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Effect of salicylic acid and methyl jasmonate on growth and secondary metabolites in *Cuminum cyminum* L.

Abdol Rahman Rahimi[1*], Asad Rokhzadi[1], Sheno Amini[2], Ezzat Karami[1]

[1]*Department of Agronomy and Plant Breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Kurdistan, Iran*

[2]*Young Researchers and Elites Club, Sanandaj Branch, Islamic Azad University, Sanandaj, Kurdistan, Iran*

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Abstract

In order to study the effect of salicylic acid (SA) and Methyl jasmonate (MeJa) on growth, yield and essential oil (EO) quantity and quality of cumin (*Cuminum cyminum* L.), the plants were sprayed with concentration of 0 (control: distilled water), 0.01, 0.1 and 1 mM of SA and MeJa. Results showed that the lowest concentrations of SA (0.01 and 0.1 mM) resulted in significant promotion of plant height and number of branches and umbels per plant. Fruit yield and EO yield significantly increased by the application of 0.1 mM SA. The EO percentage was increased by SA and MeJA application; however the increase of EO was more evident by applying the SA treatments. Twenty-two compounds were identified in cumin EO by GC-MS and GC analysis and the major compounds were γ-Terpinene-7-al, Cumin aldehyde, α-Terpinene-7-al, ρ-Cymene and β-pinene respectively. The α-Terpinene-7-al was more affected by treatments, and it considerably reduced by 1 mM SA and MeJa.

*__Corresponding Author:__ Abdol Rahman Rahimi ✉ abdolrahmanrahimi60@gmail.com

Introduction

Cumin (*Cuminum cyminum* L.) is a herbaceous and annual medicinal plant belonging to the Apiaceae family (Kafi *et al*., 2002). Cumin is originated from Iran, Egypt, Turkistan and East Mediterranean and it is widely cultivated in Iran, China, India, Morocco, South Russia, Japan, Indonesia, Algeria and Turkey (Neamatollahi *et al*., 2009 in Tuncturk and Tuncturk, 2006). Iran is one of the most important cumin exporters in the world market, and about 52% of the world cumin exportation come from Iran (Kafi *et al*., 2006 in Rezvani Moghaddam *et al*., 2007).

Cumin seed is generally used as a spicy plant to flavor food in different food preparations (Kafi *et al*., 2002). It is also used as an essential ingredient in soup, cheese, sausages, candies and cakes (Rezaei Nejad, 2011). Moreover, cumin is used as therapeutic purposes for dyspepsia, jaundice, diarrhea, diuretic, carminative and antispasmodic (Dhandapani *et al*., 2002 in Mohammad pour *et al*., 2012).

Plant growth and development are regulated by action and balance of different groups of growth regulators, which promote or inhibit such processes (Prins *et al*., 2010). Some growth regulators or plant hormones stimulate plant growth and terpene biosynthesis in a number of aromatic plant species and result in beneficial changes in quality or quantity of terpenes (Farooqi and Shukla, 1990 in Prins *et al*., 2010; Sharafzadeh and Zare, 2011; Zheljazkov *et al*., 2010).

Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature which enhances plant resistance to pathogens and other stresses (Rao *et al*., 2000). In addition to provide resistance to plant diseases; SA also has been found to induce tolerance to than some abiotic stresses such as drought (Hayat *et al*., 2008), heat (Larkindale and Huang, 2004), salinity (Shakirova *et al*., 2003), chilling (Taşgín *et al*., 2003), heavy metal (Metwally *et al*., 2003; Choudhury and Panda, 2004) and UV radiation (Rao and Davis, 1999). Moreover, SA plays role in the regulation of some physiological processes such as seed germination, fruit yield, glycolysis, flowering in thermogenic plants, nutrient uptake and transport, photosynthetic rate, stomatal conductance and transpiration (Hayat *et al*., 2010).

Methyl jasmonate (MeJA) and jasmonic acid (JA) are cellular regulators that play an important role in plant development and physiological processes such as seed germination, root growth, flowering, ripening, senescence, photosynthesis, the formation of gum and bulb, defense response against pathogens and insect attack, plant response to wound and abiotic stresses (Maciejewska *et al*., 2004; Choi *et al*., 2005; Kim *et al*., 2009; Warabieda *et al*., 2010). In addition, MeJa induces or increases the biosynthesis of many secondary metabolites that play important roles in plant adaptation to particular environments (Choi *et al*., 2005).

Due to inadequate data about the effect of SA and MeJA on cumin, the present investigation was designed to study the effect of exogenously foliar application of SA and MeJA on growth, yield and the EO composition in cumin.

Materials and methods

In order to study the effect of SA and MeJA on growth, yield and EO quantity and quality of cumin (*Cuminum cyminum* L.) this experiment was conducted at research farm of Islamic Azad University, Sanandaj Branch (35°10′N, 46°59′E; 1393 m above sea level) in spring 2011. Some of the soil physicochemical characteristics were: sand 24%, silt 33%, clay 43%, pH 7.8, organic carbon 0.68%, electrical conductivity 0.49 dS m−1, and available P and K 9.3 and 340 mg L^{-1}, respectively.

Cumin seeds were sown by hand on 6 April 2011. Each experimental plot contained 5 sowing rows 3 m in length with 10 cm space between plants on each row. Until plant establishment irrigation was performed every 4 days and the next turns of irrigation times were done dependent on the weather conditions every 7-12 days. At 4-6 leaves plant stage the plots were thinned with a 10 cm intra row space and the plots were kept weed-free by hand. The treatments included control (distilled water) and concentrations 0.01, 0.1 and 1 mM of SA and MeJA. Plants were sprayed with treatments at flowering stage twice in one week interval.

At the stage of seed ripening plants were harvested and plant height, number of branches and umbels per plant and fruit yield (kg/ha) were measured. EO of seeds was extracted by hydrodistillation using the Clevenger device for 3 h. The EOs obtained was expressed as an EO percentage (ml/100gr). EO yield (l/ha) was calculated by multiplying EO percentage and fruit yield. The EO's was dried over anhydrous sodium sulfate and kept at 4°C until analysis.

GC-MS analysis was performed on the EOs of cumin using an Agilent 7890A-GC and Agilent 5975C-MS. The chromatographic column for the analysis was an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The helium gas was used at 1ml/min as carrier gas. Oven temperature was programmed from 60°C to 210°C at a rate of 3°C /min then raised from 210°C to 240°C at a rate of 20°C/min and held at 240°C for 8.5 min. The temperature of injector and MS detector was 280°C. The EO components were identified with Chemstation software.

Treatments were arranged in a randomized complete block design (RCBD) with three replications. The statistical calculations were performed with SAS software (2001) and means were compared using Duncan's test ($p \leq 0.05$).

Results

Growth and yield

Results showed that the lowest concentrations of SA (0.01 and 0.1 mM) resulted in significant promotion of morphological traits of cumin, so that the highest rate of plant height and the largest number of branches and umbels per plant were recorded by the application of 0.01 and 0.1 mM of SA which statistically differed from control treatment (Table 1). Besides the greatest amount of seed yield (776.7 kg/ha) was obtained as the result of SA application with 0.1 mM concentration, showing a significant increase about 77% compared with control. Moreover, mean comparisons indicated that all concentrations of MeJA had no significant effect on growth and yield parameters of cumin (Table 1).

Table 1. Mean values of the effect of salicylic acid (SA) and Methyl jasmonate (MeJA) on growth and fruit yield of cumin.

Treatments	Plant height (cm)	Number of branches	Number of umbels	Fruit yield (kg/ha)
SA 1mM	21.7 ^{bc}	4.6 ^{ab}	21.8 ^{bc}	474.9 ^{b}
SA 0.1mM	23.9 ^{a}	4.9 ^{a}	32.3 ^{a}	776.7 ^{a}
SA 0.01mM	23.3 ^{ab}	4.9 ^{a}	28.4 ^{ab}	624.7 ^{ab}
MeJA 1mM	22.1 ^{abc}	4.8 ^{ab}	25.6 ^{abc}	611.3 ^{ab}
MeJA 0.1mM	22.8 ^{bc}	4.4 ^{ab}	18.6 ^{c}	434.3 ^{b}
MeJA 0.01mM	20.5 ^{c}	4.4 ^{ab}	18.2 ^{c}	441.1 ^{b}
Control	20.3 c	4.3 ^{b}	18.4 ^{c}	439.0 ^{b}

Means with the same letters in each column do not significantly differ by Duncan's test ($p \leq 0.05$).

Essential oil (EO)

As shown in Fig. 1, the EO percentage was generally increased as the result of SA and MeJA application as compared with control treatment; however the increase of EO was more evident by applying the SA treatments, so that the greatest amount of EO was recorded by the treatment of 1 mM SA which was statistically higher than control treatment.

Essential oil yield was improved by the application of SA treatments compared with control. The highest rate of EO yield was obtained at 0.1 mM level of SA application, and a descending trend in EO yield was found with increasing the level of SA to 1 mM. Application of MeJA had no significant effect on EO yield compared with control treatment; however a slight increase in EO yield was shown by the treatment of 1 mM MeJA in comparison with control (Fig. 2).

Chemical composition of the EO

The components of EO were given in Table 2. Twenty-two compounds (97.76% to 99.51% related to treatments type) were identified and quantified in EO by GC-MS and GC analysis respectively. The major compounds were γ-Terpinene-7-al, Cumin aldehyde, α-Terpinene-7-al, ρ-Cymene and β-pinene respectively and other compounds were below 1%.

The results showed that the EO components were affected by the SA and MeJA. From the results (Table 2) in all treated plants, γ-Terpinene-7-al was lower than control. The α-Terpinene-7-al was increased with application of 1mM of SA and 0.1 mM of MeJA, while other concentration of SA and MeJA decreased it. Moreover, the plants treatment with 1mM of SA and 0.1 mM and 0.01 mM of MeJA led to increase in Cumin aldehyde. The treatments of 0.1 mM and 0.01 mM MeJA resulted in decrease in γ-Terpinene, while other treatment increased it. Furthermore, in the plants which were sprayed with both SA and MeJA in all concentrations, ρ-Cymene and β-pinene was enhanced compared to control (Table 2).

Table 2. The effect of salicylic acid (SA) and Methyl jasmonate (MeJA) on percentage of essential oil composition in cumin.

Compound	SA			MeJA				
	1 mM	0.1 mM	0.01 mM	1 mM	0.1 mM	0.01 mM	control	RI[a]
α-Thujene	0.16[b]	0.13	0.12	0.16	0.11	0.09	0.08	925
α-Pinene	0.21	0.16	0.15	0.19	0.12	0.11	0.09	932
Sabinene	0.41	0.32	0.3	0.36	0.28	0.23	0.24	971
Myrcene	0.60	0.56	0.55	0.65	0.5	0.45	0.44	989
α-Phellandrene	0.58	0.44	0.39	0.39	0.28	0.32	0.29	1004
α-Terpinene	0.14	0.1	0.1	0.11	0.08	0.08	0.08	1015
Limonene	0.17	0.2	0.25	0.18	0.2	0.13	0.16	1027
β-Phellandrene	0.30	0.17	0.19	0.16	0.13	0.18	0.13	1028
1,8-Cineole	0.14	0.09	0.09	0.12	0.1	0.09	0.09	1030
cis-Sabinene hydrate	0.05	0.04	0.05	0.04	0.1	0.05	0.06	1065
trans-Sabinene hydrate	0.12	0.13	0.15	0.13	0.24	0.16	0.17	1096
trans-Sabinol	0.16	0.11	0.11	0.13	0.15	0.15	0.13	1136
Terpinene-4-ol	0.25	0.16	0.16	0.2	0.62	0.26	0.28	1175
cis-dihydro Carvone	0.86	0.57	0.51	0.78	0.65	0.59	0.56	1192
ρ-Mentha-1,4-dien-7-ol	0.27	0.24	0.25	0.15	0.19	0.2	0.23	1327
β-Acoradiene	0.32	0.2	0.18	0.19	0.16	0.18	0.16	1471
β-pinene	3.30	3.48	3.45	4.22	2.83	2.7	2.36	977
ρ-Cymene	9.19	9.09	8.75	10.06	7.86	8.55	7.55	1025
γ-Terpinene	9.95	12.37	11.47	12.29	9.49	9.59	9.61	1060
Cumin aldehyde	29.19	24.6	24.99	25.38	28.17	28.72	27.8	1247
α-Terpinene-7-al	18.59	14.71	15.43	16.49	17.71	16.49	16.79	1288
γ-Terpinene-7-al	24.47	31.64	31.82	25.38	29.32	30.1	31.96	1294
Total major components	94.69	95.89	95.91	93.82	95.38	96.15	96.07	-
Total components	99.44	99.51	99.46	97.76	99.29	99.42	99.26	-

[a] Retention Index.

[b] The bold data were increased by treatments.

Discussion

Our results suggested positive response of the green cumin to 0.1 mM of SA for growth parameters, yield and EO yield. The spray of plants with 0.1 mM concentration of SA increased both seed yield (significantly) and biosynthesis of EO (insignificantly). Thus, the increase in EO yield could be related to increase in both seed yield and EO percentage, but the effect of seed yield was found to be greater. Unlike, the higher concentration of SA (1 mM) had a week effect on growth and yield; it was also more effective on accumulation and biosynthesis of EO in plant. Corresponding results were reported by Gharib (2006) in basil (*Ocimum basilicum*) and marjoram (*Majorana hortensis*); he showed an increase in EO percentage and EO yield in treated plants with SA. They stated that the increment in EO yield might be due to the increase in vegetative growth, nutrients uptake or changes in leaf EO gland population and biosynthesis of monoterpins. Hesami *et al* (2012) with treatment of coriander by different concentration of SA in two irrigation interval, reported that when plants are treated by lowest dose of SA, growth and yield of coriander was increased; they also cleared that the application of SA in suboptimal conditions of water availability may improve the growth and productivity of the plant. Moreover, in previous studies we showed that 0.1 mM SA had more positive effects on biosynthesis of EO in coriander compared with some macro and micro elements and control, but it had no significant effects on yield and growth parameters (Rahimi *et al*., 2009). Ram *et al* (1997) by application of SA (100 ppm) in *Pelargonium graveolens*, *Mentha arvensis* and *Cymbopogon martini* cleared that SA had no effect on the herbage and EO yield.

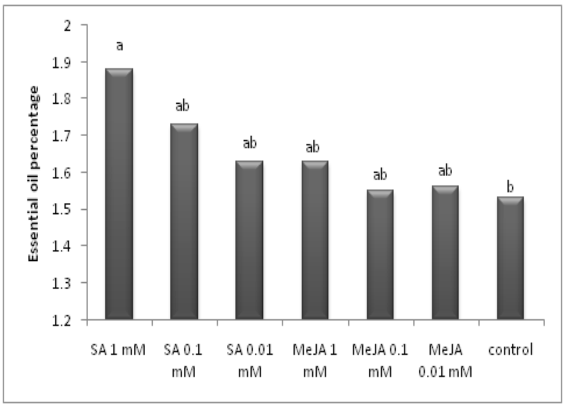

Fig. 1. The effect of salicylic acid (SA) and Methyl jasmonate (MeJA) on essential oil percentage of cumin. Means with different letters are significantly different by Duncan's test ($p \leq 0.05$).

In addition, MeJA did not have significant effect on any of evaluated traits for growth or EO but the highest values of these parameters obtained by 1mM. It seems that the higher concentration of MeJA required for enhancement these parameters. In other hand, Raouf Fard *et al* (2012) reported a significant increase in EO of *Agastache foeniculum* after 24 h of treatments with 0.1 mM of MeJA. Zheljazkov *et al* (2010) cleared the positive effect of MeJA on biosynthesis of EO in peppermint (*Mentha piperita* L.).

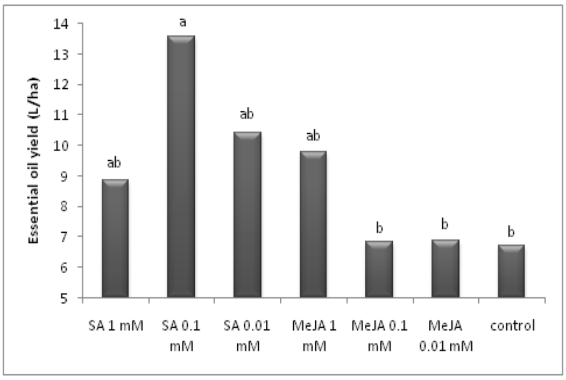

Fig. 2. The effect of salicylic acid (SA) and Methyl jasmonate (MeJA) on essential oil yield of cumin. Means with different letters are significantly different by Duncan's test ($p \leq 0.05$).

Moreover, our results showed the γ-Terpinene-7-al, Cumin aldehyde, α-Terpinene-7-al, ρ-Cymene and β-pinene as main components in cumin EO. Similarly, the compounds such as p-mentha-1,4-dien-7-al, cumin aldehyde, γ-Terpinene, β-pinene and ρ-Cymene by Iacobellis *et al* (2005) and cumin aldehyde, α-Terpinene-7-al, γ-Terpinene, γ-Terpinene-7-al, ρ-Cymene and β-pinene by Pajohi Alamoti *et al* (2012) reported in cumin EO as major components. These reports are nearly similar to our findings but some minor differences in quality or quantity of components compared with previous studies could be related to differences in genotype, environmental agronomic conditions, time of harvesting (Imelouane *et al*., 2009), different parts of plant, storage condition (Hudaib *et al.*, 2002), age of the plant, method of drying and method of extraction of the EO (Jerković *et al.*, 2001).

The compounds such as Cumin aldehyde, menthone derivatives, γ-Terpinene and ρ-Cymene are responsible for the odor and biological effects in EO of cumin (Lis-Balchin *et al.*, 1998 in Pajohi Alamoti *et al.*, 2012). In this case, Cumin aldehyde known as the most important effective on the biological activity especially for the antimicrobial activity (Farag *et al.*, 1989; Helander, 1998; Derakhshan *et al.*, 2010). So, the main compounds are as one of the quality parameters in the EO of cumin. In this study, it seems that Cumin aldehyde and Terpinene derivatives such as α-Terpinene-7-al, γ-Terpinene-7-al and γ-Terpinene play a greater role in EO quality of cumin. Among different treatments, the use of SA at the concentration of 1 mM increased all major components, except for the γ-Terpinene-7-al; it also resulted in the highest amount of Cumin aldehyde and the lowest amount of γ-Terpinene-7-al. Other concentrations of SA and the highest rate of MeJA (1 mM) although increased β-pinene, ρ-Cymene and γ-Terpinene but with reduce in content of Cumin aldehyde, α-Terpinene-7-al and γ-Terpinene-7-al resulted in reduction of the total amount of major compounds. Among of major compounds, α-Terpinene-7-al was more affected by the treatments and it considerably was reduced by both SA and MeJa at 1 mM concentration compared with lower concentrations and control. So far, no study on the effect of SA and MeJA on EO composition in cumin could be traced from the literature available; but some studies suggested that SA or MeJA have got an effect on the quality and quantity of EO composition and terpene**s** in plants studied (Gharib, 2006; Li *et al.*, 2007; Zhao *et al.*, 2010; Zheljazkov *et al.*, 2013; Rahim Malek *et al.*, 2012; Raouf Fard *et al.*, 2012; Rowshan and Bahmanzadegan, 2013).

The exact mechanism of the effect of SA and MeJA on secondary metabolites synthesis is not completely understood for us. However, according to previous studies it seems that MeJA influence gene regulation and enzymes activity in metabolic pathway involved in synthesis of secondary metabolites (Rodriguez-Saona *et al.*, 2001; Kim *et al.*, 2006; Li *et al.*, 2007). Rodriguez-Saona *et al* (2001) reported the increase of some terpenes in cotton plants treated with MeJA. They also stated that MeJA activates multiple biosynthetic pathways related to the synthesis of cotton volatiles and it can directly and systemically induce the emission of volatiles. Li *et al* (2007) revealed many of transcripts displaying high similarities to the known enzymes and peptide linked to the formation of secondary metabolites in sweet basil that it was affected by MeJA. They also have not identified genes directly involved in the pathway for terpenoid production, especially geranyl diphosphate synthase (GPPS) that it is directly associated with the formation of linalool. They also described the PAL (phenylalanine ammonia-lysase) enhancement for the likely reason in the increase of eugenol. Moreover, biosynthesis of Secondary metabolite in plant is done for adaptation to stresses and normalizes the plant physiological activities (Omidbaigi, 2005). SA, jasmonic acid (JA), and ethylene-dependent signaling pathways regulates plant responses against to both abiotic and biotic stresses (Gharib, 2006). Thus the relationship between salicylic acid and jasmonic acid in regulation plant responses to biotic and abiotic stresses (Rao *et al.*, 2000) might describe the role of these plant growth regulators in secondary metabolites synthesis and changing in EO composition.

Conclusion

Our study demonstrated that SA especially at concentration of 0.1 mM had a greater promoting effect on growth, yield and EO yield in cumin. Thus, there are a good potential for used in SA as a tool for enhancement of EO content or EO yield in the cultivation of cumin. Moreover, we suggest more research regarding the time and number of spraying for both SA and MeJA and higher concentrations of MeJA to verify the effects of this plant growth regulator on improvement the quantity and quality of crop plants.

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