World Journal of Agricultural Sciences 6 (5): 595-600, 2010
ISSN 1817-3047

Effect of Indole Acetic Acid, Gibberellic Acid and Kinetin on Vegetative Growth, Flowering, Essential Oil Pattern of Chamomile Plant (*Chamomile recutita* L. Rausch)

Fatma Reda, M.S.A. Abd El- Wahed and Karima M. Gamal El Din

Department of Botany, National Research Centre, P.O. Box, 12622 Dokki, Giza, Egypt

Abstract: Chamomile(*Chamomile recutita* L. Rausch) plants were foliarly treated with IAA,GA3 and K at three concentrations(25,50 and 100 mgL^{-1}) of each which improved vegetative growth (Plant height, number of branches, fresh and dry weight)especially at 50mgL^{-1} IAA,GA_3 100mgL^{-1} and at 50mgL^{-1}K. Total carbohydrate, total nitrogen and crude protein content were increased at the same levels mentioned for vegetative growth.Flower heads number and their fresh and dry weight increased at treatments 50mgL^{-1}IAA,50and 100$mgL^{-1}$$GA_3$ and 50mgL^{-1}K. Essential oil percent in flower heads was significantly increased at stage II of full- flowering period, especially at treatment 50 mgL^{-1}K.Gas liquid chromatography of essential oil in flower–heads (stage II) revealed that the major components, i.e. farnesene, bisabolol oxide B, α-bisabol, chamozulene and bisabolol oxide A percentages varied according to the concentration of IAA, GA_3 and K. The most pronounced increases were obtained in chamozulene at treatment 100mgL^{-1} and α -bisabolol oxide A at 100mgL^{-1}IAA or K.

Key words: Chamomile · Essential oil · IAA · GA_3 · K · Vegetative growth · Flowering

INTRODUCTION

The essential growth regulators (Whether endogenous or exogenously applied) are known to regulate growth and development of different plant organs [1]. Indole acetic acid (IAA) is one of the auxins biosynthesized within plant organs and affects many physiological processes, mainly required for cell-elongation. On the other hand, another prominent phytohormone, Gibberellic acid (GA_3), has the potential control on growth and flowering process. In addition, GA_3 application increased petiole length, leaf area and delayed petal abscission and color fading (senescence) by the hydrolysis of starch and sucrose into fructose and glucose [2, 3]. Gibberellic acid also affected stem–elongation and biosynthesis of biochemical constituents and secondary metabolites. Essential oil of chamomile was increased by foliar spray of benzyladenine (Belong to cytokinins as kinetin) [4]. Talaat and Gamal El-Din [5] reported that vegetative growth especially number of branches and umble (per plant) fresh and dry weight of fennel plants were increased as a result of exogenous spray with IAA (40 mg L^{-1}) or Kinetin (50 mg L^{-1}). Talaat and Gamal El Din [6] reported that number of leaves of sugar beet was significantly increased as a result of presowing seed treatment with indole butyric acid (40 and 60 mg L^{-1}) or with benzyladenine (20, 40 and 60 mg L^{-1}) or with gibberellic acid (40 and 60 mg L^{-1}promoted number of leaves.

Chamomile, *chamomilla recutita* (L.) Rausch (syn. *Matricaria chamomilla* L.) is known to contain several of sesquiterpene essential oil which mainly exists in flower-head. The flowers of chamomile and their extractable essential oil could be used as pharmaceutical, beverage and cosmotic industries [7]. The flowers extract are used as inflammatory, antiseptic, spasmolytic and sedative [8].

In the current literature, little could be traced concerning foliar spray of GA_3, IAA or K on growth,flowering and essential oil in chamomile flowers head. Therefore, the objectives of the present study aimed to reveal and compare the effect of these growth regulators (IAA, GA_3 and K) on vegetative growth, flowering, quantity and quality of main essential oil at two stages of chamomile plants flowering grown at the same location to find the most favourable treatment of these growth regulators in this concern.

Corresponding Author: Fatma A. Reda, Botany Department, National Research Centre, Dokki, Giza, Egypt.

MATERIALS AND METHODS

This study was carried out in the Experimental Farm of Research Centre, Dokki, Giza, Egypt. The layout of the experiment was complete randomized with three replicates per each treatment. Seeds of chamomile, *chamomilla recutita* (L.)Rausch (syn. *Matricaria chamomilla* L.) were grown in pots, 30cm diameter. The pots were filled with sand and loamy clay soil (Mechanical analysis =sand 24%, silt 44% and clay 29%). Seeds were sown at October, 26, 2008 and October, 22, 2009 growing seasons respectively. Then, after three weeks from sowing, seedlings were thinned to four seedlings per pot. Fertilization of the plants was carried out at the rate of 2.5g of calcium superphosphate (15% P_2O_3), 2g calcium nitrate (15.5% N) and 1g potassium sulphate (48-52%K_2O) per pot.The fertilizers were applied two weeks after thinning and after two weeks from 1st application.

Growth Regulators Treatments: Freshly prepared aqueous solution of indole acetic acid (IAA), Gibberellic acid and Kinetin (Kn) were foliarly applied twice, 1st after one month from sowing and 2nd spray seven days after the1stone wetting agent (Tepol, 1 m L^{-1}) was added to the freshly prepared solutions of growth regulators before spraying on plant foliage till running (20 ml/plant) using plastic automizer. Three concentrations of IAA, GA_3 and K were 25, 50 and 100 mg L^{-1} from each growth regulator. In addition,untreated plants were sprayed with distilled H_2O+wetting agent to serve as control.

Sampling, Growth and Flowering Criteria Determination: Two samples were drawn from all treatments with three replicates (4 plants/each replicate) at two physiological stages, full-vegetative growth and full -flowering Plant height, number of branches, fresh and dry weight of herb (Over ground aerial vegetative parts include stem, branches and leaves) were determined. In addition, at full-flowering sample, number of flower-heads and their fresh and dry weight were determined. For essential oil determinations,the flower heads were collected twice per week during two successive months at early morning,then air dried in shade.The flower–heads of each month were combined together, to represent 1st and 2nd stage.

Biochemical Constituents Determination: Total carbohydrates content(represented as%)was determined in the aerial vegetative parts of plant samples at full-vegetative stage according to the procedure of Dubois *et al.* [9]. Total nitrogen content (given as%) in the same plant samples of full-vegetative stage according to the procedure of A.O.A.C. [10]. Crude protein percentage was calculated by multiplying N content by 6.25.Essential oil percent was determined in the flower-heads of each treatment at the two flowering stages by steam hydro-distillation according to Guenther [11] and British Pharmacopcia [12]. The distillate oil was collected in a graduated tube and the volume of volatile oil was measured directly. Essential oil percent was calculated. Essential oil constituents were determined using Gas Liquid Chromatography (GLC) analysis for the obtained oil of different treatments after dehydrated over anhydrous Na_2SO_4Hewlett Pachard (Hp 6890 series) GC system, USA was used for the identification. The temperature program adopted to start at 60°C, final temperature 190°C and the detector's temperature was 280°C. Column (30×530µm), capillary Zb5, film thickness, 0.5 µm. The flow of carrier gases nitrogen and hydrogen or air was 30 ml/min. Essential oil compounds were identified by matching of their mass spectra with those recorded in the MS Library. In addition, the identification of these compounds was achieved by matching their retention times with those of authentic samples injected under the same conditions.

Statistical Analysis: The average of the obtained data of the two seasons was subjected to statistical analysis using F test according to the procedure of Snedecor and Cochran [13]. Least significant difference (L.S.D.) at 0.05 level was calculated to compare the mean values of determined criteria for different treatments.

RESULTS AND DISCUSSION

Vegetative Growth, Carbohydrate, Nitrogen and Crude Protein Contents: The obtained data given in Table1 indicated that plant height, number of branches, fresh and dry weights of the herb increased at 50 mg L^{-1} IAA, 100 mg L^{-1} GA_3 or 50 mg L^{-1} K with superiority of the last treatment. In support, Reda *et al.* [4] reported that foliar application of 50 mgL^{-1} benzyladenine caused stimulatory effects on vegetative growth, flower-heads number of chamomile plants grown in field at Giza, Egypt. However, at both treatments of 50 and 100 mg L^{-1} GA_3 or K, plant growth was significantly increased as compared with control plants. In this respect,Talaat and Gamal El Din [6] working on sugar beet reported that vegetative growth of this plant was effectively increased at treatments 60mgL^{-1} IBA, 40 mg L^{-1} BA and 40 mg L^{-1} GA_3. Gibberellic acid was documented to increase cell-wall elongation [1, 14].

Table 1: Effect of indole acetic acid(IAA),Gibberellic acid(GA_3)or Kinetin (K) treatments on vegetative growth of chamomile plants at full vegetative stage (Values are means of two seasons, 2008/2009 and 2009 / 2010)

Growth regulators treatments mg L^{-1}	Plant height (cm)	No. of branches/plant	Fresh weight of shoot (g/plant)	Dry weight of shoot (g/plant)
IAA				
25	27.0	12.5	17.10	1.89
50	28.5	19.0	18.35	2.22
100	27.5	16.5	15.94	1.93
GA_3				
25	28.5	13.5	14.56	1.81
50	31.0	14.0	16.63	1.89
100	33.5	18.0	17.80	1.94
Kinetin				
25	24.5	13.5	17.53	1.78
50	26.6	18.0	23.45	2.48
100	31.5	14.5	18.48	1.79
Control(untreated)	25.0	13.0	13.60	1.60
LSD 0.05	2.9	2.8	2.23	0.17

Table 2: Effect of indole acetic acid(IAA),Gibberellic acid(GA_3)or Kinetin (K) treatments on flowering vegetative growth and flower - heads of chamomile plants at full- stage(Values are means of two seasons; 2008/2009 and 2009/ 2010)

	Vegetative growth				Flower - heads		
Growth regulators treatments mg L^{-1}	Plant height (cm)	No. of branches	Fresh wt. (g/plant)	Dry wt. (g/plant)	No. of heads/plant	Fresh wt. (g/plant)	Dry wt. (g/plant)
IAA							
25	63.5	18.5	69.18	13.85	58.0	3.58	0.77
50	68.5	25.5	73.93	18.18	61.5	4.22	0.81
100	64.2	19.0	70.05	14.22	54.0	3.33	0.72
GA_3							
25	67.7	23.5	65.46	13.55	53.0	4.83	0.82
50	72.0	29.7	93.09	22.65	80.0	6.52	1.20
100	74.0	32.0	97.54	23.73	81.0	7.81	1.44
Kinetin							
25	63.7	26.0	79.25	19.28	98.1	6.31	1.16
50	64.0	27.3	80.46	19.57	103.0	6.63	1.22
100	65.0	27.0	79.58	19.36	102.0	6.26	1.20
Control(untreated)	62.0	15.0	4.66	13.21	51.0	4.00	0.74
LSD 0.05	4.0	3.3	4.24	1.81	4.2	0.46	0.15

Table 3: Effect of indole acetic acid(IAA),Gibberellic acid(GA_3)or Kinetin (K) treatments on total carbohydrate%, total nitrogen% and crude protein% of chamomile plants at full vegetative stage (Values are means of two seasons; 2008/2009 and 2009 / 2010)

Growth regulators treatments mg L^{-1}	Total carbohydrate (%)	Total nitrogen (%)	Crude protein (%)
	IAA		
25	11.4	4.5	28.13
50	11.9	5.0	31.25
100	8.9	4.4	27.50
	GA_3		
25	9.0	4.5	28.13
50	11.5	4.6	28.75
100	12.7	5.7	35.63
	Kinetin		
25	12.7	4.7	29.38
50	13.2	5.5	34.38
100	9.2	5.4	33.75
Control (untreated)	8.4	4.0	25.00
LSD 0.05	2.2	0.60	3.75

Table 2 show that the significant increases in number of branches, fresh and dry weight of the herb in the following descending order, treatment 100 mg L^{-1} GA_3, 50 mg L^{-1} IAA. These increases in plant growth could mainly be interpreted by their corresponding increases in plant height and number of branches (Tables 1 and 2). In addition, the data in Table 3 indicated that significant increases in total carbohydrate (%), total nitrogen (%) as well as crude protein (%) were obtained at the same promoting levels of growth regulators which enhanced plant growth. Abou-Dahab *et al.* [15] found that IAA (50 mg L^{-1}) caused pronounced increase in carbohydrate content in the leaves of *Digitalis lanata* Ehrh. plants.

Table 2 show the data of number, fresh and dry weight of flower-heads which were increased at treatments 50 and 100 mgL^{-1} K, 100 mgL^{-1} GA_3 and at 50 mg L^{-1} IAA, especially former ones.

Table 4: Effect of indole acetic acid(IAA),Gibberellic acid(GA_3)or Kinetin (K) treatments on essential oil percent and oil components in flower heads of chamomile plants (2008/2009 and 2009/ 2010)

Growth regulators	Essential oil%		Major components of chamomile oil%				
treatments (mg L^{-1})	Stage I	Stage II	Farnesene	Bisabolol oxide B	α - Bisabolol	Chama-zulene	α-Bisabolol oxide A
				IAA			
25	0.25	0.43	4.65	1.44	4.84	10.38	54.23
50	0.29	0.49	4.55	1.04	4.55	8.27	57.55
100	0.13	0.37	3.96	0.84	5.22	6.95	64.97
				GA_3			
25	0.27	0.51	4.01	0.57	8.37	7.35	61.58
50	0.32	0.53	3.71	0.59	8.18	8.63	58.28
100	0.13	0.43	2.68	0.88	7.24	17.63	57.42
				Kinetin			
25	0.21	0.57	6.50	0.79	6.94	11.10	54.27
50	0.25	0.67	7.87	1.12	5.70	12.26	52.90
100	0.18	0.40	4.57	0.80	4.57	12.60	62.86
Control(untreated)	0.14	0.37	3.64	0.85	6.53	11.55	53.86
LSD 0.05	0.04	0.03	-	-	-	-	-

This might be attributed that cytokinins (like K) are a group of growth regulators which could modify the flowering through their role in cell division and branching [1]. Rady and Youssef [16] reported that application of kinetin was the most suitable regulating substance for enhanced growth of leaves derived from callus of *Lavandula officinalis*.

Essential Oil Percent: Data given in Table 4 indicated that the greatest oil percent in flower heads of all treatments was obtained at full-flowering (stage II).The highest increase in essential oil percent were obtained at treatments 50 mg L^{-1} IAA, GA_3 and K at both stages I and II of full-flowering period as well as at treatment 25 mg L^{-1} of these growth regulators. Shedeed *et al.* [17] reported that essential oil percent and yield of peppermint plants were pronouncedly increased as a result of treatment 2.0 mg L^{-1} kinetin.

The increases in essential oil content at full-flowering (stage II) could be interpreted that flowers of chamomile had prochamazulene bearing and prochamazulene free glandular hairs which increased during flowering [18] and reached a maximum at full-flowering and then declined [2]. It could be suggested that growth regulators used in this study (IAA, GA_3 and K) controlled the biosynthesis of essential oil from mevalonic acid through the main metabolic pathway. In support of this suggestion, Cseke and Kaufmann [19] concluded that the control of biosynthetic pathways leading to the production of specific metabolites as essential oils are controlled by enzymes, which in turn are mainly affected by growth regulators. Auxins regulated the physiological processes through enzymatic reactions, cell structure, nucleic acids synthesis and consequently other metabolic pathways [20]. Indole acetic acid (IAA) folairly-applied to lentil plant caused pronounced decreased of flower abscission [21]. To identify and determine the components of chamomile essential oil, full-flowering stage (stage II) was chosen, as it was proved that the biosynthesis of important therapeutical components of chamomile oil was related to flower formation stages [4, 22, 23]. Data in Table 4 revealed that the major detected components of chamomile in the flower–heads of the control and treated plants were farnesene (3.64%), biosabolol oxide β (0.85%), α-biosabolol (6.53%), chamazulene (11.55%) and α– biosabolol oxide A (53.86%). It could be concluded that the highest area percent was α-biosabolol oxide A (= 53-64%) followed by that chamazulene (=7 -17%) at all treatments. In support, Piccaglia and Marotti [24], Reda and Gamal El-Dine [25] and Gupta *et al.* [27] reported the same pattern of oil constituents in flower-heads of chamomile.

The present results in Table 4 indicated that no qualitative changes in oil components in flower –heads were obtained as a result of all applied growth regulators treatments.However, quantitative increases or decreases occurred as a result of IAA, GA_3 and K treatments. Pronounced highest increases in chamazulene (17.63%) and in α-biosabolol oxide (64.97) at treatment 100 mg L^{-1} GA_3 100 mg L^{-1} IAA, respectively. However, treatments 50 and 100 mg L^{-1} IAA as well as treatments 25 and

50 mg L^{-1} GA_3 resulted in variable decreases in chamozulene. In this concern, Pino *et al*. [26] reported that the main components in the oil of chamomile were α-biosabolol oxide A (43.89%) and chamazulene (2.4%). IT could be concluded that both growth regulators and their levels quantitively affected the major components of chamomile essential oil especially the most therapeutically important components i.e. chamazulene and α-biosabolol oxide A.

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