International J. Seed Spices 3(2), July 2013:34-41

Effect of foliar application of plant growth regulators on growth, yield and essential oil components of Ajwain (*Trachyspermum ammi* L.)

Yogita Rohamare *, T. D. Nikam and K. N. Dhumal

Department of Botany, University of Pune

Pune - 411007. (M.S.), India.

Abstract

A field experiment was carried out to investigate the effects of foliar application of gibberellic acid (GA_3) and Naphthalne Acetic Acid on growth, yield and essential oil components of Ajwain during 2009-2011. The experiment was carried out in a completely randomized block design with three replications at College of Agriculture, Shivajinagar, Pune. The results revealed that foliar application of PGRS significantly enhanced the vegetative characters i.e. plant height, no. of branches and leaf area of ajwain in addition to photosynthetic pigments, total carbohydrate, essential oil percentage, essential oil yield/plant and thymol content. Ajwain essential oil composition was mainly characterized by presence of thymol, p-cymene and γ terpinene.

Key words : Ajwain, PGRs, growth, yield, essential oil components.

Introduction

Ajwain (*Trachyspermum ammi* L.) belongs to family Apiaceae. Its centre of origin is Egypt and India. Ajwain is native to India, but is also cultivated in arid and semi-arid regions of world like Iran, Egypt, Pakistan and Afghanistan. It is widely distributed in northern part of India in the states like Rajasthan, Gujarat, Uttar Pradesh, Punjab, Tamil Nadu and Andhra Pradesh. Ajwain is erect, glabrous or minutely pubescent branched annual herb which grows up to 75-80cm in height. Stem is striate, leaves are distant, and 2-3 innately divided, segments linear. Flowers are white in colour. The seeds are small and yellowish brown in colour.

Among traditional potential herbs, Ajwain is widely used for curing various diseases in both humans as well as animals. Ajwain is also known by other names in literature as owa, omum, Bishop's weed, carom, or Ethiopian cumin. A number of medicinal and therapeutic properties have been ascribed to various parts of this plant. Ajwain seed essential oil contains about 40-50% thymol which is a strong germicide, anti-spasmodic and fungicide. Thymol is used as an ingredient of deodorants, mouthwashes, toothpastes and many pharmaceutical preparations (John Zachariah, 10; Krishnamoorthy and Madalageri, 11; Malhotra and Vijay, 12). Essential oils are used in the food and pharmaceutical industries due to their therapeutic, antimicrobial and antioxidant activities.

The aroma and fragrance industry is a billion dollar world market which grows annually. Essential oils comprise the majority of compounds used by these industries. The essential oil production depends not only on genetic factors and the developmental stage of plants, but also on environmental factors. It is desirable to develop techniques of agronomical management to improve essential oil products and their specific compounds. Among other factors influencing essential oil production are plant growth regulators or plant hormones. Endogenous levels as well exogenous application could affect essential oil production and chemical composition (Prins *et al*., 20). The environmental factors can influence biochemical pathways and physiological processes that alter plant metabolism such as essential oil biosynthesis (Sangwan *et al.,* 21).

Recently, the need of essential oil bearing medicinal plants has increased exponentially both in developing and developed countries and is expected to enhance tremendously in the foreseeable future (Farooqi and Sharma, 6). Plant growth regulators (PGRS) have been defined as one of the main factors influence plants growth and their primary and secondary metabolites pool. In recent research, plant growth regulators (PGRs) have been shown to improve herb yield in basil (Arularasu and Sambandamurthi, 3), and coriander (Badgujar and Warhal, 4; Verma and sen, 25) and fenugreek (Deore and Bharud, 5). The review of literature states that there is scarcity of research work carried on seed spices especially ajwain. Thus in present investigation an attempt has been made to study the effect of foliar spray of different concentrations

*Corresponding author: Email: yogitaskandekar@gmail.com

of Naphthalene Acetic Acidand Gibberellic Acid on vegetative growth, yield and essential oil components in ajwain.

Materials and Methods

The authentic seeds of ajwain var. NRCSS AA-2 were procured from National Research Centre on Seed Spices, Tabiji Farm, Ajmer (Rajasthan), India. The field experiment was set up in the research field of College of Agriculture, Shivajinagar, Pune in a completely randomized block design (CRBD) with seven treatments and three replications. The soil properties were pH (7.5), EC (0.22), Available N_2 (213 kg/ha), P_2O_5 (10.61 kg/ha) and K_20 (362 kg/ha). The seeds of ajwain were sown during rabi season in the first week of December directly in rows. Two weeks after germination the plants were thinned to maintain a distance of 45 x 30 cm. Foliar spray of GA_3 and NAA at 25, 50 and 100 mg L^{-1} concentrations were made on field grown plants (45 DAS) with 15 days interval till flowering. Distilled water sprayed plants were treated as control. Standard inter-cultivation practices like irrigation, weeding, manuring were followed during the entire crop development. The plants of each treatment and control were harvested separately when seeds turn brownish. The harvested plants were further sundried for 2-3 days and seeds were separated out by gentle beating the sundried plants with wooden stick or by rubbing over each other. The seeds were then stored in sealed plastic bags till essential oil determination.

Analysis of physiology, growth and yield of ajwain plants

The biochemical analysis of photosynthetic pigments was carried out by Arnon, (2) method and total carbohydrate (Hedge and Hofrieter, 8) content was carried at flowering stage (90 DAS). The data on growth attributes like plant height, number of branches $plant^{-1}$ and leaf area was recorded at (120 DAS). The yield parameters like number of umbels, seed yield and dry biomass per plant were determined after harvesting.

Essential oil determination

The essential oils were extracted by hydro-distillation at 65^{O}C temperatures for 3 hours from 100 gm of seed powder in an all-glass type Clevenger apparatus. The amount of oil obtained from the seeds was calculated as:

Essential Oil (% v/w) = Observed volume of oil (ml)/Weight of sample (g) × 100

The essential oil was dried over anhydrous sodium sulphate and preserved in sealed glass vials at 4°C prior to the further analysis.

Determination of essential oil components by Gas Chromatography

The major constituents of the essential oil, namely p- cymene, γ- terpinene (Sigma Aldrich Fluka, GC grade), and thymol (Qualikems, New Delhi) were analyzed using Gas Chromatography (Shimadzu GC-2014) equipped with a flame ionization detector and a RTX-5 fused silica column, (30 m length × 0.25 mm inner diameter × 0.25 ìm film thickness). The oven temperature held at 60^{o}C for 5 min then programmed to 210^{o}C at rate of 15^{o}C min. Injector and detector (FID) temperatures were 250^{o}C. Nitrogen was used as the carrier gas with as linear velocity of 36.2 cm/s. The sample size was 1.0 µl for all the measurements. The identification of active constituents was based on the retention time. The active constituents were quantified as percent contents, comparing their peak with the peaks obtained from the authentic standards.

Statistical analysis

The data were analyzed statistically by one way ANNOVA model using SPSS-20 statistical software. Means were compared using Duncan's Multiple Range Test (DMRT) at *P* d" 0.05.

Results and discussions

Effect of foliar spray of PGRS on growth of ajwain plants

All the growth attributes like plant height, number of leaves and leaf area were significantly enhanced by exogenous application of plant growth regulators as compared to the unsprayed plants (Table 1). Highest plant height was noted at 100 mg L^{-1} GA_3 (95.49 cm) and that in control it was 81.15 cm. GA_3 also enhanced the number of primary branches and leaf area as compared to NAA and control. Gul *et al.,* (7) noted that GA3 foliar spray enhanced the height and ornamental wealth of *Araucaria heterophylla* plants. Same results for the positive effects of GA_3 on plant growth and development have been reported by Santos *et al.* (22) in *Ocimum* spp and Mishriky (13) in celery (*Apium graveolens*). Verma and sen (25) also reported the enhanced growth attributes with foliar application of GA_3 and NAA in coriander. The increased herb growth due to PGRS application could be due to the stimulation of cell division and elongation while increasing plasticity of cell wall (Mohammed, 15; Paleg, 17).

Effect of foliar PGRS on physiology of ajwain plants

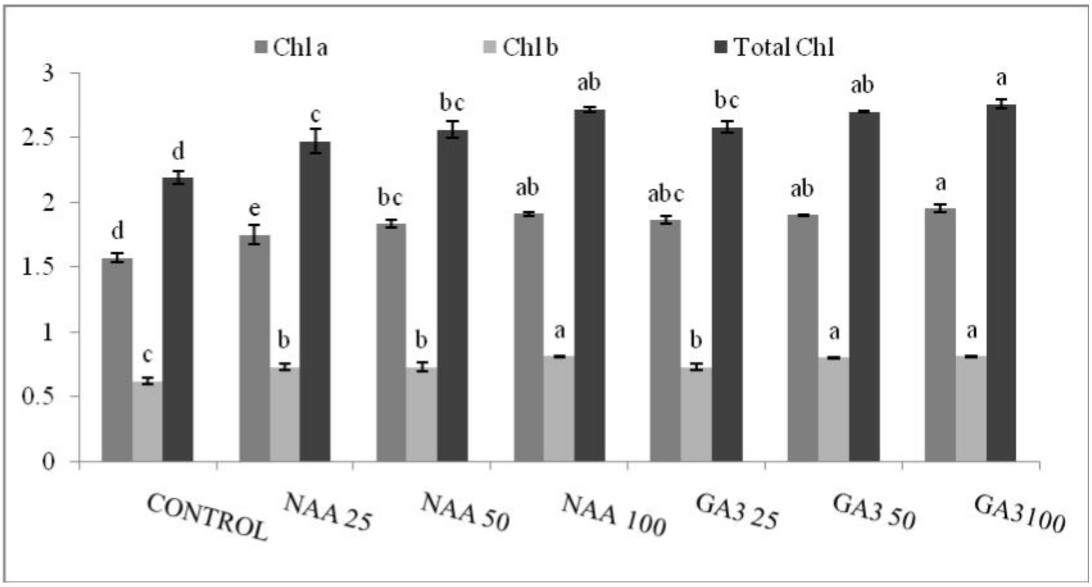
The application of foliar PGRs improved the photosynthetic pigments and total carbohydrate content of ajwain significantly over the control. The highest chlorophyll content was obtained at 100 mg L^{-1} GA_3 followed by NAA. There was about 26.04 % and 24.21% increase in total chlorophyll content by 100 mg L^{-1} GA_3 and NAA respectively as compared to control (Figure 1). Foliar application of 100 mg L^{-1} NAA and GA_3 resulted in 32.7 % and 26.8 % increase in total carbohydrate content as compared to control (Figure 2). Increase in leaf chlorophyll content might be responsible for increased photosynthesis, ultimately enhanced the total

Table 1: Effect of foliar sprays of Plant growth regulators on plant height, number of primary branches and leaf area of (*Trachyspermum ammi* L.) ajwain plants

Treatment (mgL^{-1})	**Plant height (cm)**	**No. of primary branches**	**Leaf area (sq. cm)**
Control	81.15±0.71c	11.71±0.36d	16.18±0.18d
NAA 25	86.45±0.73b	12.78±0.4bcd	18±0c
50	87.48±0.75b	13.59±0.3bc	19.79±0.41b
100	88.48±0.75b	14.01±0.01ab	20.93±0.52ab
GA_3 25	88.57±1.29b	12.71±0.36cd	21.31±0.66ab
50	92.91±0.91a	13.87±0.47abc	21.85±0.6a
100	95.49±0.76a	14.94±0.53a	22.66±0.88a
SEm	0.99	0.25	0.51
p- value	0.000	0.001	0.000

Values are means of three replicates ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Figure 1: Effect of foliar sprays of Plant growth regulators on photosynthetic pigments ($mg\ g^{-1}$ fr wt) of (*Trachyspermum ammi* L.) ajwain plants



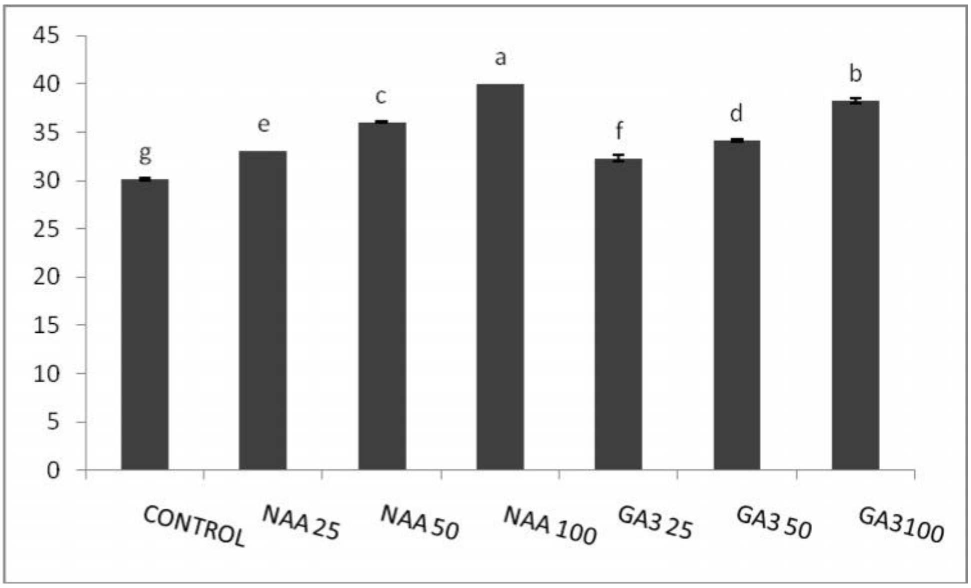
Values are means of three replicates. Vertical bars represent ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Table 2: Effect of foliar sprays Plant growth regulators on yield attributes of (*Trachyspermum ammi* L.) ajwain plants

Treatment (mg L^{-1})	Number of umbels/ plant	Seed yield/plant (gm)	Dry biomass/plant (gm)
Control	172.33±1.45f	53.33±0.33e	64.88±0.47c
NAA 25	191.27±0.9d	58.64±0.64d	77.44±0.29b
50	201.33±0.88c	62.72±0.28c	81.64±2.2ab
100	214.22±1.35a	68.34±0.33a	83.16±1.17a
GA_3 25	184.54±2.32e	57.97±0.55d	76.97±1.51b
50	198.34±0.33c	61.93±0.52c	79.91±0.96ab
100	207.32±0.88b	66.66±0.89b	80.38±3.29ab
SEm	2.96	1.09	1.37
p- value	0.000	0.001	0.000

Values are means of three replicates ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Figure 2: Effect of foliar sprays of Plant growth regulators on total carbohydrate content (mg g^{-1} fr wt) of (*Trachyspermum ammi* L.) ajwain



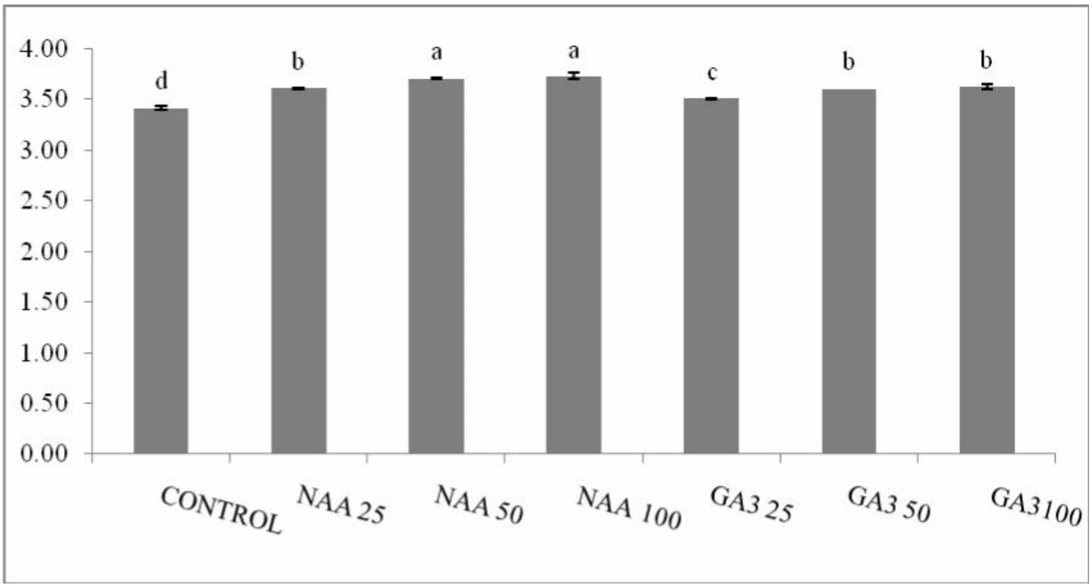
Values are means of three replicates. Vertical bars represent ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Table 3: Effect of foliar sprays Plant growth regulators on essential oil components (percent) of (*Trachyspermum ammi* L.) ajwain

Treatment (mgL^{-1})	p-Cymene	γ-Terpinene	Thymol	others
Control	26.2±0.2a	28.71±0.29a	41.81±0.43f	3.28±0.36
NAA 25	24.07±0.07b	26.69±0.31b	47.25±0.25d	1.99±0.01
50	23.11±0.11c	24.96±0.04c	49.24±0.24c	2.69±0.31
100	18.18±0.18d	17.62±0.62e	60.25±0.25a	3.95±1.05
GA3 25	26.02±0.02a	27.07±0.07b	43.15±0.15e	3.75±0.25
50	23.11±0.11c	24.32±0.32c	49.21±0.21c	3.36±0.64
100	18.2±0.2d	19.32±0.32d	59.31±0.31b	3.18±0.82
SEm	0.69	0.86	1.5	0.23
p- value	0.000	0.000	0.000	NS

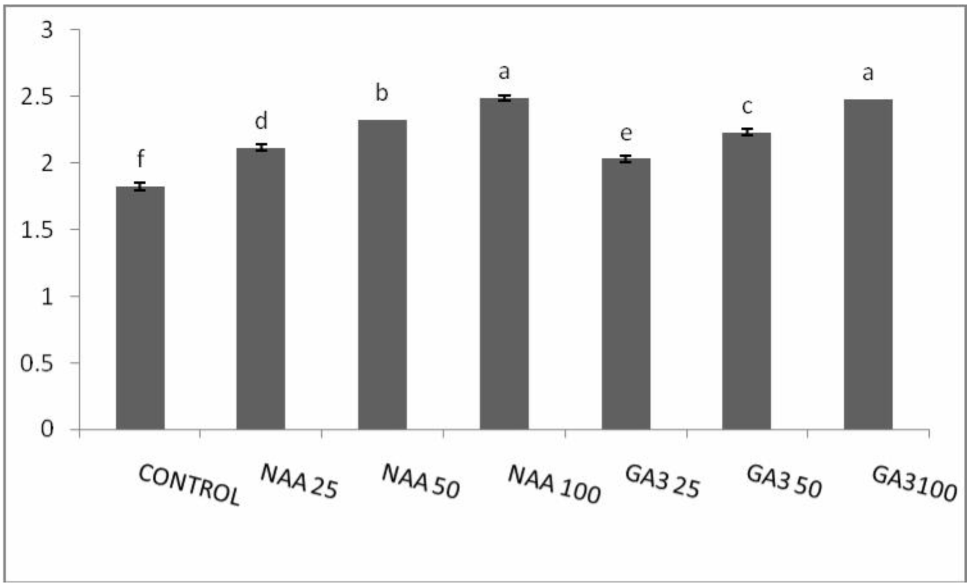
Values are means of three replicates ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Figure 3: Effect of foliar sprays of Plant growth regulators on essential oil content (percent) of (*Trachyspermum ammi* L.) ajwain seeds



Values are means of three replicates. Vertical bars represent ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

Figure 4: Effect of foliar sprays of Plant growth regulators on essential oil yield (mg $plant^{-1}$) of (*Trachyspermum ammi* L.) ajwain



Values are means of three replicates. Vertical bars represent ± SE. Within column, values followed by different letters are significantly different ($p < 0.05$) according to DMRT

carbohydrate content. It is likely that increased photosynthetic CO_2 fixation might provide more carbohydrates for metabolism and for export to sink (Ali, *et al.,* 1; Yu *et al.,* 26).

Effect of PGRS on yield and essential oil components of ajwain plants

Foliar application of PGRs has significantly improved the yield attributes like number of umbels, seed yield and dry biomass per plant over control (Table 2). Application of 100 mg L^{-1} NAA emerged as the best treatment to enhance the yield attributes followed by GA_3. Foliar PGRs application also gave the most improved values for essential oil percentage and essential oil yield $plant^{-1}$ (Figure 3 and 4). The promotion of essential oil production of medicinal and aromatic plants, as affected by several plant growth regulators, has been reported by various workers (Misra and Srivastava, 14; Naeem *et al.,* 16; Sangwan *et al*., 21). The positive effect of foliar PGRs on essential oil production might be attributed to the improved overall plant growth and metabolism as revealed in present study (Figure 3 and 4). It seems that PGRs might have enhanced the intrinsic genetic potential of the ajwain plants to produce additional herbage yield with improved quantity of essential oil through enhanced plant growth, photosynthesis and overall plant metabolism in the present study. These results are in agreement with the findings of Idrees *et al.,* (9), Naeem *et al.,* (16) Shukla *et al*., (23) and Srivastava and Sharma (24) regarding various medicinal plants as influenced by different plant growth regulators. Zhang *et al.* (27) verified that after GA application (14 μM) there was a 400% increase in the concentration of artemisinin compared to control plants.

As a result of PGRs application on the foliage of ajwain, there was observed a progressive increase in the content of thymol in the essential oil as compared to the control (Table 3). Foliar spray of plant growth regulators induced substantial increase in thymol content but there was decrease in the p- cymene and γ- terpinene level. The increase in thymol content can be attributed to conversion of γ- terpinene to p- cymene and further to thymol (Poulose and Croteau, 18). In *Salvia officinalis* L. the chemical composition changed with gibberellic acid (100 mg L^{-1}) application, with a significant reduction of β-tujone and α-humulene in relation to the control plants (Povh and Ono, 19).

Conclusion

Ajwain growth and essential oil production were stimulated with increasing concentration of exogenously applied plant growth regulators. Thymol remained the major component of essential oil with all the treated and control plants. It is worthy to mention that the content of this component with special industrial interest was enhanced by foliar sprays of plant growth regulators.

Acknowledgments

The financial support for this work was provided by University Grants Commission, New Delhi. The authors thank National Research Centre on Seed Spices, Tabiji Farm, Ajmer (Rajasthan) for kind supply of seed materials. Authors are also thankful to Director, Institute of Bioinformatics and Biotechnology, University of Pune and Dnyaneshwar for their help in GC analysis.

References

1. Ali, Q., Athar, H., and Ashraf, M. 2008. Modulation of Growth, Photosynthetic Capacity and Water Relations in Salt Stressed Wheat Plants by Exogenously Applied 24_Epibrassinolide, *Plant Growth Regul.* **56**:107–116.
2. Arnon, D. T. 1949. Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**:1-15.
3. Arularasu, P. and Sambandamurthi, S. 1999. Effect of gibberellic acid, nitrogen and spacing on herbage yield and oil yield in *tulsi* (*Ocimum sanctum* L.). *South Indian Horticult.* **47**:370–372.
4. Badgujar, C. D. and Warhal, K.N. 1988. Effect of seed soaking and wrapping on growth and yield of vegetable coriander. *J. Maharashtra Agricultural Univ.* **13**:344–365.
5. Deore, B. P. and Bharud, R.W. 1990. Growth, yield and storability of fenugreek as influenced by foliar spray of growth substances. *J. Maharashtra Agricult. Univ.* **15**:208–210.
6. Farooqi, A. H. A. and Sharma, S. 1988. Effect of Growth Retardants on Growth and Essential Oil Content in Japanese Mint, *Plant Growth Regul.* **7**:39–45.
7. Gul, H., Khattak, A.M. , Amin, N. 2006. Accelerating the growth of (*Araucaria heterophylla*) seedlings through different gibberellic acid concentrations and nitrogen levels. *J. Agric. Biol. Sci*., **1**: 25-29.
8. Hedge, J. E. and Hofreiter, B. T. 1962. In: Whistler, R. L., Be Miller, J. N, editors. Methods in carbohydrate chemistry. New York: Academic Press.
9. Idrees, M., Khan, M. M. A., Aftab, T. and Naeem, M. 2010. Synergistic Effects of Gibberellic Acid and Triacontanol on Growth, Physiology, Enzyme Activities and Essential Oil Content of *Coriandrum sativum* L. *The Asian Australasian J. Plant Sci. Biotechnol.* **4**:24–29.
10. John Zachariah, T. 2008. 16. Ajwain In: Chemistry of spices (Eds: Parthasarthy, V. A., T. Chempakam and T. John Zachariah, CAB International, 312-318.
11. Krishnamoorthy, V., and Madalageri, M. B. 1999. Bishop weed (*Trachyspermum ammi*): an essential crop for north Karnataka. *Journal of Medicinal and Aromatic Plant Sciences.* **21:** 996-998.
12. Malhotra, S. K. and Vijay O. P. 2004. II Particular herbs and spices: Ajwain. In: Peter, K.V.(ed.) Handbook of Herbs and Spices. Woodhead Publishing ltd., Cambridge, UK, **2**: 107-116.
13. Mishriky, J. E. 1990. Response of celery (*Apium graveolens* var. Dulce) to foliar application of gibberelic acid (GA_3). *Bull. Faculty Agricult. Cairo Univ., Egypt* **41**:777–784.
14. Misra, A. and Srivastava, N. K. 1991. Effects of the Triacontanol Formulations “Miraculan” on Photosynthesis, Growth, Nutrient Uptake, and Essential Oil Yield of Lemongrass (*Cymbopogon flexuosus*) Steud, Watts. *Plant Growth Regul.***10:**57–63.
15. Mohammed, H. M. 1975. Effect of GA_3, calcium, kinetin and ethylene on growth and cell wall composition of pea epicotyls. *Plant Physiol.* **56**:622–625.
16. Naeem M., Khan, M. M. A., Moinuddin, Idrees, M. and Aftab T. 2011. Triacontanol_Mediated Regulation of Growth and Other Physiological Attributes, Active Constituents and Yield of *Mentha arvensis* L. *Plant Growth Regul.* **65**:195–206.
17. Paleg, L.G. 1965. Physiological effect of gibberellins. *Ann. Rev. Plant Physiol.* **16**:291–322.
18. Poulose, A. J. and Croteau, R. 1978. Biosynthesis of aromatic monoterpenes-Conversion of ã- terpinene to p- cymene and thymol in *Thymus vulgaris* L. *Archives of Biochemistry and biophysics.* **187**: 307-314.
19. Povh, J. A. and Ono, E. O. 2007. Efeito do ácido giberélico na composição do óleo essencial de *Salvia officinalis* L. *Publ UEPG Biol Health Sci* **13:**7-10.
20. Prins, C. L., Vieira, I. J. C. and Freitas, S.P. 2010. Growth regulators and essential oil production. *Braz. J. Plant Physiol*. **22**: 91-102.

21. Sangwan, N. S., Farooqi, A. H. A., Shabih, F. and Sangwan, R.S. 2001. Regulation of essential oil production in plants. *Plant Growth Regul*. **34**: 03-21.

22. Santos, B., Morales-Payan, M. J. P., Stall, W.M., Dusky, J.A. 1998. Effects of nitrogen and gibberellic acid combination on basil growth. *Soil Crop Sci. Soc. Florida Pro*., **57**: 99-10.

23. Shukla, A., Farooqi, A. H. A., Shukla, Y. N., and Sharma, S. 1992. Effect of Triacontanol and Chlormequat on Growth, Plant Hormones and Artemisinin Yield in *Artemisia annua* L. *Plant Growth Regul.***11:**165–171.

24. Srivastava, N. K. and Sharma, S. 1991. Effect of Triacontanol on Photosynthetic Characters and Essential Oil Accumulation in Japanese Mint (*Mentha arvensis* L.). *Photosynthetica.* **25**: 55–60.

25. Verma, P. & Sen, N. L. 2008 The Impact of Plant Growth Regulators on Growth and Biochemical Constituents of Coriander (Coriandrum sativum L.) Journal of Herbs, Spices & Medicinal Plants. **14:** 144-153.

26. Yu, J. Q., Huang, L. F., Hu, W. H., Zhou, Y. H., Mao, W. H. and Ye, S. F. 2004. A Role for Brassinosteroids in the Regulation of Photosynthesis in *Cucumis sativus. J. Exp. Bot.* **55:**1135–1143.

27. Zhang, Y. S., Ye, H. C., Liu, B. Y., Wang, H. and Li, G. F. 2005 Exogenous GA_3 an flowering induce the conversion of artemisinic acid to artemisinin in *Artemisia annua* plants. *Russian J Plant Physiol* **52**: 68-73.

Received : February 2013; Revised : May 2013; Accepted : June 2013.