

## EFFECT OF PUTRESCINE AND ATONIK ON GROWTH AND SOME BIOCHEMICAL CONSTITUENTS AS WELL AS ESSENTIAL OIL COMPOSITION OF CHAMOMILE PLANT (*Chamomilla recutita* L., Rausch.).

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### ABSTRACT

The experiments were conducted during two growing seasons, 2002-2003 and 2003-2004 at experimental Farm area of National Research Centre, Dokki, Cairo, Egypt to study the effect of putrescine in absence and presence of atonik on chamomile plant.

Putrescine in absence or presence atonik significantly enhanced each of the following characters: Plant height, number of branches, shoot fresh and dry weights/plant during vegetative and flowering stages as well as flower head characters (flower number, fresh and dry weights of flowers/plant) during flowering stage. The combined treatment of putrescine at 25 mg/l and atonik at 60 ml/l gave the highest fresh weight of plant during vegetative stage as well as putrescine 50 mg/l during flowering stage. The highest values of flower heads (flower number, fresh and dry weights/plant) were obtained at atonik 120 ml/l. Nitrogenous compounds (total N and crude protein) of chamomile leaves significantly increased with putrescine concentration (50 mg/l). While, atonik concentration (120 ml/l) gave the highest contents of free amino acids, phenols as well as indoles. The combined treatment of putrescine 100 mg/l and atonik 120 ml/l gave the highest essential oil percentage of flower head. Regarding essential oil yield/plant, atonik at 120 ml/l proved to be more effective in this respect. The essential oil percentage and yield per plant of the first collection were higher than the second collection.

The major components of chamomile essential oil which were identified by gas chromatography were farnesene, bisabolol oxide  $\beta$ ,  $\alpha$ -bisabolol, Chamazulene and bisabolol oxide A. However the percentage of essential oil constituents were concentration dependent.

**Keywords:** Putrescine, Atonik, Growth, Biochemical constituents, Chamomilla.

### INTRODUCTION

The diammine putrescine occurred widely in the higher plants. It was suggested to be involved in a variety of growth and developmental processes such as cell division (Bagni *et al.*, 1982 and Bueno and Matilla, 1992), dormancy breaking and germination of seeds (Bonneau *et al.*, 1994), tuberization (Bagni *et al.*, 1982), flower induction and development (Tarengi and Martin-Tanguy, 1995), embryogenesis (Mengoli *et al.*, (1989), fruit set and growth (Biasi *et al.*, 1991), (Crisosto *et al.*, 1988), senescence (Kao, 1994, Uphold and Van Staden, 1991) and plant morphogenesis (Hausman *et al.*, 1995).

The interaction of polyamines with the macromolecules was responsible for physiological effects on plant growth and development (Smith, 1985). Whereas, protective roles of polyamines had been postulated under adverse environmental conditions as exogenously added polyamines. They

were effective in preventing chlorophyll loss (Cohen *et al.*, 1984) or inhibiting RNase and protease activities (Kaur-Sawhney and Galston, 1982).

Serafini-Fracassini, (1991) suggested polyamine to be activators of protein process, that was related to genes encoding (Imai *et al.*, 2004), despite the high expression levels of transgenes in the extracellular space. This might be due to the amount of polyamine levels or a different compartmentalization of the recombinant proteins and the bulk amount of endogenous polyamines (Rea *et al.*, 2004). So, Abd El-Wahed and Gamal El-Din (2004) found that spermidine improved plant growth parameters of chamomile plant during the vegetative stage. Biochemical constituents of leaves (total sugars, phenols and indoles) were increased as well as essential oil and constituents of essential oil in the flowers.

Atonik is as a new type of plant stimulator supporting germination, rooting, growth and pest resistance, it is neither a growth hormone nor a pesticide (Wojdya, 2004). Application of atonik was effective in increasing leaf area, number of boll set, dry weight of shoot, root and leaves of cotton (Abdel-Al, 1998). The longest shoots were obtained after spraying atonik solution (Daping *et al.*, 2004). Thus, it is a growth stimulator with positive effect on biochemical and physiological processes in sugar beet plants (Cerny *et al.*, 2002). So, it promoted bud bursting 4-6 days earlier and advanced blooming of 3-5 days of *Satsuma mandarin* plant (Hong Xiang *et al.*, 2003). On the other hand, Atonik application had no significant effects on the total and marketable yield of daughter bulbs and quality of forced tulip plants (Hetman *et al.*, 2004).

Growth, fresh and dry weights and essential oil yield with its terpenoid constituents of *Mentha piperita* L. were improved by application of polyamines (Youssef *et al.*, 2002).

So, chamomile plants are traded as fresh or dried herbs or as oil distilled forms. Therefore, the aim of the present study was to study the effect of putrescine and atonik on growth, some biochemical constituents and essential oil productivity of chamomile plant.

## **MATERIALS AND METHODS**

The present study was conducted in two successive seasons 2002-03 and 2003-04 at the Experimental Farm area of the National Research Centre, Dokki, Cairo, Egypt to study the effect of putrescine or atonik separately on growth, flowering and some biochemical constituents of chamomile plant (*Chamomilla recutita* L., Rausch.).

Chamomile seeds were germinated in pots filled with soil mixture of loamy soil and sand (1:3). Chamomile seedlings were transplanted after 45 days to soil with six seedlings/pot at 23 and 20 October 2002 and 2003 respectively, thinned to four seedlings/pot in earthen ware pot No.30 filled with loamy soil in which calcium superphosphate fertilizer (15.5 P<sub>2</sub>O<sub>5</sub>) was added presowing at the rate of 6g/pot. Amonium nitrate (33.5% N) and potassium sulphate (48-52% K<sub>2</sub>O) at the rate of 12 g/pot were added during vegetative growth stage (30 days from transplanting). Chamomile plants were

sprayed by the following concentrations (25, 50, 75 and 100 mg/l) of putrescine ;  $\text{NH}_3\text{-(CH}_2\text{)}_3\text{-NH}_3$  or atonik [Sodium 5-nitroguaiacolate + sodium 1-nitrophenolate + 4-nitrophenolate] solutions at the vegetative stage before flower-budding after 70 or 75 days from transplanting. A third group of plants were sprayed by both bioregulators separately to study the combined effect of them on chamomile. Putrescine treatments were sprayed at the first then atonik at the second times. Moreover, the control plants were sprayed with distilled water.

**Growth and flowering characters measurements:**

Measurements of growth characters were taken for plant height, branch number, shoot fresh and dry weights/plant at two physiological stages (vegetative and flowering stages). Flower head number, flower [fresh and dry] weights/plant were determined at full flowering stage. The open flowers head were picked every three days at ray flower stage. The flowers head were collected along the first period (45 days) and the second period (45 days) during the dates (January, February and March).

**Biochemical constituents determination:**

Samples from fresh herbs of each treatment were taken and dried at 70°C for constant weight. Total sugar content was determined according to Dubois *et al.* (1956). Moreover, the following parameters were also determined: Nitrogen and protein percentage (A.O.A.C., 1970), total free amino acids (Plummer, 1978) total phenols (Danial and George, 1972) and total indoles (Bentley, 1961). Essential oil content of air-dried flowers was determined at all flowers collected according to British pharmacopoeia (1980) dehydrated over anhydrous sodium sulfate then kept at refrigerator (-4°C) till GLC analysis.

**Identification of essential oil constituents:**

Essential oil of chamomile flowers of the first collection was analyzed by GLC using a Hewlett Packard, (HP 6890 series) GC system, U.S.A. using capillary column Zb5 (30 m x 53-0  $\mu\text{m}$ ), 0.5 $\mu\text{m}$  film thickness. Oven temperature was programmed at 60°C for 2 min. from 60°-190°C at rate of 4 ml/min. and finally 250°C (15 min) with  $\text{N}_2$ :  $\text{H}_2$ : Air at 30:30:300 ml/min. The temperature of the detector (FID) was maintained at 280°C. Identification of the oil components was based on the comparison of the Rts of the separated compounds with those of standard compounds that injected under the same conditions and confirmed for the major compounds by their relative retention indices.

**Statistical analysis:**

The design of the experiment was arranged as complete randomized block with three replicates. Combined analysis of the average values of the two seasons was carried out and the values of LSD were calculated as described by Snedecor and Cochran (1980).

## RESULTS AND DISCUSSION

### Effect of putrescine and atonik on growth characters:

Putrescine, atonik and their combinations led to significant increases of growth characters as plant height, number of branches, fresh and dry weights of shoot per plant during vegetative and flowering stages as compared with control as shown in Table (1). The increment in chamomile growth characters was related to each bioregulator (putrescine and atonik) concentration applied alone or in combinations. Putrescine (25 mg/l) and atonik (60 ml/l) combination gave the highest values of growth characters during vegetative and flowering stages.

The increases of fresh and dry weights of shoot that resulted from the treatment 25 mg/l putrescine and atonik (60 ml/l) were 86.3 and 74.1% and 152.4 or 52.3% respectively compared with control during vegetative and flowering stages. Both bioregulators and their combination had promotive effect on growth characters during the development of chamomile plant. Putrescine or atonik application decreased growth characters with increasing their concentrations. Increasing atonik concentration applied in combinations decreased growth characters during vegetative and flowering stages. This might be due to peroxidases control on endogenous auxin level (Pedreno *et al.*, 1990), lignification (McDougall, 1992) and catalyze of the formation of covalent cross-links after oxidation of cell wall ester and ether bound phenolic acids thus preventing cell expansion (Fry, 1986). It could be concluded that the favourable concentrations of putrescine and atonik concentrations were 25 mg/l and 60 ml/l, respectively when applied at vegetative stage. In this respect, Abd El-Wahed (2000), Habba *et al.*, (2001), Abd El-Wahed and Gamal El-Din (2004) who mentioned that spermidine (another polyamine) stimulated vegetative growth characters as plant height, leaf area, plant fresh and dry weights of maize, wheat and chamomile plants. Also, putrescine was essential for growth, fresh and dry weight of *Mentha piperita* (Youssef *et al.*, 2002) and *Datura sp* (Youssef *et al.*, 2004) because polyamines could be activators of physiological processes in plant (Imai *et al.*, 2004). So, atonik application was more effective in increasing leaf area, dry weight of shoot and leaves of cotton and sugar beet (Abdel Al, 1998, Cerny *et al.*, 2002 and Daping *et al.*, 2004).

### Effect of putrescine and atonik on flowering characters:

The presented data in Table (1) show that flower head number and fresh and dry weights of chamomile flowers were significantly increased during flowering stage by putrescine, atonik and their combinations at different concentrations compared with their control. The head flower characteristics were surpassing with putrescine and atonik application alone than their combinations. This effect was accompanied with concentration of both bioregulators. Whereas, the increase of atonik concentration in the combination reduced head flower characteristics. Putrescine concentration (50 mg/l) gave the highest head flowers number. Putrescine (120 mg/l) and atonik (120 ml/l) had similar effect on fresh and dry weights of head flowers.

**Table (1): Effect of putrescine and atonik and their combination on chamomile growth characters (combined analysis of two seasons).**

Bioregulators treatment	Vegetative stage			Flowering stage			Flower heads/plant				
	Plant height (cm)	Branches number	Shoot weight (g)	Plant height (cm)	Branches number	Shoot weight (g)	Number	Weight (g)			
			Fresh			Dry		Fresh	Dry		
Control	21.0	9.0	24.21	3.11	46.0	10.0	33.48	12.02	22.0	2.0	0.38
Putreccine 25 mg/l	36.7	18.3	70.35	7.27	58.0	19.5	44.58	13.28	44.0	4.29	0.81
Putreccine 50 mg/l	21.7	22.3	57.38	6.67	46.5	22.3	72.62	17.35	59.5	4.90	0.93
Putreccine 100 mg/l	30.0	9.0	31.91	3.97	56.0	10.5	70.95	17.31	34.5	4.38	0.83
Atonik 60 ml/l	26.0	7.0	18.24	3.74	60.7	10.5	59.43	16.74	24.0	2.02	0.38
Atonik 120 ml/l	23.5	8.3	29.17	4.10	53.7	14.7	62.24	17.58	44.5	4.91	0.93
Putreccine 25 mg /l+ Atonik 60 ml/l	36.75	19.0	75.10	7.85	68.7	22.7	45.1	15.44	57.5	4.85	0.92
Putreccine 25mg/l+ Atonik 120 ml/l	22.7	12.3	29.7	3.41	46.7	22.0	34.49	12.67	52.0	3.96	0.75
Putreccine 50mg/l + Atonik 60 ml/l	22.3	11.3	39.91	4.20	51.0	15.5	46.84	14.73	35.0	3.66	0.69
Putreccine 50mg/l + Atonik 120 ml/l	35.7	10.5	29.8	3.50	54.7	13.0	41.02	13.09	22.5	2.05	0.39
Putreccine 100mg/l + Atonik 60 ml/l	27.3	9.7	27.77	3.91	60.0	11.2	64.77	17.31	26.5	2.46	0.83
Putreccine 100mg/l + Atonik 120 ml/l	27.0	9.0	26.51	3.29	57.0	10.0	55.03	15.00	26.0	2.08	0.39
LSD at 5%	3.37	1.38	3.83	0.8	5.75	1.01	5.53	1.10	6.23	0.33	0.06

The increases of head flower characteristic had been evaluated by 170.0% for head flower number, 145.5% for fresh weight and 144.7% for dry weight, respectively compared with control. This effect shows that head flower number was related to number of branches. These results showed that chamomile plant responded positively to putrescine or atonik applied alone more than their combinations. This might be due to polyamine relation to flowering process that was observed by many investigators. In cherry flower buds, polyamines were presented in all stages of bud development (Wang *et al.*, 1985). So, atonik promoted bud bursting 4-6 days earlier and advanced blooming of 3-5 days of *Satsuma mandarin* plant (Hong Xiang *et al.*, 2003).

**Effect of putrescine and atonik on some biochemical constituents in chamomile leaves:**

**Total sugars:**

Data presented in Table (2) show that total sugars content in chamomile leaves significantly increased with bioregulators (putrescine or atonik) applied singly compared with control at the vegetative stage. The effect was a concentration dependent. In addition, the combined treatment of both bioregulator increased total sugars content in the leaves but tended to decrease as atonik concentration was raised. However, atonik concentration at 120 ml/l proved to be more effective in this respect. These results showed that bioregulators play an important role on carbohydrate metabolism in plant. Whereas, polyamines were able to bind with pectic and polysaccharides (D'Oraci and Bagni, 1987). In the same trend Tipirdamaz *et al.*, (1995) found that polyamine application significantly increased  $\alpha$  amylase activity. Whereas, osmotic stress caused by higher sugar concentration found to enhance polyamine levels (Flores & Galston, 1984). Both carbohydrates and polyamines were related in potato (Mikizel, 1990), maize (Abd El-Wahed, 2000), chamomile (Abd El Wahed and Gamal-El-Din, 2004). Phenolic compounds application increased total carbohydrates in rosemary plant (Rashad, 2003). Atonik improved sugars content of sugar beet (Gerny *et al.*, 2002).

**Nitrogenous compounds:**

Data given in Table (2) show that spraying putrescine, atonik and their combinations significantly increased the nitrogenous compounds (total nitrogen and crude protein) in chamomile leaves compared with control during vegetative stage. Increases in total nitrogen and crude protein were related to both bioregulators concentration as single or their combination. Putrescine concentration at 50 mg/l showed the most beneficial effect on total nitrogen and protein percentage. However, free amino acids content significantly decreased with spraying chamomile plant by bioregulators (Putrescine, atonik and their combinations) compared with control. Decreasing free amino acids content in chamomile leaves was observed at all treatments except atonik at 120 ml/l. It appeared from these results that free amino acids content in chamomile leaves was decreased with increasing atonik concentration in the bioregulators combination. This appeared in increasing protein percentage and decreasing free amino acids in chamomile leaves. Whereas, polyamines linked to particular

proteins (Folk, 1980). Covalently bound polyamine protein complexes had been found in *Helianthus tuberosus* (Grandhi *et al.*, 1992). But, putrescine application increased free amino acid in *Datura stramonium* leaves (Youssef *et al.*, 2004). In the same trend phenolic compounds influenced protein synthesis and enzymatic activity (Leather and Einhellig, 1988). Atonik led to increase of total N and N use efficiency in wheat plant (Farahat, 2002) and formation of amino acids in *Cathranthus roseus* leaves (El-Mergawi and Abd El-Wahed, 2003).

**Table (2): Effect of putrescine and atonik on some biochemical constituents in chamomile leaves at vegetative stage (combined analysis of two seasons).**

Bioregulators Treatment	Total sugars (%)	Total nitrogen (%)	Free amino acids (mg/g)	Crude protein (%)	Phenols (mg/g)	Indoles (mg/g)
Control	9.8	4.1	29.2	0.0	14.3	9.7
Putrescine 25 mg/l	14.5	4.6	19.6	25.6	25.8	10.8
Putrescine 50 mg/l	13.7	5.8	20.6	28.8	23.2	10.4
Putrescine 100 mg/l	14.8	4.4	10.1	27.5	21.1	10.5
Atonik 60 ml/l	12.4	4.4	25.8	27.5	24.9	10.7
Atonik 120 ml/l	15.9	4.6	32.1	28.8	27.5	11.6
Putrescine 25mg/l + Atonik 60 ml/l	15.3	4.6	17.3	28.8	19.7	10.1
Putrescine 25mg/l + Atonik 120 ml/l	14.9	4.6	17.5	28.8	21.8	10.3
Putrescine 50mg/l + Atonik 60 ml/l	14.9	5.3	20.3	33.1	23.3	11.3
Putrescine 50mg/l + Atonik 120 ml/l	12.0	5.0	17.3	31.3	23.3	10.2
Putrescine 100mg/l + Atonik 60 ml/l	15.3	4.7	22.8	29.4	23.4	10.1
Putrescine 100mg/l + Atonik 120 ml/l	14.4	5.3	12.7	33.1	22.4	9.7
LSD at 5%	1.2	0.5	2.7	2.1	3.6	0.4

**Total phenolic compounds:**

Data presented in Table (2) show that putrescine, atonik and their combinations spraying on chamomile plant significantly increased total phenolic compounds in leaves compared with control. It decreased with increasing putrescine concentration only, but gave the opposite trend with atonik application. This effect appeared in increasing phenolic compounds in chamomile leaves with increasing atonik concentration in the bioregulators combination. Although there was a decrease in total phenolic compounds with putrescine and atonik combination compared with atonik treatments only. However, atonik (120 ml/l) proved to be more effective in this respect. These results showed that putrescine and atonik combination had low effect on amino acids conversion to phenolic compounds in chamomile leaves. Phenolic acids had been known to conjugate with polyamines and modulating the free levels of these plant growth substances. Thus, the effect of L- $\alpha$ -aminoxy- $\beta$ -phenylpropionic acid found on polyamines (putrescine, spermine and spermidine) (Martin-Tanguy, 1985). Suggestion that an interaction between phenolic acids and polyamines was involved in the mechanism of growth promotion by L- $\alpha$ -aminoxy- $\beta$ -phenylpropionic acid (Mader and Hanke, 1997). So, phenolic compounds application increased it in the vegetative organs of rosemary plant (Rashad, 2003).

**Total indoles:**

Data presented in Table (2) show that putrescine, atonik and their interaction significantly affected total indoles content of chamomile leaves. This appeared in increasing indoles content during vegetative stage. Atonik concentration (120 ml/l) gave the highest total indoles content in chamomile leaves. This showed that total indoles increased with increasing atonik and decreasing putrescine concentration. These results showed that the effect of bioregulators acted in contrast trend as showing indoles in the leaves with the combination treatment (putrescine 100 mg/l, atonik 120 ml/l). These results showed that putrescine and atonik application had a role on the balance of phytohormones in plants. But, ester of IAA and amide forms were differed according to the plant type. (El-Mergawi and Abd El-Wahed, 2004). Whereas, phenols decreased IAA oxidase activity and increased diamine oxidase/polyamine oxidase and polyphenol oxidase activity (Nag *et al.*, 2001).

**Effect of putrescine and atonik on essential oil content of chamomile flower heads:**

The presented data in Table (3) show essential oil percentage and yield of air-dried chamomile flower heads. It was evident that treated plants with the applied bioregulators (putrescine and atonik) as single or combined tended to show an increase in the essential oil percentage and yield at collection dates of chamomile flowers along flowering period. Both oil percentage and yield increases were related to both bioregulators application. The combined treatment (putrescine 100mg/l and atonik 120 ml/l) achieved the highest oil percentage of flower heads at the first and second collections. While, atonik concentration (120 ml/l) gave the highest oil yield. Increased essential oil percentage as compared with that of control was ranged between (24.3-124.3%) at first collection and (16.7-116.7%) at the second collection. While, the increase of oil yield was ranged (114.3-457.1%) at the first collection and (400.0-500.0%) at the second collection. These results reflected that both bioregulators had a role on essential oil biosynthesis or translocation from herb to flower heads. The considerable increases of oil percentage and yield at the 1<sup>st</sup> collection might be due to increase flower development at this time. The highest oil content was obtained at first collection during 45 days of flowering period with the highest concentration of the combined bioregulators. Essential oil content in air dried flower heads decreased with advancing plant age.

These results agreed with those obtained by Letchamo *et al.*, (1993) and Salmon, (1994) who found that the essential oil content was decreased with increasing harvest frequency and the lowest concentration was found in the fourth harvest of all genotypes of chamomile. Abd El-Wahed and Gamal El-Din (2004) reported that essential oil of chamomile flower heads was high during the second month of the collection period. Therefore, the production of essential oils and aromatic compounds from plants were diverse physiological, biochemical and genetic regulation. Biochemical and metabolic processes comprised the molecular mechanisms that regulate carbon flow through the biosynthetic routes as well as the turn rate of the revalent terpenoid and/or phenylpropanoid metabolism. Phenylpropanoid were the



precursors of polyamines (Smith, 1985). This appeared in increasing essential oil percentage and yield of chamomile flower heads with spermidine application (Abd El-Wahed and Gamal El-Din, 2004).

**Table (3): Effect of putrescine and atonik on essential oil yield and it's constituents of air dried flower heads of chamomile during flowering period.**

Bioregulators Treatment	Oil (%)		Oil yield/plant (g)		Major oil constituents (%)				
	1 <sup>st</sup> collection	2 <sup>nd</sup> collection	1 <sup>st</sup> collection	2 <sup>nd</sup> collection	Farnesene	Bisabolol Oxide β	α-Bisabolol	Chamazulene	Bisabolol oxide A
Control	0.37	0.24	0.007	0.005	6.15	6.08	11.35	11.45	55.64
Putrescine 25 mg/l	0.46	0.38	0.020	0.016	10.82	4.01	14.55	11.31	46.19
Putrescine 50 mg/l	0.49	0.28	0.024	0.014	4.06	4.10	13.89	7.97	55.75
Putrescine 100 mg/l	0.71	0.32	0.031	0.014	7.18	1.30	0.66	6.39	61.08
Atonik 60 ml/l	0.75	0.48	0.015	0.010	3.94	1.67	6.05	8.31	60.72
Atonik 120 ml/l	0.79	0.58	0.039	0.030	6.18	4.13	16.05	11.74	53.05
Putrescine 25mg/l + Atonik 60 ml/l	0.58	0.42	0.028	0.020	4.71	2.40	10.09	12.35	61.69
Putrescine 25mg/l + Atonik 120 ml/l	0.65	0.47	0.026	0.019	4.61	2.70	12.68	12.48	59.88
Putrescine 50mg/l + Atonik 60 ml/l	0.74	0.33	0.027	0.012	4.69	3.23	7.20	10.59	64.87
Putrescine 50mg/l+ Atonik 120 ml/l	0.78	0.35	0.016	0.007	5.83	0.57	0.12	10.06	60.41
Putrescine 100 mg/l+ Atonik 60 ml/l	0.82	0.34	0.020	0.008	7.74	0.80	3.16	11.70	54.92
Putrescine 100mg/l + Atonik 120 ml/l	0.83	0.52	0.017	0.011	23.64	2.20	0.63	12.29	20.14
LSD at 5%	0.05	0.04	0.001	0.001					

**Effect of putrescine and atonik on essential oil constituents:**

The data presented in Table (3) show the terpenic constituents of essential oil from chamomile flowers treated with bioregulators (putrescine, atonik and their combinations). Bioregulators effect on terpenic constituents of chamomile oil was varied according to bioregulators application and their concentrations. All terpenoid constituents were varied in their response of putrescine, atonik and their combination comparing with control. Whereas, putrescine application increased the main terpenic constituents (farnesene, α-bisabolol, and bisabolol oxide A). The increases in the oil constituents with putrescine application were 74.9, 28.2 and 9.8%, respectively. While, atonik application stimulated α-bisabolol (41.4%), Chamazulene (3.4%) and bisabolol oxide A (9.1%) and for putrescine and atonik combinations, the increase rate was for farnesene (284.4%), chamazulene (9.0%) and bisabolol oxide A (16.6%). The results reflected the effect of the treatments on all constituents of chamomile oil. It might be due to the effect of treatments on biosynthesis of terpenoid compounds, that might be attributed to their specific reactions and isomerisation of cyclic monoterpenes, sesquiterpenoids,

phenylpropanoids of chamomile oil (Sangwan *et al.*, 2001). These results agreed with those of Abd El-Wahed and Gamal El-Din, (2004) who found that spermidine application on chamomile plant stimulated some terpenic constituents of essential oil. Also, polyphenol oxidases catalyze the oxygen dependent oxidation of phenols to quinines. The quinonid products were highly reactive molecules which could covalently modify and cross-link a variety of cellular nucleophiles, resulting in formation of melanin (Mayer and Harel, 1979 and Mayer 1987).

It could be concluded that putrescine in absence and presence of atonik enhanced *chamomilla recutita* L., Rausch. growth parameters. Atonik (120 ml/l) tended to induce the flowers formation more than the other treatments

## REFERENCES

- Abdel Al, M.H. (1998). Response of Giza 85 cotton cultivar to the growth regulators pix and atonik. *Egyptian J. Agric. Res.*, 76(3):1173-1180.
- Abd El-Wahed, M.S.A. (2000). Effect of stigmaterol, spermidine and sucrose on vegetative growth, carbohydrate distribution and yield of maize plants. *Egypt J. Physiol. Sci.*, 24 (2-3): 225-239.
- Abd El-Wahed, M.S.A. and K.M. Gamal El-Din (2004). Stimulation of growth, flowering and biochemical constituents of chamomile plant (*Chamomilla recutita*, Rausch.) with spermidine and stigmaterol application. *Egypt. J. Hort. Sci.*, 1 (Accepted for publication).
- A.O.A.C. (1970). Kjeldahl nitrogen determination "Official Methods of Analysis of Association Agriculture Chemists" 11<sup>th</sup> Ed. Assoc. Official Agric. Chemists, Washington.
- Bagni N.; D. Serafini-Fracassini and P. Torrigiani (1982). Polyamines and cellular growth processes in higher plants. In: Wareing PF (ed) *Plant Growth Substances*, pp 473-482. New York: Academic press.
- Bentley, J.A. (1961). *Encycl. Plant Physiol.* Ed. By Rubland, W. Vol. XIV: 513-520, Springer-Verlag, Berlin.
- Biasi, R ; G. Costa and N. Bagni (1991). Polyamine metabolism as related to fruit set and growth. *Plant Physiol Biochem.*, 29: 497-506.
- Bonneau, L.; M. Carre and J. Martin-Tanguy (1994). Polyamines and related enzymes in rice differing in germination potential. *Plant Growth Regul.*, 15:75-82.
- British Pharmacopoeia (1980). Vol. II. Volatile oil in drugs, A108- A112, Printed in England for Her Majesty's Stationary Office at the Univ. Press, Cambridge.
- Bueno. M. and A. Matilla (1992). Effect of spermine and abscisic acid on mitotic divisions in isolated embryonic axes of chick pea seeds (*Cicer arietinum* L.). *Cytobios* 71: 151-155.
- Cerny, L; V. Pacuta; J. Feckova and J. Golian (2002). Effect of year and Atonik application on the selected sugar beet production and quality parameters. *J. Central Eur. Agric.*, 3(1): 15-22.

- Cohen E.; S.M. Arad; Y.H. Heim and Y.Mizrahi (1984). Polyamine biosynthetic enzymes in the cell cycle of *Chlorella*. *Plant Growth Regul.*, 15: 83-92.
- Crisosto C.H.; P.B. Lombard; D. Sugar and V. Polito (1988). Putrescine influence on ovule senescence, fertilization time, and fruit set in "Comice" pear. *J. Am. Soc. Hort. Sci.* 113: 708-712.
- Danial, H.D. and C.M. George (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *J. Am. Soc. Hort-Sci.*, 17:651-654.
- Daping, Z; T. Dan and D. Lixia (2004). Effect of applying atonik plant growth liquid on the growth to citrus plant materials. *South China Fruits*, 33(12) 18.
- D'Oraci, D. and N. Bagni (1987). *In vitro* interactions between polyamines and pectic substances. *Biochem. Biophys. Res. Commun.* 18: 1159-1163.
- Dubois, M.; K.S. Gilles, J. Hamilton; R. Rebersand; F. Smith (1956). Colormetric methods for determination of sugar and related substances. *Anal. Chem.*, 28: 350-356.
- El-Mergawi, R.A. and M.S.A. Abd El-Wahed (2003). Influence of salicylic acid on *Cathranthus roseus* growth and vincristine formation under different water regimes. *Egypt. J. Hort.*, 30 (3-4): 179-196.
- El-Mergawi, R.A. and M.S.A. Abd El-Wahed (2004). Diversity in salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize. *Egypt. J. Agron.* 26: 49-61.
- Farahat, M.M. (2002). Effect of amcotone and atonik with some additives on nitrogen accumulation and productivity of wheat (*Triticum aestivum* L.). *Plant. Arab Univ. Jour. Agric. Sci.*, 10(1): 193-203.
- Flores, H.E. and A.W. Galston, (1984). Osmotic stress induced polyamine accumulation in cereal leaves. *J. Exp. Bot.*, 40(25):681.
- Folk J.E. (1980). Transglutaminases. *Ann. Rev. Biochem.*, 49: 517-531.
- Fry, S.C. (1986). Cross linking of matrix polymers in the growing cell walls of angiosperms. *Ann. Rev. Plant. Physiol.*, 37: 165-186.
- Grandhi B.; S. Del Duca; D. Serafini-Fracassini and C. Dinnella (1992). Re-entry in cell cycle: protein metabolism and transglutaminase-like activity in *Helianthus tuberosus*. *Plant Physiol., Biochem.*, 30: 415-424.
- Habba, E. El.; M.M. Farahat and M.S.A. Abd El-Wahed (2001). Physiological response of wheat (*Triticum aestivum* L.) to spermidine and ethryl. *J. Agric. Sci. Mansoura Univ.*, 26(9): 5474-5487.
- Hausman J.F.; C. Kevers and T. Gaspar (1995). Auxin-polyamine interaction in the control of the rooting inductive phase of poplar shoots *in vitro*. *Plant Sci.* 110:63-71.
- Hetman, J.; H. Laskowska and W. Durlak (2004). The influence of Atonik on the yield of the bulbs and the quality of forced tulip flowers "Rococo" and "Yokohama". *Folia-Universitatis Agriculturae-Stetinensis-Agricultura*, 94: 39-43.

- Hong Xiang, Y.W.; L. Mei; G. Jieqiang; K. Fuzhi and L. Hefang (2003). Effects of Atonik on growth and fruit setting of Satsuma (*Satsuma mandarin*). J. of Fruit Sci., 20 (4): 291-94.
- Imai, K.; T. Matsuyama, Y. Hanzawa, T. Akiyama, M. Tamaoki, H. Saji, Y. Shirano and T. Takahashi (2004). Spermidine synthase genes are essential for survival of Arabidopsis. Plant Physiol., 135: 1565-1573.
- Kao C.H. (1994). Endogenous polyamine levels and dark-induced senescence of detached corn leaves. Bot. Bull. Acad. Sin., 35:15-18.
- Kaur-Sawhney, R. and A.W. Galston (1982). On the physiological roles of polyamines in higher plants. In: Sen SP (ed) Recent developments in plant sciences. Today and Tomorrow's printers, New Delhi, pp. 129-144.
- Leather, G.R. and F.A. Einhellig, (1988). Bioassay of naturally occurring allelochemicals for toxicity. J. Chem. Ecol., 14:1821-1828.
- Letchamo, W.; R. Marquard; D. Palevitch; J.E. Simon and A. Mathe (1993). The pattern of active substances accumulation in chamomile genotypes under different growing conditions and harvesting frequency. Acta Hort., 331:357-364.
- Mader, J.C. and D.E. Hanke (1997). Polyamine sparing may be involved in the prolongation of cell division due to inhibition of phenylpropanoid synthesis in cytokinin-starved. Soybean Cells. J. Plant Grow. Regul., 16: 89-93.
- Martin-Tanguy, J. (1985). The occurrence and possible functions of hydroxycinnamoyl acids amides in plant. Plant Grow. Regul., 3:381-399.
- Mayer, A.M. (1987). Polyphenol oxidases in plants-recent progress. Phytochem., 26:11-20.
- Mayer, A.M. and E. Harel (1979). Polyphenol oxidase in plants. Phytochem., 18: 193-215.
- McDougall, G.J. (1992). Changes in cell wall associated peroxidases during the lignification of flax fibres. Phytochemistry, 31: 3385-3389.
- Mengoli, M.; N. Bangi; G. Luccarini; V. Ronshi and D. Serafini-Fracasini (1989). *Daucus carota* cell culture: polyamines and effect of polyamine biosynthesis inhibitors in the pre-embryogenic phase and different embryo stages. J. Plant Physiol., 134: 389-394.
- Mikitzel, L.J. (1990). Metabolic studies of the mechanisms underlying age reduced growth potential of potato (*Solanum tuberosum* L.) seed tubers. Dissertation Abstracts. International "B" Sciences and Engineering 11: 4852B.
- Nag, S.; K. Saha, and M.A. Choudhuri (2001). Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. J. Plant Grow. Regul., 20(2): 182-194.
- Pedreno, M.A.; A. Ros-Barcelo; F. Garcia-Carmona and R. Munoz (1990). Oxidation of dihydroxyfumaric acid in the absence of H<sub>2</sub>O<sub>2</sub> by cell wall-bound peroxidases from lupine. A possible general model. Plant Physiol. Biochem., 28: 37-42.
- Plummer, D.T. (1978). An introduction to practical Biochemistry 2<sup>nd</sup> Ed. P. 144. McGraw-Hill Book Company (UK) Limited London. New York.

- Rashad, El-Sh.M. (2003). Synergistic and antagonistic effects of indole acetic acid and some phenolic compounds on vegetative growth, pigments, essential oil and some correlated components in rosemary plants. *Egypt. J. Appl. Sci.*, 18 (7): 241-263.
- Rea, G.; M.c. Pinto, R. Tavazza, S. Biondi, V. Gobbi, P. Ferrante L. DeGara, R. Federico, R. Angelini and P. Tavladoraki (2004). Ectopic expression of maize polyamine oxidase and pea copper amine oxidase in the cell wall of tobacco plants. *Plant Physiol.*, 134:1414-1426.
- Salamon, I. (1994). Changes in quantitative-qualitative composition of chamomile essential oil during the three harvests of a year. *Herba Polonica*, 40(1-2): 17-25.
- Sangwan, N.S.; A.H.A. Farooqi, F. Sabih, R.S. Sangwan (2001). Regulation of essential oil production in plants. *Plant Growth Regul.*, 34:3-21.
- Serafini-Fracassini D. (1991). Cell cycle-dependent changes in plant polyamine metabolism. In: Slocum, RD, Flores HE (eds) *Biochemistry and physiology of polyamines in plants*. CRC Press, Boca Raton, FL, pp.: 159-171.
- Smith T.A. (1985). Polyamines. *Annu. Rev. Plant Physiol.*, 36: 117-143.
- Snedecor, G.W. and W.G. Cochran (1980). *Statistical methods-6<sup>th</sup> Ed.* Iowa State Univ., Press, Ames Iowa, USA.
- Tarenghi, E. and J. Martin-Tanguy (1995). Polyamines uses induction and floral development of strawberry (*Fragaria ananassa* Duch.). *Plant Grow. Regul.*, 17: 157-165.
- Tipirdamaz, R.; M. Durusoy and S. Bozuch (1995). Effect of exogenous polyamines on alpha amylase activity during seed germination under salt stress. *Turkish J. Bot.*, 19(4): 411.
- Uphold, S.J. and J. Van Staden (1991). Polyamines and carnation senescence: Endogenous levels and the effect of applied polyamines on senescence. *Plant Growth Regul.*, 10: 355-362.
- Wang, S.Y.; M. Faust and G.L. Steffens (1985). Metabolic changes in cherry flower buds associated with breaking of dormancy in early and late blooming cultivars. *Physiol. Plant.*, 65:89-94.
- Wojdya, A.T. (2004). Effectiveness of atonik in the control of powdery mildew, black spot and rust. *Folia-Horticulturae*, 16(1): 175-181.
- Youssef, A.A.; M.S. Aly; E.N. Abou Zied; L. Iliey and S. Titiana (2002). Effect of some growth substances on mass production and volatile oil yield of *Mentha piperita* L. "Bulgaro". *Egypt. J. Appl. Sci.*, 17 (11): 610-623.
- Youssef, A.A.; R.A. El-Mergawi and M.S. Abd El Wahed (2004). Effect of putrescine and phenylalanine on growth and alkaloid production of some *Datura* species. *J. Agric. Sci. Mansoura Univ.*, 29(7): 4037-4053.

تأثير البتروسين والأتونك على النمو وبعض المكونات البيوكيماوية وإنتاجية  
الزيت الطيار لنبات البابونج  
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تم إجراء التجارب بأرض التجارب الزراعية بالمركز القومي للبحوث بالدقى - القاهرة - جمهورية مصر العربية حيث تم رش نباتات البابونج بمنظم النمو الأتونك بتركيزات (صفر، ٦٠، ١٢٠ مل/لتر) في وجود أو عدم وجود البتروسين بتركيزات (صفر، ٢٥، ٥٠، ١٠٠ مجم/لتر) في مرحلة النمو الخضري. وكانت مواعيد الرش للبتروسين بعد ٧٠ يوم من الزراعة والأتونك بعد ٧٤ يوم من الزراعة وقد صممت التجربة في نظام القطاعات الكاملة العشوائية بثلاث مكررات في سنوات الدراسة ٢٠٠١/٢٠٠٢ - ٢٠٠٢/٢٠٠٣ م وتتلخص النتائج كالتالى:

- ١- أظهرت معاملات البتروسين منفردا أو مع الأتونك زيادة معنوية في صفات النمو (ارتفاع النبات، عدد الأفرع، الوزن الغض، الوزن الجاف/نبات) أثناء مرحلتى النمو الخضري والإزهار، وأعطت المعاملة بالبتروسين ٢٥ مجم/لتر + الأتونك ٦٠ مل/لتر أفضل وزن خضري/نبات في مرحلة النمو الخضري بينما كان تركيز البتروسين ٥٠ مجم/لتر أفضل للمعاملات في مرحلة الإزهار.
- ٢- تم الحصول على زيادة معنوية في صفات الإزهار (عدد الإزهار، الوزن الغضن، السوزن الجاف/نبات) عند إضافة البتروسين بتركيز ٢٥ ملجم/لتر.
- ٣- زاد محتوى الأوراق من النيتروجين الكلى، البروتين بإضافة البتروسين بتركيز ٥٠ مجم/لتر بينما انخفض محتوى الأوراق من الأحماض الأمينية معنويا تحت تأثير إضافة المعاملات مقارنة بالكنترول.
- ٤- أعطى تركيز الأتونك ١٢٠ مل/لتر أفضل محتوى من الأحماض الأمينية الحرة والفينولات والاندولات للأوراق.
- ٥- زادت نسبة الزيت الطيار بالأزهار معنويا بإضافة المعاملات وكان التركيز ١٢٠ مل/لتر من الأتونك أفضل للمعاملات تأثيرا على زيادة محتوى الأزهار من الزيت الطيار كنسبة أو إنتاجية/نبات.
- ٦- اختلفت استجابة المكونات الرئيسية لزيت شيح البابونج (فارنيسين، يس أبولول أوكسيد ب، بس أبولول أوكسيد أ، الفابس أبولول، كامازولين)، للمعاملات المضافة. أتضح من النتائج أن معاملة البتروسين ٢٥ مجم/لتر + الأتونك ٦٠ مل/لتر أعطت أفضل وزن غض في مرحلة النمو الخضري وتركيز الأتونك ١٢٠ مل/لتر أعطى أفضل النتائج للتا للحصول على أعلى محصول من الزيت الطيار/نبات الأزهار.