STIMULATION OF GROWTH, FLOWERING, BIOCHEMICAL CONSTITUENTS AND ESSENTIAL OIL OF CHAMOMILE PLANT *(CHAMOMILLA RECUTITA* L., RAUSCH) WITH SPERMIDINE AND STIGMASTEROL APPLICATION

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Summary. Experiments were conducted during two growing seasons (2001/02 and 2002/03) at the greenhouse to study the effect of spermidine and stigmasterol on growth, flowering, biochemical constituents and essential oil content of chamomile plant *(Chamomilla recutita* L., Rausch). Different concentrations (25, 50, 75 and 100 mg/1) of spermidine and stigmasterol were applied during the vegetative stage. Both bioregulators (especially at 100 mg/1) improved plant growth parameters (plant height, branches number, fresh and dry weight) during the vegetative stage. Spermidine was more effective than stigmasterol. This effect was consequential during the flowering stage. However, at that stage, fresh and dry weights, flowers number and weight, were more affected by stigmasterol application than by spermidine. At both stages, spermidine and stigmasterol, at the concentration of 100 mg/1 strongly affected growth. Consequently, the biochemical constituents of leaves (total sugars, phenols and indoles) were increased. Essential oil content of flowers was significantly improved by the application of the both bioregulators at 100 mg/1. Stigmasterol was more effective than spermidine in increasing essential oil content and plant yield at the three collections.

The major components of chamomile essential oils, identified by gas liquid chromatography (GLC) were farnesene, bisabolol oxide β, α bisabolol, chamazulene and bisabolol oxide A. Their percentage rates varied according to the concentrations of spermidine and stigmasterol applied.

Abbreviations: GLC (gas liquid chromatography)

INTRODUCTION

Chamomile *(Chamomilla recutita* (L.) Rausch) is one of the important herbal medicines as it is used for the treatment of many diseases (Simpson, 2001). The main constituents of the essential oil of chamomile belong to the sesquiterpenes - the precursor of stigmasterol found either free or as conjugated compound in the plant cell (Grunwald, 1982). Stigmasterol is a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport (Genus, 1978). The biological functions of sterol conjugates, such as fatty acids or glucoside esters and sterylacylglucoside, were much less than the sterol storage forms as contents in the oil bodies of chamomile plant. It was suggested that the interaction of sterols with phospholipids stabilized the membrane permeability (Grunwald, 1982). Sterols play an important role in plant development including cell expansion, vascular differentiation, etiolation and reproductive development (Clouse and Sasse, 1998 and Abd El Wahed *et al.,* 2001). Similar to the effect of brassinosteroids, both sitosterol, stigmasterol and atypical sterols, involved in the regulatory function of plant development and gene expression. Sterols are essential for the normal plant growth and development like the brassinosteroids (He *et al.,* 2003).

Spermidine is one of the major polyamine forms in plants. They are able to bind with several negatively charged molecules, such as DNA (Basu *et al.,* 1990, Pohjanpelta and Holtta, 1996), proteins (Apelbaum *et al.,* 1988), membrane phospholipids and proteins (Schuber *et al.* 1983 and Tassoni *et al.,* 1996) and pectic polysaccharides (D'Oraci and Bagni, 1987). Polyamines are involved in protein phosphorylation (Ye *et al.,* 1994) and post transcriptional modification of DNA (Basu *et al.* 1990). Polyamines were localized in the vacuoles, mitochondria and chloroplasts (Slocum, 1991), and detected in thylakoid membranes of spinach and photosystem II (Borrell *et al.,* 1995). Growth, fresh/dry weights and essential oil (yield with its terpenoid constituents of *Mentha piperita* L.) were improved by the application of polyamines (Youssef *et al.,* 2002).

The present aims to study the influence of spermidine and stigmasterol on the stimulation of vegetative growth, flowering and flowers essential oil of chamomile plants.

MATERIALS AND METHODS

The present study was conducted in two successive seasons: 2001-02 and 2002-03, at the greenhouse of the National Research Centre; Dokki, Cairo, Egypt, to study spermidine and stigmasterol stimulation of 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 transferred after 45 days to a pot with six seedlings on 19 and 21 October, 2001 and 2002, respectively, thinned to a pot with four chamomile seedlings, transferred into earthen pot No30 filled with loamy soil in which calcium superphosphate fertilizer (15.5% P_2O_5) was added presowing at 6g/pot. Amonium nitrate (33.5% N) and potassium sulphate (48-52% K_2O) at 12g/pot were added during vegetative growth stage (30 days from planting). Chamomile plants were treated by spraying with 25, 50, 75 and 100 mg/l of spermidine or stigmasterol solutions and control (distilled water) at the vegetative stage before flower budding.

Growth characteristics measurements:

Measurements of growth characteristics for plant height, branches number, fresh and dry weight, were taken at two physiological stages (vegetative and flowering stage). Flowers number and plant weight were determined during the flowering stage.

Biochemical constituents of plants:

Samples from fresh herbs of each treatment were taken and dried at 70°C for constant weight. Total sugars content was determined according to Dubois *et al.* (1956); total free amino acids – according to Plummer (1978); total phenols – according to Danial and George, 1972; and total indoles – according to Bentley, 1961. Essential oil content of air-dried flowers-heads collected during three successive months (January, February and March) was determined according to British pharmacopoeia (1980). They were dehydrated over unhydrous sodium sulfate and kept at refrigerator till GLC analysis.

Identification of essential oil constituents:

Essential oil of chamomile flowers was analyzed by GLC using a Hewalett Pakard, HP 6890 system U.S.A. with capillary column Zb5 (30 m x 53-0 Urn), 0.5 μm film thickness. Oven temperature was programmed at 60°C for 2 min, then from 60° to 190°C at the rate of 4 ml/min, and finally at 250°C (for 15 min) with N_2:H_2:Air at the ratio of 30:30:300 ml/min. The temperature of the detector (FID) was maintained at 280°C. Identification of the components was based on the comparison between the Rts of the separated compounds and those of standard compounds under the same conditions.

Statistical analysis:

The design of the experiment was arranged as a 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 according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of spermidine and stigmasterol on growth characteristics:

Results in Table 1 indicate that spraying chamomile plants with each bioregulator (spermidine or stigmasterol) strongly affects growth characteristics such as plant height, number of branches, fresh and dry weights, during vegetative and flowering stages compared to the control. An increase in spermidine and stigmasterol concentrations (up to 100mg/l) significantly enhanced growth characteristics. Spermidine and stigmasterol concentration of 100 mg/1 resulted in highest values of the growth parameters during two physiological stages (vegetative and flowering). The increment of fresh weight that resulted from the treatment with spermidine and stigmasterol was evaluated to be 83.4% or 75.9% and 81.1% or 75.9%, respectively, for plant dry weight compared to the control during vegetative stage. Fresh weight increments were 41.35 or 79.2% and 43.6% or 82.0%, respectively, during the flowering stage. Results presented in Table 1 show that both bioregulators had stimulation effect on growth characteristics during both physiological stages. This effect might be due to spermidine and stigmasterol concentrations in plant tissue during the differential growth stages of chamomile plants. Stigmasterol treatment (at 100 mg/1) was surpassing in all treatments for criteria of growth during flowering stage. From these results it could be concluded that spermidine and stigmasterol had a favorable effect at the concentration of 100 mg/1. Similar results were reported by Abd El-Wahed (2000), Abd El Wahed *et al* (2001), who mentioned that both stigmasterol and spermidine stimulated vegetative growth characteristics (plant height, leaf area, plant fresh and dry weight) and net assimilation rate of maize and vascular bundles differentiation of wheat by application of sterol compounds. Putrescine was essential for growth, fresh and dry weight of *Mentha piperita* (Youssef *et al.,* 2002).

Similar to the effect of brassinosteroids, both typical (Sitosterol and Sigmasterol) and atypical sterols play a regulatory function in plant development (He *et al*., 2003).

Effect of spermidine and stigmasterol on flowering characteristics:

Data given in Table 1 show that gradual increase in all growth parameters during flowering stage was obtained by increasing the concentrations of spermidine and

Table 1: Effect of spermidine and stigmasterol on chamomile plant growth characteristics (average values for both seasons: 2001/02 and 2002/03) at growth and flowering stages.

Treatments		Vegetative Stage				Flowering stage					
Bioregulators	Concentration mg/l	Plant height (cm)	Branches number	Plant weight (g)		Plant height (cm)	Branches number	Plant weight (g)		Flower heads/ Plant	
				Fresh	Dry			Fresh	Dry	Number	Weight (g)
Spermidine	Control	40.0	14.0	19.96	7.97	43.1	19.5	45.17	13.47	102.5	13.65
	25	43.3	18.0	22.8	9.65	50.8	21.5	50.72	17.49	132.8	16.71
	50	46.3	21.5	30.48	12.05	53.1	27.5	58.88	18.56	133.2	18.01
	75	47.0	23.0	33.49	13.22	57.4	29.5	60.54	19.14	167.7	19.25
	100	53.0	25.3	36.60	14.43	61.3	31.0	63.84	19.34	173.7	19.97
Stigmasterol	25	42.3	17.0	23.39	9.34	44.8	21.0	49.06	16.92	133.0	16.13
	50	42.3	22.8	24.47	9.77	49.3	25.0	64.19	19.45	162.8	18.15
	75	47.0	25.0	25.47	10.07	53.1	26.5	68.59	20.78	165.3	22.21
	100	49.0	25.8	35.10	14.02	53.3	28.0	80.92	24.52	182.3	25.57
L.S.D. 5%		2.1	2.86	2.49	1.05	1.59	1.23	3.85	3.38	3.38	2.08

stigmasterol. Spermidine and stigmasterol application significantly increased flower heads number and weight of the chamomile plant. Their trend of increases, for both spermidine and stigmasterol spraying, were accompanied by an increase in their concentrations. On the other hand, the application of spermidine and stigmasterol on chamomile during vegetative stage, led to an increase in the number of branches per plant. This resulted in an increase of the number and weight of flower heads per plant during flowering stage. The highest flower heads number and weight values of the chamomile plant were obtained by the application of 100 mg/1 stigmasterol. The increment in flowers number and plant weight was 69.5% and 77.9% for spermidine and stigmasterol, respectively. These results show that chamomile plant responds to spermidine and stigmasterol application positively. This could be due to the stigmasterol role in flowers differentiation and their development, since, flower heads yield was affected by the plant age. It reached a maximum of 134 days after planting and thereafter decreased (Emongor and Chweya, 1989).

Relations between polyamine and flowering process have been observed by many investigators. In Cherry flower buds, polyamines that increase rapidly with the onset of active metabolism were detected in all stages of bud development (Wang *et al.,* 1985). Conjugated polyamines are known to be associated with the physiology of flowering metabolite synthesis (Slocum and Galston, 1985). Spermidine availability could act as a limiting factor in sexual development of tobacco (Martin-Tanguy, 1997). Spraying *Calendula afficinalis* plants with brassinosteroid significantly promoted their flowering characteristics (Shalaby and Talaat, 1998). It might be due to the brassinosteroid effect on plants sex differentiation. Its application to staminate inflorescence of *Luffa cylindrical* induced bisexual and pistillate flowers (Clouse and Sasse, 1998).

Effects of spermidine and stigmasterol on some biochemical contents in chamomile leaves:

Total sugars:

Data presented in Table 2 show that the increase of total sugars content in leaves was significant when spermidine and stigmasterol at 50 and 25 mg/l, respectively, were applied. All concentrations of stigmasterol and 50, 70 and 100 mg/l spermidine increased sugar content compared to the treatment with the control. Spermidine or stigmasterol (at 50 and 100 mg/1) increased notably sugar content in chamomile leaves. This proved that bioregulators were sufficient to produce the majority of sugar content in vegetative organs. Furthermore, bioregulators play an important role in metabolism and translocation processes in plant. Polyamines, are able to bind with pectic and polysccharides (D'oraci and Bagni, 1987). On the other hand, epibrassinosteroide affected the regulation of the hydrolytic and transport activities of tonoplast photohydrolases (Ozolina *et al.,* 1999). Significant increase in maize

leaves was obtained with stigmasterol application (Abd El-Wahed, 2000), whereas, sugars were also found to repress expression of the gene encoding of the key glyoxylate cycle malate synthase and isocitrate lyase enzymes in cucumber cells and in a mesophyll protoplast transient experession system (Graham *et al.,* 1994).

Total free amino acids content:

Data given in Table 2 show that spraying the plants with spermidine or stigmasterol significantly decreased total free amino acids content compared to the control (except for the spermidine treatment at 75 mg/1). Free amino acids content decreased under both bioregulators application, especially at the concentration of 100 mg/1. Decrease rates ranged from 5.0% to 51.1% and 19.9% to 66.7%, respectively. This shows that spermidine acts as a source of nitrogen (Smith, 1985) for nitrogenous compounds. It could be due to the bioregulator‘s effect on translocation processes from leaves to flowers, linking or converting to other plant biosubstances. In this respect, polyamines including spermine and spermindine, linked with particular proteins (Flok, 1980). Convalently bound polyamine-protein complexes have been reported in *Helianthus tuberosus* tubers (Dinnella *et al., 1992* and Grandhi *et al.* 1992), in young tobacco internodes, leaves and ovaries, especially in meristematic areas

Table 2: Effects of spermidine and stigmasterol on total sugars, free amino acids, indoles and phenols content in chamomile leaves at vegetative stages (Mean values of both seasons, 2001/02 and 2002/03).

Treatments		Total sugars %	Free amino Acids (mg/g)	Phenols (mg/g)	Indoles (mg/g)
Bioregulators	Concentration mg/1				
Spermidine	Control	10.4	14.1	17.2	9.5
	25	10.7	10.4	29.0	11.3
	50	13.3	11.3	24.3	11.2
	75	11.5	13.4	22.0	11.2
	100	10.8	6.9	15.2	10.4
Stigmasterol	25	11.8	4.7	17.5	10.4
	50	12.0	10.5	17.9	11.1
	75	13.3	11.3	22.2	10.6
	100	14.9	8.2	17.3	10.4
L.S.D. at 5%		1.18	1.82	3.39	0.89

(Sawhney and Appeluthite, 1992). That was one of the supposed mechanisms through which polyamine could regulate cell division and related growth processes. In addition, decrease in free amino acids content might be indirectly due to the brassinosteroid effect on enzymatic activities of membrane potential DNA, RNA and protein synthesis (Mandava, 1998).

Total phenolic compounds:

Data presented in Table 2 show significant differences in phenolic compounds content of chamomile plant as a result of spermidine or stigmasterol application. All spermidine treatments, except at 100 mg/1, significantly increased phenolic content of chamomile leaves compared to the control. Application of stigmasterol increased insignificantly phenols, and at 75 mg/l it showed significant increment. Spermidine treatment (at 25 mg/1) was more effective than the stigmasterol one. It could be due to the convertion of spermidine into another substence in the plant. It could derive from sugars, free amino acids, phenolic compounds and essential oil. That might be due to the accumulation of high phenolic compounds content in the plant tissue. 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). It had been proposed that some phenolic compounds may act as regulators of auxin transport (Jacobs and Rubery, 1988). Moreover, sitosterol increased the total number of the phenolic compounds in rice plant during developmental stages (Abd El-Wahed *et al.,* 2003).

Total indoles:

Data presented in Table 2 show that spermidine and stigmasterol significantly affected total indoles content of chamomile leaves. This was shown by the increased indoles content of the leaves during flowering stage. Spermidine and stigmasterol effects were significantly variable compared to the control. Furthermore, spermidine and stigmasterol concentrations (25 and 50 mg/1) resulted in the highest values of indoles content in chamomile leaves. This showed that spermidine and stigmasterol treatment enhanced phytohormones which can play an important role as signals and regulators of growth and development of plant endogenous polyamine (Shunquan *et al.,* 2001). Auxin was required to induce organogenesis both in the vegetative tomato meristem and in the *Arabidopsis* inflorescence meristem (Reinhardt *et al*., 2000). In the same time, at appropriate concentrations, brassinolide stimulated all growth characteristics. (Yang *et al.,* 2003).

Effect of spermidine and stigmasterol on essential oil content of chamomile flowers:

Data presented in Table 3 show essential oil content in air dried chamomile flower heads. It was evident that treated with bioregulators plants tended to increase their essential oil average content at the three collection dates of chamomile flowers along three months. 100 mg/1 concentration of spermidine and stigamasterol induced highest oil content of chamomile flower heads. Increase rates of essential oil were 2.0%-51.0% or 17.7%-43.1% at the first collection; 14.8%-26.0% or 32.8%-57.4% at the second collection and 10%-30% or 26.7%-70.0% at the third collection. The considerable decreases of oil content in chamomile flowers at the prior and the final collection might be attributed to the lower development rate of flowers at these stages. The highest essential oil content was obtained at the second collection during the second month of the flowering period, applying the highest concentrations of both bioregulators, as shown in Table 3. Highest oil content of flowers (0.96%) resulted

Table 3: Effect of spermidine and stigmasterol on essential oil content in air dried flower heads of chamomile at three successive collections, during flowering period (three months)

Treatments		1st collection	2nd collection	3th collection	Oil yield ml/plant
Bioregulators	Concentration mg/1	%	%	%	
Spermidine	Control	0.51	0.61	0.30	6.4
	25	0.52	0.70	0.33	8.7
	50	0.59	0.73	0.35	10.1
	75	0.74	0.75	0.36	11.7
	100	0.77	0.77	0.39	12.8
Stigmasterol	25	0.60	0.81	0.38	9.7
	50	0.67	0.89	0.40	11.8
	75	0.69	0.90	0.42	14.9
	100	0.73	0.96	0.51	18.7
LSD at 5%					2.1

from stigmasterol application at 100 mg/1. Spermidine and stigmasterol stimulated the formation of chamomile flower heads. Essential oil content of dried chamomile flowers decreased significantly with plant age (Emonogor and Chweya, 1989). The same results were obtained by Lechamo *et al.,* 1993, and Salaman, 1994, who found that the essential oil content was decreased by the increase of the harvest frequency. Lowest concentration was found in the fourth harvest of all genotypes of chamomile.

Spermidine and stigmasterol caused significant increment of essential oil yield Table 3. This increment was caused by both bioregulators concentrations. At the concentration of 100 mg/1, spermidine and stigmasterol caused the highest yield of essential oil. Plant yield increment was 100% and 192.2% for spemidine and stigmasterol, respectively. These results show that chamomile essential oil yield is related to plant heads weight and oil content. It might be due to both regulators effect on bioprocesses in the plant. These results are in accordance with Sangwan *et al.* (2001) who found that the production of essential and aromatic oils from plants is a diverse physiological, biochemical, genetically regulated process. Biochemical and metabolic processes comprise the molecular mechanisms that regulate carbon flow through the biosynthetic routes, as well as the turnover rate of the revelant terpenoid and/or phenylpropanoid metabolism (Smith, 1985, Chappell, 1995). In addition, lipid bodies are made up of a spheroidal core of neutral lipid triacylglycerols, sterol esters or poly droxyalkanoates (Murphy and Vance, 1999).

Essential oil constituents:

Terpenic constituents of the essential oil from chamomile flowers treated with bioregulators (spermidine and stigmasterol) are shown in Table 4. All terpenic constituents of chamomile essential oil varied according to bioregulators application and their concentrations. Therefore, spermidine application increased with 68% the main terpenic constituents (bisabolol oxide B, chamazulene and bisabolol A) compared to their content in the untreated plant. The increase of oil constituent rate under spermidine was 79.3%, 18.5% and 38.1%. Stigmasterol application stimulated farnesene, bisabolol oxide B, chamazulene and bisabolol oxide A by 5.7%, 22.7%, 10.5% and 46.3%, respectively. It might be concluded that the application of the two bioregulators caused considerable variations of the five major components of the essential oil distilled from chamomile flower heads. It might be due to their effect on biosynthesis of terpenoid compounds, that may be attributed to their specific reactions on the enzymatic pathways responsible for physiological actions and isomerisation of cyclic monoterpenes sesquiterpenoids, phenylpropanoids (Sangwan *et al.,* 2001) of chamomile essential oil.

From the results obtained we concluded that, at the observed growing stages, growth of chamomile plants, chemical composition including sugars, free amino acids, indoles, phenols essential oil yield and its constituents, are influenced by the

Table (4): Mean values of the major essential oil constituents of chamomile flower heads under spermidine and stigmasterol application.

Treatments		Farnesene	Bisabolol oxide B	α-Bisabolol	Chamazulene	Bisabolol oxide A
Bioregulators	Concentration mg/l					
Spermidine	Control	7.75	6.08	9.28	12.08	49.51
	25	3.76	7.45	2.44	10.66	66.56
	50	3.77	3.30	5.59	7.42	68.38
	75	6.01	1.25	8.86	9.96	58.63
	100	4.91	10.90	2.19	14.32	57.49
Stigmasterol	25	3.99	2.94	5.26	7.35	72.42
	50	4.47	5.26	11.39	12.84	55.58
	75	8.19	2.70	5.61	11.83	62.51
	100	6.43	5.75	5.07	13.35	57.86

treatment with spermidine and stigmasterol. At the same time, the application of spermidine and stigmasterol at 100 mg/1 improved chamomile growth, oil quantity and quality by increasing the level of farnesene, bisabolol oxide B, α-bisabolol, chamazulene and bisabolol oxide A.

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