Influence of Gibberellic Acid, Indole Butyric Acid, and Methanol on Morpho-physiological and Phytochemical Traits in *Thymus vulgaris* L.

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Abstract

Background: Plant growth regulators (PGRs) have important roles in many processes such as germination, seedling growth, nutrition uptake, morphogenesis, ripening, etc.

Objective: This study aimed to evaluate the effect of gibberellic acid, indole butyric acid, and methanol as plant growth regulators on morpho-physiological and phytochemical features in *Thymus vulgaris* L.

Methods: The farm experiment based on a randomized complete block design was performed at 2014. The treatments were included G_1M_1 (GA_3 50 ppm + methanol 20 %), G_1M_2 (GA_3 50 ppm + methanol 40 %), G_2M_1 (GA_3 100 ppm + methanol 20 %), G_2M_2 (GA_3 100 ppm + methanol 40 %), I_1M_1 (IBA 50 ppm + methanol 20 %), I_1M_2 (IBA 50 ppm + methanol 40 %), I2M1 (IBA 100 ppm + methanol 20 %), I_2M_2 (IBA 100 ppm + methanol 40 %), $G_1I_2M_2$ (GA_3 50 ppm + IBA 100 ppm + methanol 40 %), $G_1I_2M_2$ (GA_3 100 ppm + IBA 50 ppm + methanol 40 %), and control (distillate water).

Results: The results showed that the combination of GA_3 or IBA along with methanol changed significantly leaf length and width, number of branches, leaf dry weight, stem dry weight, plant dry weight, seed weight, essential oil content and thymol amount. The highest values of the most traits were **observed in I_1M_1 treatment.**

Conclusion: Methanol combination with one of the GA_3 or IBA can improve morpho-physiological and phytochemical traits of thyme (*Thymus vulgaris* L.). Thus, the most effective PGRs combination was related to GA_3 50 ppm + Methanol 20 % and IBA 50 ppm + Methanol 20 %.

Keywords: ***Thymus vulgaris* L., Essential oil, Gibberellic acid, Indole butyric acid, Methanol, Thymol**

Introduction

Thyme (*Thymus vulgaris* L.) is a bushy perennial herb shrub with many branches of the Lamiaceae family. It is an aromatic evergreen plant which has a tall between 20 to 50 cm. Inflorescence clusters in thyme has composed of the purple or pink flower [1, 2]. Thyme is native to the Mediterranean especially the western regions as well as cultivated in southern Europe for commercially [3]. The essential oil of thyme flowering branches (0.4 %) have composed of two main phenolic compounds, which include thymol and carvacrol. Thyme and its essential oil due to having some properties such as anti-inflammatory, antibacterial, antioxidant, disinfectant, and carminative are using in the pharmaceutical, food, and cosmetic industry [1, 2].

A critical issue in medicinal plant production is an increasing plants biomass amount and secondary metabolite content without using harmful chemical materials such as chemical fertilizers, herbicides, and pesticides [4]. Plant growth regulators (PGRs) interfered in some features of plant growth and development. Also, these compounds have important roles in many processes such as germination and seed dormancy breaking, seedling growth, nutrition uptake, morphogenesis, and ripening. Plant growth regulators are organic, natural or synthetic compounds, which can change or control at least one specific physiological process in a plant. These compounds called as plant hormones when produced within the plant [5]. The Environmental Protection Agency (EPA) has defined the plant regulator as "any substance or mixture of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise, alter the behavior of plants or their produce" [6]. In general, these compounds are existent the low concentrations in plants and are active at the synthesis place, or transferred to distant tissues [7]. Recent researches have shown plant growth regulators (PGRs) can improved biomass and secondary metabolite biosynthesis in many medicinal plants like *Trigonella foenum-graecum* L., *Ocimum gratissimum* L., C*hamomile recutita* L., and *Catharanthus roseus* L. [8, 9, 10, 11].

Gibberellins as a group of diterpenoids make up a class of plant hormones, which can ameliorate the plant growth and development. Gibberellic acid (GA_3) has many physiological effects on plants and it has demonstrated GA_3 has an inductive effect on plant growth and secondary metabolite biosynthesis [12, 13, 14]. Application of GA_3 leads to increase the growth of intact common chicory (*Cichorium intybus* L.) and the accumulation of coumarin at its hairy root culture [15]. The activities of some important enzymes in plants such as carbonic anhydrase, nitrate reductase, and rubisco were increased by GA_3 foliar application [8, 16, 17]. Furthermore, it has claimed that gibberellic acid increases the biosynthesis of several secondary metabolites like steroids, terpenoids, and anthocyanins [18].

Other groups of plant hormones are auxins [19]. Auxins have several physiological effects on the plants included cell elongation and differentiation, apical dominance, root initiation, fruit development, and, etc. [13, 20]. Indole-3-acetic acid (IAA) is the main

detectable auxin in plants [21]. Moreover, the presence of indole-3-butyric acid (IBA) in plant tissues has demonstrated [22]. Also, IAA stimulated leaf growth in *C. jwarancusa* [23].

Recent studies revealed that yield and growth of C_3 crops enhanced with methanol spraying, in addition, it has reported that methanol can act as a carbon source for plants [24, 25, 26, 27]. Carbon dissipation during photorespiration is approximately equal 25%, therefore, methanol spray due to carbon increment can decrease the photorespiration [27]. Moreover, methanol size is smaller than CO_2, therefore methanol is absorbing into the plant and metabolized to CO_2 [26]. This study aimed to evaluate the effect of gibberellic acid, indole butyric acid, and methanol as plant growth regulators on morpho-physiological and phytochemical features of *Thymus vulgaris* L.

Materials and Methods

This experiment was performed at the research farm of Medicinal Plants Institute, ACECR. The treatments investigated as a randomized complete block design with three replications during growth season of 2014. The soil farm sampling was done before plowing and its physico-chemical features presented in table 1. Rooted cuttings had been planted in rows 50 cm apart with inter-row spacing of 20 cm apart. Plants were harvested in the blooming stage. Experimental units consist of six rows of 3m length. Irrigation and other field practices had been done as needed.

Treatments were included GA_3 50 ppm + methanol 20 %v/v (G_1M_1), GA_3 50 ppm + methanol 40 %v/v (G_1M_2), GA_3 100 ppm + methanol 20 %v/v (G_2M_1), GA_3 100 ppm + methanol 40 %v/v (G_2M_2), IBA 50 ppm + methanol 20 %v/v (I_1M_1), IBA 50 ppm + methanol 40 %v/v (I_1M_2), IBA 100 ppm + methanol 20 %v/v (I_2M_1), IBA 100 ppm + methanol 40 %v/v (I_2M_2), GA_3 50 ppm + IBA 100 ppm + methanol 40 % ($G_1I_2M_2$), GA_3 100 ppm + IBA 50 ppm + methanol 40 %v/v ($G_1I_2M_2$), and control (distillate water).

All the treatments were sprayed in three times consist 5 April, 20 April and 10 May 2014. Plants were cut at a height of 10 cm above soil levels and dried in a shaded area. The investigated traits were plant height, leaf length, leaf width, branches number, leaf dry weight, stem dry weight, plant dry weight, essential oil content, and thymol percentage.

Table 1- Some physico-chemical properties of soil (0-30 depth) in the experimental farm

EC	pH	OM	N	P	K	Fe	Mn	Zn	Cu	Texture		
(dS/m)		(%)	(%)	(ppm)	(ppm)	(ppm)	ppm	ppm	ppm	Sand (%)	Silt (%)	Clay (%)
2.71	8.2	0.82	0.07	48.9	33.6	4.81	11.2	0.6	0.7	79	13	8

Essential oils analysis

Hydro distillation method was used for thyme essential oil extraction. 100 g powder of dried thyme was weighed precisely and then the essential oil was extracted by clevenger apparatus for 3 hours. The acquired essential oil was dehydrated by adding anhydrous sodium sulfate and stored at 4 °C till it was analyzed [28].

Qualitative and quantitative analyses of oils were performed by Shimadzu gas chromatography model 15A, equipped with a FID detector and fused silica capillary column (OV-101, 25m × 0.2 mm). GC analytical conditions were: injector temperature: 230 ◦C, oven temperature: 175 ◦C (isothermal), and detector temperature: 230 ◦C. The thymol percentage was computed from GC (FID) peak areas with using the area normalization method [29, 30].

Statistical analysis

All the data were subjected to statistical analysis (one-way ANOVA) using SAS software ver. 9.1. The difference between treatments means were compared by Duncan's Multiple Range Test at 5% probability.

Results

Analysis variance results presented in Table 2. The result indicated that the PGRs combinations had a significant effect on the investigated traits except the plant height (Table 2). Utilization different concentrations of GA_3 and IBA with the methanol 20 or 40 percent increased the thyme leaf length (15 to 30%). However, high concentrations of methanol with different levels of GA_3 and IBA slightly enhanced the leaf length; hence, they had not a significant difference with control. Therefore, the highest and lowest leaf length obtained in G_1M_1 (6.02 mm) and $G_2I_1M_2$ (4.37 mm), respectively. The maximum leaf width observed in I_1M_1 (2.64 mm) and its minimum acquired in the $G_2I_1M_2$ (1.67 mm). Generally, increasing the GA_3 and IBA concentration at each level of methanol had no significant effect on enhancing the leaf dimensions. However, increasing the methanol concentration decreased the amount of leaf length and width compared to control. Also, concurrent use GA_3, IBA, and methanol reduced the value of leaf length and width. (Table 3). The foliar application of PGRs combinations had a significant ($P \leq 0.01$) effect on thyme branch number and they increased among 23 to 70 %. The maximum and minimum branches number obtained in G_1M_2 (13.63) and control (8) (Table 3).

Use of the PGRs combinations had a significant effect ($P \leq 0.01$) on the dry weight of leaf, stem, and whole plant (Table 2). Compared to control, the PGRs increased the leaf dry weight from 14.5 to 36 percent excepted to treatments of G_2M_2, $G_2I_1M_2$, and $G_1I_2M_2$. The maximum and minimum amount of the leaf dry weight obtained in I_1M_1 (165.2 g m^{-2}) and $G_2I_2M_2$ (118.6 g m^{-2}), respectively. Increasing the GA_3 and IBA concentration up to 100 ppm at the each methanol level caused to reduce the leaf dry weight, but was not significant. Increasing the methanol concentration in both concentrations of GA_3 had no significant effect on the leaf dry weight. However, the amount of leaf dry weight was reduced by increasing methanol

Table 2- Analysis of variance for effects of GA_3, IBA, and methanol on some traits of thyme

S.O.V.	df	Mean of Squares								
		Plant height	Leaf length	Leaf width	Number of branches	Leaf dry weight	Stem dry weight	Plant dry weight	Essential oil	Thymol
Replication	2	20.1 ^{ns}	0.85 ^{ns}	0.03 ^{ns}	1.99 ^{ns}	2.31 ^{ns}	2.7 ^{ns}	3.01 ^{ns}	0.03 ^{ns}	0.82 ^{ns}
PGRs	10	13.9 ^{ns}	0.73**	0.29 **	8.21 *	7.28**	12.3 **	7.45**	019 **	246.3 **
Error	20	22.82	0.26	0.11	3.35	0.48	0.59	0.65	0.002	7.27
CV (%)		15.08	9.42	14.99	15.67	4.95	4.51	7.41	9.04	13.46

ns: non-significant differences; *: significant at P<0.05; **: significant at P<0.01

Table 3- Means comparison of GA_3, IBA, and methanol effects on some traits of thyme

Plant growth regulators	Leaf length (mm)	Leaf width (mm)	Number of branches	Leaf dry weight (g m^{-2})	Stem dry weight (g m^{-2})	Plant dry weight (g m^{-2})
Control	4.63 ^{bc}	1.92 ^{cd}	8 ^{c}	121.1 ^{f}	163.8 ^{de}	284.6 ^{f}
G_1M_1	6.02 ^{a}	2.58 ^{ab}	11.54 ^{ab}	152.5 ^{bc}	181.7 ^{c}	334.2 ^{bc}
G_2M_1	5.71 ^{a}	2.25 ^{abcd}	13.07 ^{ab}	142.2 ^{cde}	174.4 ^{cd}	316.6 ^{cd}
G_1M_2	5.5 ^{ab}	2.21 ^{abcd}	13.63 ^{a}	140.4 ^{cde}	186.2 ^{bc}	326.7 ^{bc}
G_2M_2	5.32 ^{ab}	1.95 ^{bcd}	10.17 ^{abc}	130.7 ^{ef}	159.5 ^{ef}	290.2 ^{ef}
I_1M_1	5.76 ^{a}	2.64 ^{a}	13.1 ^{ab}	165.2 ^{a}	211 ^{a}	366.2 ^{a}
I_2M_1	5.73 ^{a}	2.44 ^{abc}	11.97 ^{ab}	162.2 ^{ab}	174.8 ^{cd}	337 ^{b}
I_1M_2	5.53 ^{ab}	2.29 ^{abc}	11.07 ^{abc}	146.4 ^{cd}	197.0 ^{ab}	343.4 ^{b}
I_2M_2	5.49 ^{ab}	2.28 ^{abc}	10.77 ^{abc}	138.7 ^{de}	167.1 ^{de}	305.9 ^{de}
$G_2I_1M_2$	4.37 ^{c}	1.67 ^{d}	9.83 ^{bc}	118.6 ^{f}	133.1 ^{g}	251.7 ^{g}
$G_1I_2M_2$	5.25 ^{abc}	1.89 ^{cd}	10.64 ^{abc}	126.1 ^{f}	147.6 ^{f}	273.7 ^{f}

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability

concentration in each level of IBA.

The stem dry weight was increased with some of PGRs foliar application. Among the treatments, G_1M_1 (181.7 g m^{-2}), G_1M_2 (186.2 g m^{-2}), I_1M_1 (211 g m^{-2}), and I_1M_2 (197 g m^{-2}) led to increasing the stem dry weight compared to control (163.8 g m^{-2}). In contrast, the stem dry weight was significantly decreased in G2I1M2 (133.1 g m^{-2}) and $G_1I_2M_2$ (147.6 g m^{-2}) in compared to control. The stem dry weight did not show a significant change with increasing the GA_3 concentration up to 100 ppm in both methanol levels. In contrast, the stem dry weight was decreased a both methanol levels, while IBA concentration increased.

Use of methanol 20% with different concentrations of gibberellic acid or indole butyric acid (50 and 100 ppm) increased the plant dry weight. With increasing the methanol concentration up to 40 %, the gibberellic acid 50 ppm and both IBA concentrations significantly enhanced the plant dry weight. Therefore, the highest and lowest amount of plant dry weight were obtained in I_1M_1 (366.2 g m^{-2}) and $G_2I_1M_2$ (251.7 g m^{-2}), respectively (Table 3). At the methanol 20 %, the plant dry weight had no significant change by increasing

GA_3, but raising the IBA concentration caused to reduce the plant dry weight. In addition, increasing the IBA and GA_3 concentration at the methanol 40 % reduced the plant dry weight.

Combination of GA_3 and IBA along with different concentrations of methanol had a significant ($P \leq 0.01$) effect on the essential oil and thymol content (Table 2). Excluding I_2M_2 and $G_1I_2M_2$, the essential oil percentage were significantly increased in other PGRs compared to control treatment. The highest essential oil content was obtained in G_1M_1 (1.66 %) which had no significant difference with G_2M_1 (1.62 %) and I_1M_1 (1.6 %). Moreover, the lowest content of the essential oil observed in I_2M_2 (1.1 %), $G_1I_2M_2$ (1.6 %), and the control (1.74 %). In each concentration of GA_3 and IBA, increasing the methanol concentration up to 40 % caused to decrease the essential oil content (Figure 1). Compared to the control, the thymol content was significantly increased by foliar application of G_1M_1, G_2M_1, G_2M_2, and I_1M_1. Simultaneous, the methanol 40 % with either the 50 or 100 ppm concentration of IBA was significantly reduced the thymol content in compared to the control. Thus, the highest and lowest thymol content was related to G_1M_1 (63 %) and I2M2 (31.1 %), respectively. Excepted to 100 ppm concentration of GA_3, the thymol content in both IBA concentrations and GA_3 50 ppm was reduced with increasing the methanol concentration (Figure 2).

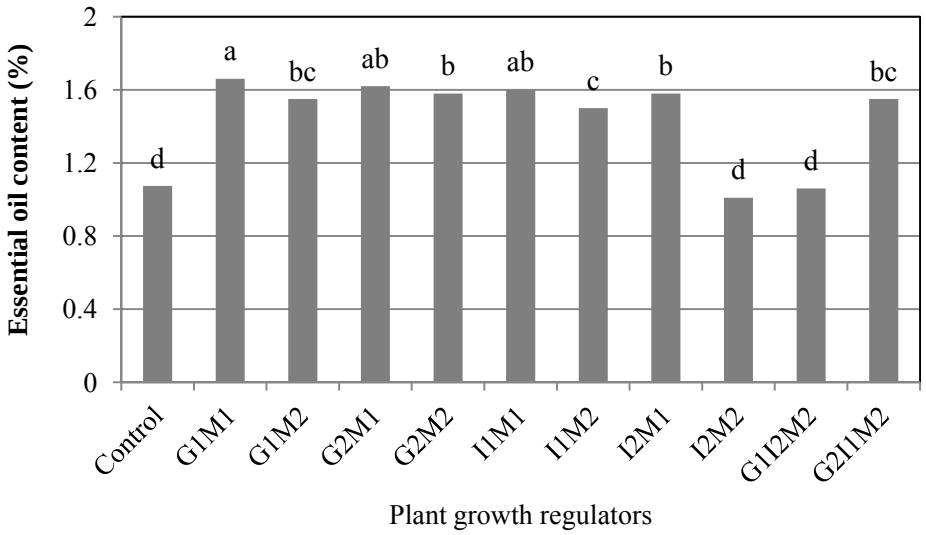

Figure 1- Effect of different plant growth regulators on essential oil content. **G1M1: GA_3 50 ppm + methanol 20 %, G_1M_2: GA_3 50 ppm + methanol 40 %, G_2M_1: GA_3 100 ppm + methanol 20 %, G_2M_2: GA_3 100 ppm + methanol 40 %, I_1M_1: IBA 50 ppm + methanol 20 %, I_1M_2: IBA 50 ppm + methanol 40 %, I_2M_1: IBA 100 ppm + methanol 20 %, I_2M_2: IBA 100 ppm + methanol 40 %, $G_1I_2M_2$: GA_3 50 ppm + IBA 100 ppm + methanol 40 %, $G_2I_1M_2$: GA_3 100 ppm + IBA 50 ppm + methanol 40 %.**

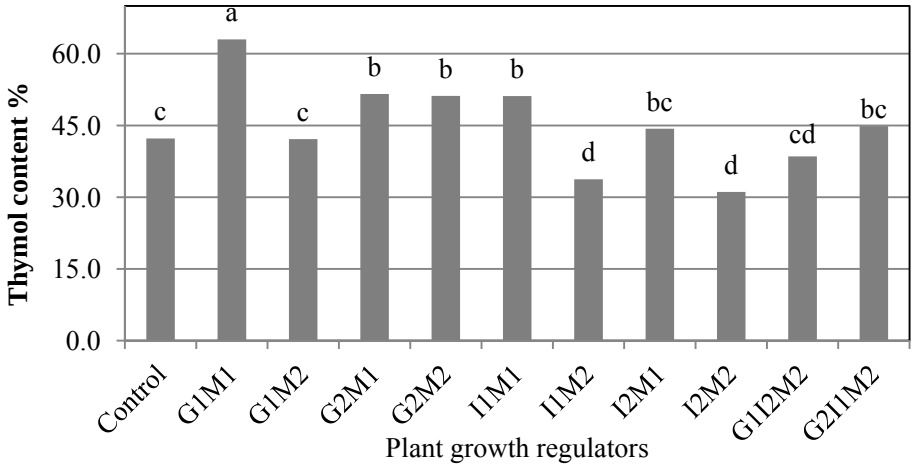

Figure 2- Effect of different plant growth regulators on thymol content. G1M1: GA_3 50 ppm + methanol 20 %, G_1M_2: GA_3 50 ppm + methanol 40 %, G_2M_1: GA_3 100 ppm + methanol 20 %, G_2M_2: GA_3 100 ppm + methanol 40 %, I_1M_1: IBA 50 ppm + methanol 20 %, I_1M_2: IBA 50 ppm + methanol 40 %, I_2M_1: IBA 100 ppm + methanol 20 %, I_2M_2: IBA 100 ppm + methanol 40 %, $G_1I_2M_2$: GA_3 50 ppm + IBA 100 ppm + methanol 40 %, $G_2I_1M_2$: GA_3 100 ppm + IBA 50 ppm + methanol 40 %.

Discussion

The results showed that the combination of GA_3 or IBA as plant growth regulator along with methanol could change some morph-physiological and phytochemical traits of thyme included the leaf length and width, number of branches, and leaf, stem and plant dry weight, and content of essential oil and thymol. This finding confirmed by previous studies [8, 10, 11, 31].

We found that application of GA_3 or IBA accompanied with methanol increased the length and width leaf. Salehi Sardoei (2014) explained that the leaf length of *Aloe barbadensis* was increased by spraying various concentrations of GA_3 [32]. Plant hormones may be involved in leaf expansion and development [33]. Because, PGRs can stimulate cell division and elongation via increasing the plasticity of cell wall [34]. Leaf area is a function of leaf dimension and leaf number [35]. Using these mixtures of PGRs had a high impact on the branches number compared to control but was no a significant difference among them. Therefore, it can deduce that the thyme branches number strongly was affected by PGRs induction. Increasing the number of branches was reason for raise the number of leaves and leaf area. This result was supported by other studies [20, 31].

The dry weight of leaf, stem, and plant were improved by using some of PGRs. It was remarkable that the PGRs mixtures were effective for increasing the leaf dry weight over than the stem dry weight. Compared to control treatment, the leaf dry weight was increased on average 17.5 percent under PGRs applications. But the stem dry weight was enhanced on average 5.8 percent. These results have compliance with other author's findings [10, 11, 31, 36]. The PGRs can cause a change in some metabolic pathways, photosynthesis

function, yield, and morphological characteristics of plants. Moreover, it has described that PGRs can improve the plant growth and development through increasing protein synthesis, nitrate reductase activity, uptake water and mineral nutrition, production and transfer of carbohydrates, photosynthesis efficiency and pigment biosynthesis, etc. [8, 11, 20]. IAA-treated plants exhibited higher values of dry weight and chlorophyll content than the control [16, 20, 37]. Also, it has demonstrated that the methanol application on plants could improve plant yield and dry weight due to inhibiting carbon waste in the photorespiration process [27].

Spraying the PGRs improved the essential oil and thymol content of thyme. These results was confirmed with some author's studies [20, 36]. Reda et al., (2005) reported similar results for increasing the essential oil yield of *Thymus vulgaris* L. after GA application [38]. Also, it has been expressed the essential oil content of *Salvia officinalis* was increased after spraying GA_3 (100 ppm). Moreover, it has been revealed that this increase accompanied by a reduction of α-thujone as a major compound [9]. Another study showed that the application of IAA increased the essential oil yield of *Ocimum basilicum* L., but GA caused to the yield reduction. In addition, the methyl chavicol amount was decreased in all treatments [39]. Several authors have described that this alteration in the essential oil content has linked to the glandular trichome numbers. Application of gibberellic acid is responsible for a decrease the number of these structures in *Lavandula dentata*, *Thymus mastichina* and *Picea abies* [40, 41, 42]. Therefore, alteration the essential oil content after spraying the PGRs is describable via considering changing the length and width leaf and the leaves glandular trichome density.

Some studies have shown that the monoterpenes and methyl chavicol content in the basil essential oil was changed by spraying of methyl jasmonate [43, 44]. Rodriguez-Saona et al., (2001) discovered that the monoterpenes contents in *Gossypium hirsutum* L. were varied by GA application [45]. The authors concluded that this shift could link to amount expression of the genes responsible for regulating the monoterpenes biosynthesis pathway. Thus, the numerous enzymes activity which plays a critical role in the biosynthesis of secondary metabolites such as the lipoxygenase, cinnamic acid 4-hydroxylase, prephenate dehydrogenase, polyphenol oxidase, phosphatase, etc. Bagheri et al., (2014) claimed that the foliar application of methanol 10 and 20 % significantly increased essential oil content of lavender [46]. Also, the various concentrations of methanol application on *Satureja hortensis* L. increased essential oil content. The highest amount of essential oil obtained at methanol 30%. These results were in conformity with the obtained results of present study. The alcohols via increasing the plant photosynthetic activity, cytokine production, nitrate reductase activity and decreased photorespiration can change the plant growth and phytochemical content [47].

Conclusion

Methanol combination with one of the GA_3 or IBA could improve morpho-physiological and phytochemical traits of thyme (*Thymus*

vulgaris L.). However, simultaneous use of GA_3, IBA, and methanol has a negative effect on the most features of thyme. In general, the best treatment was GA_3 50 ppm + Methanol 20 % or IBA 50 ppm + Methanol 20 %.

References

1. Hosseinzadeh S, Jafarikukhdan A, Hosseini A and Armand R. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. *International Journal of Clinical Medicine* 2015; 6: 635-642.

2. Prasanth Reddy V, Ravi Vital K, Varsha PV and Satyam S. Review on *Thymus vulgaris* Traditional Uses and Pharmacological Properties. *Med. Aromat Plants* 2014; 3: 164.

3. Christopher B. RHS A-Z Encyclopedia of Garden Plants. Dorling Kindersley, United Kingdom. 2008, pp: 1136.

4. Bybordi A. Effect of foliar applications of Iron and Zinc on the yield and quality of white qom and red ray onion varieties in grown Khosrowshahr Regions. *Pajouhesh and Sazandegi* 2007; 74: 153-160.

5. Kreuser, B. Effective Use of Plant Growth Regulators on Golf Putting Greens. *Green Section Record* 2015; 53 (7): 1- 10.

6. Khan MS, Zaidi A and Musarrat J. Microbial strategies for crop improvment. Springer-Verlag Berlin Heidelberg. London, New York. 2009, pp: 371.

7. Davies P.J. Plant Hormones Biosynthesis, Signal Transduction, Action. Springer Dordrecht Heidelberg, London, New York. 2010, pp: 835.

8. Dar T.A., Uddin M, Khan M.M.A., Ali A., Hashmi N and Idrees M. Cumulative effect of gibberellic acid and phosphorus on crop productivity, biochemical activities and trigonelline production in *Trigonella foenum-graecum* L. *Cogent Food & Agriculture* 2015; 1: 1-14.

9. Hazzoumi Z, Moustakime Y and Joutei K.A. Effect of gibberellic acid (GA), indole acetic acid (IAA) and benzylaminopurine (BAP) on the synthesis of essential oils and the isomerization of methyl chavicol and trans-anethole in *Ocimum gratissimum* L. *Springer Plus*. 2014; 3: 321-328.

10. Fatma reda M.S.A., El-washed A and Gamal El Din, K.M. Effect of indole acetic acid, gibberellic acid and kinetic on vegetative growth, flowering, essential oil pattern of chamomile plant (*chamomile recutita* L. Rausch). World journal of agriculture science. 2010; 6 (5): 595 - 600.

11. Muthulakshmi S and Pandiyarajan V. Effect of Iaa on the Growth, Physiological and Biochemical Characteristics in *Catharanthus roseus* (L). G. Don. *International Journal of Science and Res*. 2015; 4 (3): 442 - 48.

12. Jones A. M. P., Saxena P.K. and Murch S.J. Elicitation of Secondary Metabolism in *Echinacea purpurea* L. by Gibberellic Acid and Triazoles. *Eng. Life Sci*. 2009; 9: 205 - 210.

13. Taiz L and Zeiger E. Plant physiology (5th ed.). Sunderland, MA: Sinauer Associates. 2010, pp: 782.

14. Yuan L. and Xu D.Q. Stimulation effect of gibberellic acid short-term treatment on leaf photosynthesis related to the increase in

RuBisCO content in broad bean and soybean. *Photosynthesis Res.* 2001; 68: 39 - 47.

15. Bais H.P., Sudha G., George J. and Ravishankar G.A. Influence of Exogenous Hormones on Growth and Secondary Metabolite Production in Hairy Root Cultures of *Cichorium intybus* L. *cv.* Lucknow Local. *In Vitro Cell Dev–pl*., 2001; 37: 101 - 132.

16. Afroz S., Mohammad F., Hayat S. and Siddiqui M. H. Exogeneous application of gibberellic acid counteracts the ill effects of sodium chloride in mustard. *Turkish Journal of Biology* 2005; 29: 233 - 236.

17. Aftab T., Khan M.M.A., Idrees M., Naeem M., Singh M. and Ram M. Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. *Journal of Plant Interactions* 2010; 5: 273 - 281.

18. Srivastava N.K. and Srivastava A.K. Influence of gibberellic acid on $14CO_2$ metabolism, growth, and production of alkaloids in *Catharanthus roseus*. *Photosynthetica* 2007; 45: 156 - 160.

19. Sharma H and Kumar A. Effect of plant growth regulators and chemical fertilizers on growth and productivity of Chlorophytum tuberosum and Pergularia daemia. *Journal of Medicinal Plants Res*. 2011; 5 (13): 2647-2651.

20. Khan A.F., Mujeeb F., Aha F. and Farooqui, A. Effect of plant growth regulators on growth and essential oil content in palmarosa (*Cymbopogon martinii*). *Asian J. Pharm. Clin. Res*. 2015; 8 (2): 373-376.

21. Georg E.F., Hall M.A. and De Klerk G.J. Plant Propagation by Tissue Culture 3rd Edition. Springer. Netherlands. 2008, pp: 175 - 204.

22. Santner A., Calderon-Villalobos L.I.A. and Estelle M. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* 2009; 5 (5): 301 - 308.

23. Ansari SH, Qadry JS, Jain VK. Effect of plant hormones on the growth and chemical composition of volatile oil of *Cymbopogon jwarancusa* (Schutt). *Indian J. Foresty* 1988; 11: 143-5.

24. Makhdum MI, Malik MNA, Din SU, Ahmad F and Chaudhry FI. Physiology response of cotton to methanol foliar application. *Journal of Researches in Sciences* 2002; 13: 37 - 43.

25. Li Y, Gupta J and Siyumbbano AK. Effect of methanol on soybean photosynthesis and chlorophyll. *J. Plant Nutr*. 1995; 18: 1875-1880.

26. Gout E, Aubert S, Bligny R, Rébeillé F, Nonomura AR, Benson AA and Douce R. Metabolism of methanol in plant cells. Carbon-13 nuclear magnetic resonance studies. *Plant Physiol*. 2000; 123: 287 - 296.

27. Nadali I, Paknejad F, Moradi F, Vazan S, Tookalo M, Jami Al-Ahmadi M and Pazoki A. Effects of methanol on sugar beet (*Beta vulgaris*). *Australian Journal of Crop Sci*. 2010; 4 (6): 398 - 401.

28. European Pharmacopoeia (Third ed.). Council of Europe. Strasbourg. 1997, p: 12.

29. Shibamoto T. In: Sandra, P., Bicchi, C. (Eds.), Capillary Gas Chromatography in Essential Oil Analysis. Hüthig, Heidelberg, 1987, pp: 259.

30. Adams RP. Identification of essential oil

components by gas chromatography quadrupole mass spectroscopy. Allured Publishing, Carol Stream, IL, USA. 2004, pp: 456.

31. Shah S.H., Ahmad I. and Samiullah. Effect of gibberellic acid spray on growth, nutrient and yield attributes during various growth stages of black cumin (*Nigella sativa* L.). *Asian J. Plant Sciences* 2006; 5 (5): 881 - 884.

32. Salehi Sardoei A. Gibberellic Acid and Benzyladenine Application increase offsets in Aloe barbadensis. *European Journal of Experimental Biology* 2014; 4 (1): 646 - 650.

33. Aldesuquy H. Synergistic effect of phytohormones on pigment and fine structure of chloroplasts in flag leaf of wheat plants irrigated by seawater. *Egyptian J. Basic and Applied Sci*. 2015; 2: 310 - 317.

34. Rohamare Y., Nikam T.D. and Dhumal K.N. Effect of foliar application of plant growth regulators on growth, yield and essential oil components of Ajwain (*Trachyspermum ammi* L.). *International J. Seed Spices* 2013; 3 (2): 34 - 41.

35. Mousavi Bazaz A., Karimian Fariman Z. and Bannayan M. Modeling individual leaf area of basil (*Ocimum basilicum*) using different methods. *International Journal of Plant Production* 2011; 5 (4): 439 - 448.

36. Hassanpouraghdam M.B., Hajisamadi Asl, B. and Khalighi A. Gibberellic Acid Foliar Application Influences Growth, Volatile Oil and Some Physiological Characteristics of Lavender (*Lavandula officinalis* Chaix.). *Romanian Biotechnological Letters* 2011; 16 (4): 6322 - 6327.

37. Abdel-Latef A.A. Response of some *sorghum* cultivars to salt stress and hormonal treatment. M.Sc. Thesis, Fac. Agric. South Valley Univ. Qena, Egypt. 2002, pp: 273.

38. Reda F., Abdel Rahim E.A., El Baroty G.S.A. and Ayad. H.S. Response of essential oils, phenolic components and polyphenol oxidase activity of thyme (*Thymus vulgaris* L.) to some bioregulators and vitamins. *J. Agr. Biol*. 2005; 7: 735 - 739.

39. Salah el deen Et, M. Mahmoud. Response of Growth and essential oil content of sweet Basil (*Ocimum basilicum* L.) To some naturel hormones, *Acta Horticulturae* 1996; 426: 629-634.

40. Prins C.L., Vieira I.J.C., Freitas Braz S.P. Growth regulators and essential oil production. *J. Plant Physiol*. 2010; 22: 91 - 102.

41. Gershenzon J. Metabolic costs of terpenoid accumulation in higher plants. *J. Chem. Ecol.* 1994; 20: 1281 - 1328.

42. Erbelgin N., Krokene P., Christiansen E., Gazmend Z. and Gershenzon J. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (Picea abies) and reduces host colonization by the bark beetle Ips typographus. *Oecologia* 2006; 148: 426 -436.

43. Kim H.J., Chen F., Chen F. and Rajapakse N.C. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). *J. Agric Food Chem*. 2006; 54: 2327 -2332.

44. Li Z., Wang X., Chen F. and Kim H.J. Chemical changes and over expressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. *J. Agri. Food Chem*. 2007; 55: 706 - 713.

45. Rodriguez-Saona C., Crafts-Brandner S.J., Pare P.W. and Henneberry T.J. Exogenous methyl jasmonate induces volate emissions in cotton plants. *J. Chem. Ecol*. 2001; 27 (4): 679 - 695.

46. Bagheri H.R., Ladan Moghadam A.R. and Afshari H. The effects of foliar application of methanol on growth and secondary metabolites in lavender. International Research *Journal of Applied and Basic Sci*. 2014; 8 (2): 150 - 152.

47. Tavassoli A and Galavi M. Effect of foliar application of methanol on efficiency, production and yield of plants- A review. *Indian J. Agriculture Res*. 2011; 45 (1): 1 - 10.