrates (%)	Oil percentage (%)
Soaking					
	Control	1.56	12.5	10.74	0.18
	50	2.34	13.4	15.9	0.21
Spermedine	75	2.73	14.6	20.1	0.23
	100	3.42	16.7	22.2	0.27
	50	3.11	9.3	17.3	0.32
ATP	75	3.89	10.8	22.5	0.38
	100	3.91	15.5	25.3	0.39
Spraying					
	50	1.89	15.7	14.41	0.19
Spermedine	75	2.46	17.8	16.32	0.20
	100	3.47	19.2	18.52	0.25
	50	2.76	11.3	16.82	0.30
ATP	75	3.94	13.4	20.43	0.33
	100	3.99	15.6	23.21	0.35
LSD (0.05)		0.04	0.138	1.11	0.002

Phytohormos

The data recorded in table 4 show that the detected amounts of endogenous gibberellin (GA_3), total cytokinins and Absecic acid (ABA) content in tuberose plants significantly affected by spermidine and ATP treatments. The highest amounts of endogenous GA_3 (26.21 and 26.43ng/g) were recoded with ATP at 100 ppm as soaking or spraying treatment followed by spermidine at 100 ppm (spraying treatment) as compared with control treatment. On the other hand, application of spermidine at 100 ppm as soaking treatment resulted in the highest amount of total cytokinins (391.21ng/g fw) followed by soaking or spraying treatment of ATP which gave 382.13 and 385.00ng/g FW respectively. Also the application of ATP at 75 or 100ppm as soaking or spraying treatment resulted in the highest amounts of ABA (35.11, 37.21, 31.11 and 33.41 ng/g FW respectively) as compared with control plants.

In this respect Mahgoub *et al.* (2006)[39] reported that treated carnation plants with puterscine at the rate of 200 and 400 ppm increased the contents of endogenous promoters (IAA, GA_3 and cytokinins) in comparison with those obtained from the untreated plants. On the other hand, application of the same treatment caused dramatic reduction in the level of endogenous natural inhibitor (ABA).

It has been shown that polyamines interact in some way with all plant hormones. Polyamines like cytokinins have antisenescence activity (Altman, 1989)[40]. Polyamines have been ascribed various roles such as that of a new class of plant growth regulators, hormonal second messengers and as one of the reserves of carbon and nitrogen at least in cultured tissues (Slocum and Flores, 1991)[41].

In addition, the consequence of spermidine degradation can be the products of a precursor for other growth substances in plant (Abd El-Wahed, 2006)[42].

The results may be attributed to the major role ATP in activating most processes in plant metabolism (Mengel and Kirkly, 1982)[14]. The AMP (the hydrolytic derivative of ATP) is the main precursor of cytokinins (Jameson, 1994)[16].

Table 4: Effect of spermidine and ATP on endogenous *phytoromons* (ng/gm Fw) of *Polianthes tuberose* L. (mean of two seasons)

Treatment \ concentration (ppm)		GA3	Total cytokinines	ABA
			Soaking	
	Control	16.32	260.41	6.73
	50	18.41	280.56	15.21
Spermedine	75	22.22	385.52	22.14
	100	24.42	391.21	27.08
	50	19.75	255.1	30.00
ATP	75	24.32	367.33	35.11
	100	26.21	382.13	37.21
			Spraying	
	50	17.25	270.13	13.41
Spermedine	75	20.11	341.21	20.28
	100	22.18	356.23	24.21
	50	17.62	273.41	26.25
ATP	75	22.56	380.31	31.11
	100	26.43	385.00	33.41
LSD (0.05)		0.83	1.31	1.48

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