Saudi Journal of Biological Sciences (2014) 21, 355–365

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Changes in growth, hormones levels and essential oil content of *Ammi visnaga* L. plants treated with some bioregulators

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Received 1 August 2013; revised 28 October 2013; accepted 31 October 2013
Available online 19 November 2013

KEYWORDS

Ammi visnaga;
Phenolic compounds;
Amino acids;
Hormones;
Growth criteria;
Essential oil

Abstract The effects of foliar application of different concentrations of amino acids (tyrosine and phenylalanine) and phenolic acids (trans-cinnamic acid, benzoic acid and salicylic acid) on growth, pigment content, hormones levels and essential oil content of *Ammi visnaga* L were carried out during two successive seasons. It is clear that foliar application of either amino acids or phenolics significantly promoted the growth parameters in terms of shoot height, fresh and dry biomass, number of branches and number of umbels per plant. The increment of growth parameter was associated with elevated levels of growth promoters (IAA, GA3, total cytokinins) and low level of ABA. The greatest increase in the previously mentioned parameters was measured in plants exposed to different concentrations of phenols particularly in benzoic acid-treated plants. Such effect was concentration dependent. All treatments led to significant increments in yield seeds and oil content. Moreover, gas liquid chromatographic analysis revealed that the main identified components of essential oil were 2,2-dimethyl butanoic acid, isobutyl isobutyrate, α-isophorone, thymol, fenchyl acetate and linalool. Phenolics and amino acid treatments resulted in qualitative differences in these components of essential oil.

1. Introduction

Ammi visnaga, known as Khella, is an annual or perennial herb that belongs to the family *Apiaceae* (*Umbelliferae*). Khella is native to the Mediterranean and is cultivated in Egypt. *A. visnaga* is antiasthmatic; diuretic; lithontriptic and vasodilator. It is an effective muscle relaxant and has been used for centuries to alleviate the excruciating pain of kidney stones (Chevallier, 1996). The seeds are used as a folk medicine for diuretic and lithontriptic (Uphof, 1959). Visnaga seeds contain

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.sjbs.2013.10.008
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oil that includes the substance 'khellin', which is used in the treatment of asthma. They have antispasmodic action on the smaller bronchial muscles, dilate the bronchial, urinary and blood vessels without affecting blood pressure (Bown, 1995). Essential oil of *A. visnaga* is known for its proprieties against coronary diseases and bronchial asthma (Rose and Hulburd, 1992; Satrani et al., 2004). The major components were linalool, isoamyl 2-methyl butyrate, and isopentyl isovalerate (Khadhri et al., 2011).

Furthermore, phenolics are low molecular compounds ubiquitous in all tissues of higher plants with great significance in plant development. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, and are of great significance in plant development (Curir et al., 1990). However, their biological, ecological and agronomical significance in the rhizosphere is much less clear. Furthermore these biomolecules may contribute to soil and water conservation, weed management, mineral element nutrition, as well as they impact as signal molecule in certain symbiotic relationships, and act as defense molecules against soil pests and pathogens (Makoi and Ndakidemi, 2007). Additionally, they serve as flower pigments, act as constitutive protection agents against biotic and abiotic stress (Deladonde et al., 1996), function as signal molecules, act as allelopathic compounds, and affect cell and plant growth (Dakora 1995; Dakora and Phillips, 1996; Ndakidemi and Dakora, 2003), are important natural animal toxicants (Adams, 1989) and some may function as pesticides (Vidhyasekaran, 1988; Waterman and Mole, 1989; Beier, 1990). They are also functional components of the rhizosphere and its soil organic matter (Haider et al., 1975; Martin-Tanguy, 1997). They have long been recognized as allelochemicals for weed control (Rice, 1984, Putnam and Tang, 1986) phytoestrogens in animals (Adams, 1989) and plant defense molecules (Vidhyasekaran, 1988). In the rhizosphere, they act as important precursors for the synthesis of soil humic substances (Haider et al., 1975). Salicylic acid participates in the regulation of several physiological processes in plant such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, inhibition of ethylene biosynthesis, transpiration and photosynthesis (Khan et al., 2003; Shakirova et al., 2003). SA increases cell metabolic rate (Amin et al., 2007). The biosynthesis of salicylic acid in plants starts from phenylalanine and follows one of two known paths of synthesis which involves trans-cinnamic acid then hydroxylation of benzoic acid which is a direct precursor of salicylic acid (Raskin, 1992).

Moreover, amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins (Davies, 1982). Amino acids are particularly important for cell growth stimulation. They act as buffers which help to maintain favorable pH value within the plant cell. They protect the plants from ammonia toxicity. They can serve as a source of carbon and energy, as well as protect the plants against pathogens. Amino acids also function in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidines, alkaloids, vitamins, enzymes, terpenoids and others (Goss, 1973; Abd EL-Aziz and Balbaa, 2007). Furthermore, Hass (1975) stated that the biosyntheses of cinnamic acids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine.

A. visnaga plants are used for medicinal or culinary purposes (natural medicide) as well as it can increase the production and the chemical composition of *A. visnaga* plants by using different methods such as adding some fertilizers and some natural chemical (phenolic compounds and amino acids). So the aim of this study is to investigate the role of some phenolic substances (salicylic acid, t-cinnamic acid and benzoic acid) and amino acids (tyrosine and phenylalanine) in the growth, endogenous hormones, photosynthetic pigments, total, soluble and insoluble carbohydrates of *A. visnaga* plants as well as the essential oil content of the seeds.

2. Materials and methods

2.1. Experimental

Two pot experiments were conducted in the greenhouse of National Research Centre (NRC), Dokki, Cairo, Egypt, during two successive seasons of 2009/2010 and 2010/2011. *A. visnaga* seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Ten sterilized seeds were sown in each pot in the third week of October. Each pot was filled with 10 kg of air-dried clay soil. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) and are presented in Table 1. Eight weeks after sowing, the seedlings were thinned and three plants per pot were left. Pots (30 cm diameter) were divided into three main groups. The first group was exposed to different levels of phenolic compounds (salicylic acid, trans-cinnamic acid and benzoic acid) at concentrations 5, 10 and 20 mg L^{-1}. The second group was sprayed with different levels of amino acids (phenylalanine and tyrosine) at concentrations 50, 100 and 200 mg L^{-1}. Phenolic compounds and amino acids were applied after 30 days from the sowing date. The third group was sprayed with HO to serve as control. The experiments were conducted under natural day condition, with photoperiod 11 h ± 2 and temperature about 27 °C ± 2. All agricultural practices were conducted according to the recommendations by the Egyptian Ministry of Agriculture as follows: fertilizers were added to all pots as follows: cattle manure (2 gpot^{-1}), phosphorus (2 gpot^{-1}) as calcium super phosphate (15.5% P^2O^5), nitrogen (2.0 gpot^{-1}) as ammonium sulfate (20.5% N) and potassium (1.5 gpot^{-1}) as potassium sulfate (48% K2O). Meteorological data at Giza, Egypt during the two growing seasons are presented in Table 2.

The growth parameters of differently treated *Ammi* plants were measured after 75, 119, 180 and 210 days from sowing (stages A, B, C and D respectively). Stage A was at the vegetative growth while stage B at the beginning of flowering and stages C and D were at early fruiting and harvest time.

2.2. Vegetative growth characters

Plant height (cm), fresh and dry weights of shoot (g plant^{-1}) were recorded during the vegetative stage. Plant height (cm), number of branches and umbels (plant^{-1}), fresh and dry weights of shoots (plant^{-1}) were recorded at flowering, early fruiting and fruiting stages.

2.3. Endogenous hormones

The endogenous hormone levels were determined using the method described by Wasfy and Orrin (1975).

Table 1 Physical and chemical properties of the soil used.

Soil texture	pH	EC*	Organic C (%)	Organic matter (%)	Total N (%)	(%)Total P	Total K (%)
Sandy loam	7.2	0.6	0.9	1.9	0.3	0.1	0.1

EC*, (salinity) electric conductivity.

Table 2 Meteorological data at Giza, Egypt during the two growing seasons.

Months	1st season (2007/2008)					2nd season (2008/2009)				
	T(°C)		Rs ($MJm^{-2}d^{-1}$)	RH (%)	ETp (mmd^{-1})	T(°C)		Rs ($MJm^{-2}d^{-1}$)	RH (%)	ETp (mmd^{-1})
	Max.	Min.				Max.	Min.			
November	22.6	10.5	13.0	48.2	23.1	22.8	12.0	12.2	38.9	24.2
December	19.4	11.6	14.4	48.9	27.4	21.3.4	11.5	13.5	40.2	26.8
January	18.0	10.9	24.9	48.3	28.3	19.6	10.2	22.6	49.3	32.5
February	23.8	12.6	25.9	55.6	29.6	22.1	16.2	26.9	50.2	33.5
March	27.5	14.3	26.7	70.6	30.4	28.7	17.6	27.2	52.4	34.0
April	28.9	14.4	27.5	80.4	36.5	29.1	18.1	30.5	56.2	37.9
May	31.2	16.3	31.6	88.9	42.5	30.4	20.1	32.5	74.2	44.2
June	35.4	20.5	35.8	90.3	49.6	33.8	21.3	33.8	84.2	51.3

Monthly average. T, temperature; Rs, solar radiation; RH, relative humidity; ETp, potential evapotranspiration.

2.4. Photosynthetic pigments

Chlorophyll (Chl) *a*, Chl *b* and total carotenoid content was measured according to the method of Association of Official Agricultural Chemists (AOAC, 1970).

2.5. Total and soluble carbohydrate

Total and soluble carbohydrate contents were determined according to the method described by Dubois et al. (1956). Then, the insoluble carbohydrates were calculated.

2.6. Essential oil isolation

The ripening fruits of *A. visnaga* were collected air dried and weighted for extraction of the essential oil. Five grams of dry fruits was crushed into smaller pieces and reduced to fine powder with the aid of a mechanical grinder. The powder sample was extracted with petroleum ether (PE 40–60 °C) for 48 h at room temperature. The extract was evaporated to dryness using a rotary evaporation at reduced pressure. The essential oil was passed over dark anhydrous sodium sulfate to remove moisture. The fraction obtained was stored in a refrigerator at 4 °C in the dark to identify the chemical constituents of oil (Adams, 2007). GC–MS analysis was carried out on a Varina 3400 system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d.); Oven temperature was 40–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, injector temperature 250 °C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/min, ionization energy 70 eV; scan time 1 s; mass range 40–350 amu.

2.7. Identification of components

The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices with those of authentic compounds. Kovats, indices (Kováts, 1958) were determined by co-injection of the sample with a solution containing a homologous series of *n*-hydrocarbons, at a temperature run identical to that described above.

2.8. Statistical analysis

In this experiment, one factor was considered: different concentrations of amino acids (50, 100 and 200 mg L^{-1}), phenolic compound treatments (5, 10 and 20 mg L^{-1}) and control. The experimental design followed a complete random block design. According to Snedecor and Cochran (1980) , the average of data was statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant values were determined according the Least Significant Difference (LSD at 0.05 and at 0.01 p) by using the STAT-ITCF program (1982).

3. Results and discussion

3.1. Effect of amino acids and phenolic compounds on growth parameters

Foliar application of different concentrations of either phenols or amino acids stimulates a gradual increase in growth parameters in terms of plant height, number of branches, number of umbels fresh and dry weights and water content of *A. visnaga* shoots throughout the experimental periods. Results also, investigated that phenols stimulate all the previous morphological parameters particularly at 20 mg L^{-1} compared with those of amino acids (tyrosine and phenylalanine) throughout the experimental period (Tables 3–8). The greatest increase in all investigated morphological criteria was measured in *A. visnaga* plants exposed to 20 mg L^{-1} benzoic acid at all stages. Similar results were obtained by Balbaa and Talaat (2007)

Table 3 Changes in the values of plant height of shoot system of *Ammi visnaga* L. plants (cm plant^{-1}) treated with different concentrations of amino acids and phenolic compounds during the vegetative (I), flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Plant height (cm)			
		I	II	III	IV
Control	0	15.8 ± 0.2	51.0 ± 1.0	66.1 ± 0.3	80.0 ± 1.0
Tyrosine	50	18.7 ± 2.1	57.7 ± 2.1	67.0 ± 0.5	82.0 ± 1.0
	100	20.0 ± 2.0	59.3 ± 1.5	69.3 ± 2.1	90.7 ± 1.5
	200	24.7 ± 1.5	65.7 ± 1.5	84.0 ± 1.0	98.0 ± 1.7
Phenylalanine	50	20.0 ± 1.0	60.3 ± 1.5	69.0 ± 1.0	88.7 ± 1.5
	100	23.0 ± 0.5	62.0 ± 2.0	76.7 ± 1.2	92.3 ± 2.5
	200	25.0 ± 1.0	68.7 ± 1.5	89.0 ± 1.0	98.7 ± 1.5
Benzoic acid	5	27.1 ± 2.6	75.3 ± 1.5	80.0 ± 1.0	103.3 ± 1.5
	10	29.7 ± 2.1	80.0 ± 1.0	93.3 ± 1.2	112.7 ± 2.1
	20	33.8 ± 4.1	91.7 ± 1.5	104.3 ± 1.5	117.7 ± 1.5
Tarns-cinnamic acid	5	22.5 ± 2.8	71.3 ± 1.5	79.3 ± 2.1	100.1 ± 1.0
	10	23.2 ± 1.9	71.7 ± 1.5	87.3 ± 2.1	108.3 ± 1.5
	20	26.6 ± 0.7	80.3 ± 2.5	96.7 ± 1.5	113.7 ± 1.5
Salicylic acid	5	20.4 ± 0.9	70.0 ± 2.0	74.3 ± 0.6	89.7 ± 1.5
	10	21.5 ± 3.5	71.3 ± 2.1	81.3 ± 1.5	94.7 ± 2.5
	20	25.2 ± 1.4	74.3 ± 2.1	85.3 ± 0.6	99.0 ± 1.0
LSD at					
0.05		2.8	2.3	1.8	2.1
0.01		3.9	3.3	2.5	3.0

Table 4 Changes in the values of branch number of shoot system of *Ammi visnaga* L. plants (cm plant^{-1}) treated with different concentrations of amino acids and phenolic compounds during the flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Branch number		
		II	III	IV
Control	0	1.0 ± 0.0	3.3 ± 0.3	4.0 ± 0.5
Tyrosine	50	1.0 ± 0.0	3.3 ± 0.6	5.0 ± 0.0
	100	1.0 ± 0.0	3.7 ± 0.3	5.3 ± 0.6
	200	1.3 ± 0.6	4.7 ± 0.3	5.7 ± 1.2
Phenylalanine	50	1.0 ± 0.0	4.0 ± 0.0	5.5 ± 0.3
	100	1.7 ± 0.6	4.3 ± 0.6	6.0 ± 0.5
	200	1.7 ± 0.3	4.7 ± 0.6	7.0 ± 0.5
Benzoic acid	5	1.0 ± 0.0	5.0 ± 0.0	7.7 ± 0.3
	10	2.0 ± 0.0	5.3 ± 0.3	7.7 ± 0.6
	20	3.0 ± 0.0	6.3 ± 0.3	10.0 ± 0.0
Tarns-cinnamic acid	5	1.0 ± 0.0	4.3 ± 0.3	6.0 ± 0.5
	10	1.0 ± 0.0	5.3 ± 0.3	7.0 ± 0.5
	20	2.3 ± 0.3	5.7 ± 0.3	7.7 ± 0.6
Salicylic acid	5	1.0 ± 0.0	3.7 ± 0.6	6.0 ± 0.5
	10	1.7 ± 0.3	5.0 ± 0.0	6.3 ± 0.6
	20	1.7 ± 0.6	5.3 ± 0.3	7.7 ± 0.6
LSD at				
0.05		0.4	0.5	0.7
0.01		0.5	0.7	1.1

Table 5 Changes in the values of umbel number of shoot system of *Ammi visnaga* L. plants treated with different concentrations of amino acids and phenolic compounds during the flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Umbel number		
		II	III	IV
Control	0	3.0 ± 0.5	16.0 ± 2.0	18.0 ± 0.5
Tyrosine	50	4.3 ± 0.1	19.0 ± 1.0	21.3 ± 2.3
	100	5.5 ± 0.1	20.0 ± 0.0	24.0 ± 2.0
	200	7.5 ± 0.2	22.7 ± 4.6	27.0 ± 0.2
Phenylalanine	50	5.5 ± 0.3	19.8 ± 0.9	25.2 ± 1.8
	100	7.3 ± 0.2	21.6 ± 0.9	25.3 ± 2.3
	200	8.0 ± 0.3	25.2 ± 0.9	28.8 ± 0.8
Benzoic acid	5	9.0 ± 0.2	26.4 ± 0.5	29.5 ± 0.5
	10	10.7 ± 0.2	28.0 ± 2.0	30.7 ± 2.3
	20	13.3 ± 0.4	30.7 ± 2.3	40.0 ± 0.0
Tarns-cinnamic acid	5	6.3 ± 0.2	23.4 ± 0.7	24.0 ± 2.0
	10	7.0 ± 0.1	27.0 ± 1.0	30.7 ± 2.3
	20	11.7 ± 1.5	28.8 ± 0.4	31.3 ± 1.5
Salicylic acid	5	6.3 ± 0.2	21.3 ± 2.3	24.0 ± 2.0
	10	7.0 ± 0.2	23.4 ± 1.8	25.2 ± 1.8
	20	10.5 ± 0.2	27.0 ± 0.4	30.6 ± 0.7
LSD at				
0.05		0.6	2.3	2.2
0.01		0.8	3.3	3.1

who concluded that phenylalanine treatments significantly promoted plant height, number of branches, fresh and dry weights of rosemary plants. Abd El-Aziz et al. (2007) indicated also that foliar application of tyrosine significantly promoted plant height, number of leaves and branches, fresh and dry weights of branches and shoots and stem diameter in both cuttings of *Salvia farinacea* Plants. It was recorded that application of certain amino acids significantly increased the vegetative growth of Chrysanthemum (El-Fawakhry and El-Tayeb, 2003), peppermint plant (Refaat and Naguib 1998), datura plant (Youssef et al., 2004a,b) and *Pelargonium graveolens* (Mahgoub and Talaat 2005). Furthermore, salicylic acid caused significant increases in most growth parameters of different plant species (Abd El-Wahed et al., 2006; El-Khallal

Table 6 Changes in the values of fresh weight of shoot system of *Ammi visnaga* L. plants (g plant^{-1}) treated with different concentrations of amino acids and phenolic compounds during the vegetative (I), flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Fresh weight (g plant^{-1})			
		I	II	III	IV
Control	0	2.0 ± 0.4	15.0 ± 1.0	22.4 ± 0.6	27.8 ± 1.9
Tyrosine	50	2.4 ± 0.1	18.7 ± 1.7	28.4 ± 1.1	32.0 ± 1.8
	100	3.6 ± 0.7	23.5 ± 0.9	36.4 ± 1.9	45.4 ± 2.4
	200	5.1 ± 0.4	26.2 ± 1.3	43.8 ± 0.6	54.2 ± 1.0
Phenylalanine	50	2.5 ± 0.2	21.3 ± 2.2	31.3 ± 3.1	39.2 ± 3.4
	100	4.0 ± 1.6	26.6 ± 1.5	41.2 ± 3.3	48.0 ± 4.5
	200	7.1 ± 1.5	29.5 ± 0.9	46.1 ± 1.5	55.7 ± 2.7
Benzoic acid	5	8.1 ± 0.2	33.5 ± 2.0	49.0 ± 1.0	58.9 ± 7.0
	10	9.1 ± 1.4	34.9 ± 2.5	53.0 ± 1.2	63.3 ± 0.6
	20	10.0 ± 1.5	41.6 ± 1.0	60.6 ± 4.5	69.7 ± 4.4
Tarns-cinnamic acid	5	3.4 ± 1.2	25.8 ± 3.9	38.2 ± 3.8	46.0 ± 2.4
	10	4.5 ± 0.8	30.4 ± 4.9	44.3 ± 1.0	52.5 ± 2.0
	20	6.7 ± 0.4	39.3 ± 2.5	56.2 ± 3.8	65.2 ± 2.3
Salicylic acid	5	3.0 ± 0.4	24.2 ± 3.4	37.0 ± 3.8	43.0 ± 1.0
	10	4.5 ± 1.4	28.5 ± 1.6	43.9 ± 3.2	52.7 ± 2.5
	20	5.1 ± 0.9	31.5 ± 3.3	50.1 ± 0.4	61.7 ± 2.6
LSD at					
0.05		1.3	3.3	3.5	4.1
0.01		1.8	4.6	4.9	5.8

Table 7 Changes in the values of dry weight of shoot system of *Ammi visnaga* L. plants (g plant^{-1}) treated with different concentrations of amino acids and phenolic compounds during the vegetative (I), flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Dry weight (g plant^{-1})			
		I	II	III	IV
Control	0	0.3 ± 0.02	8.1 ± 0.02	15.6 ± 0.1	25.3 ± 0.2
Tyrosine	50	0.3 ± 0.02	9.6 ± 0.4	19.7 ± 0.1	28.8 ± 0.2
	100	0.4 ± 0.02	11.7 ± 0.03	25.1 ± 0.1	40.1 ± 0.04
	200	0.6 ± 0.02	12.4 ± 0.02	29.3 ± 0.1	47.6 ± 0.2
Phenylalanine	50	0.3 ± 0.03	10.2 ± 0.2	21.7 ± 0.2	35.1 ± 0.1
	100	0.5 ± 0.02	12.8 ± 0.1	28.1 ± 0.04	41.7 ± 0.7
	200	0.6 ± 0.02	13.8 ± 0.2	30.2 ± 0.1	45.5 ± 0.3
Benzoic acid	5	0.6 ± 0.02	15.3 ± 1.0	31.7 ± 0.8	47.2 ± 0.5
	10	0.7 ± 0.01	15.5 ± 0.3	33.0 ± 0.3	48.5 ± 0.2
	20	0.8 ± 0.02	16.6 ± 0.1	37.6 ± 0.2	52.3 ± 0.1
Tarns-cinnamic acid	5	0.3 ± 0.02	12.5 ± 0.1	25.1 ± 0.4	39.9 ± 0.1
	10	0.4 ± 0.02	13.6 ± 0.1	28.2 ± 0.2	43.6 ± 0.2
	20	0.6 ± 0.03	16.4 ± 0.03	35.0 ± 0.5	50.2 ± 0.2
Salicylic acid	5	0.3 ± 0.02	11.4 ± 0.3	25.6 ± 0.2	37.3 ± 0.2
	10	0.5 ± 0.02	13.2 ± 0.3	28.1 ± 0.1	44.5 ± 0.1
	20	0.5 ± 0.02	14.5 ± 0.1	31.4 ± 0.4	48.0 ± 0.2
LSD at					
0.05		0.0	0.3	0.4	0.4
0.01		0.0	0.4	0.6	0.5

et al., 2009; Delavari et al., 2010 and Dawood et al. 2012). The promotive effect of salicylic acid could be attributed to its bioregulator effects on physiological and biochemical processes in plants such as ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as well as increase the antioxidant capacity of plants (Raskin, 1992; Blokhina et al., 2003 and El-Tayeb, 2005).

3.2. Effect of amino acids and phenolic compounds on chemical composition

The changes of chlorophylls a and b as well as carotenoid content in response to amino acids and phenolics treatments are shown in Table 9 High pigment levels (chl a, b, carotenoids) were measured in *A. visnaga* leaves treated with phenols compared with those of amino acids. The maximum increase in

Table 8 Changes in the percentage of water content of *Ammi visnaga* L. shoots with different concentrations of amino acids and phenolic compounds during the vegetative (I), flowering (II), early fruiting (III) and fruiting (IV) stages, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Water content (%)			
		I	II	III	IV
Control	0	85.5 ± 3.6	45.6 ± 3.5	30.4 ± 1.7	8.7 ± 6.5
Tyrosine	50	87.5 ± 0.6	48.5 ± 2.5	30.7 ± 3.0	9.8 ± 5.3
	100	87.7 ± 2.5	50.1 ± 2.0	30.8 ± 3.5	11.4 ± 4.5
	200	88.6 ± 0.8	52.5 ± 2.2	33.1 ± 0.8	12.2 ± 1.4
Phenylalanine	50	85.9 ± 6.0	51.8 ± 4.0	30.5 ± 6.1	10.1 ± 7.3
	100	87.3 ± 4.2	52.0 ± 2.9	31.5 ± 5.2	12.7 ± 6.5
	200	91.6 ± 2.3	53.2 ± 0.8	34.4 ± 2.4	18.3 ± 4.0
Benzoic acid	5	92.0 ± 0.4	54.2 ± 2.4	35.3 ± 2.6	19.1 ± 9.6
	10	92.1 ± 1.4	55.4 ± 2.8	37.5 ± 4.9	22.9 ± 2.6
	20	92.4 ± 1.0	60.2 ± 0.9	37.8 ± 4.7	24.8 ± 4.5
Tarns- cinnamic acid	5	89.3 ± 3.5	50.8 ± 8.0	33.8 ± 7.0	13.2 ± 4.7
	10	90.7 ± 1.6	54.7 ± 6.7	36.3 ± 1.8	16.7 ± 3.0
	20	91.5 ± 0.9	58.1 ± 2.7	37.7 ± 2.0	23.4 ± 0.6
Salicylic acid	5	89.1 ± 1.0	52.3 ± 6.9	30.4 ± 6.9	13.3 ± 1.6
	10	89.2 ± 3.7	53.6 ± 3.7	35.9 ± 4.6	15.4 ± 4.0
	20	90.3 ± 1.4	53.6 ± 4.6	37.4 ± 0.7	22.2 ± 3.1
LSD at					
0.05		3.1	5.5	N.S	6.5
0.01		4.3	7.7	N.S	9.3

Table 9 Changes in the values of photosynthetic pigments of *Ammi visnaga* L. plants (mg L^{-1}) treated with different concentrations of amino acids and phenolic compounds during the vegetative stage, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Chl *a*	Chl *b*	Carotinoids	Chl *a* + Chl *b*	Chl *a*/Chl *b*	Chl *a* + Chl *b*/carotinoids
Control	0	2.3 ± 0.1	1.4 ± 0.4	1.5 ± 0.2	3.7 ± 0.5	1.7 ± 0.5	2.5 ± 0.6
Tyrosine	50	2.6 ± 0.1	1.5 ± 0.2	1.8 ± 0.2	4.2 ± 0.3	1.7 ± 0.1	2.4 ± 0.4
	100	4.0 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	5.9 ± 0.2	2.2 ± 0.0	2.8 ± 0.2
	200	4.9 ± 0.1	2.0 ± 0.2	2.6 ± 0.1	6.9 ± 0.2	2.4 ± 0.1	2.7 ± 0.2
Phenylalanine	50	2.9 ± 0.1	1.6 ± 0.2	1.8 ± 0.0	4.5 ± 0.3	1.8 ± 0.2	2.5 ± 0.2
	100	4.3 ± 0.1	1.8 ± 0.2	2.6 ± 0.1	6.1 ± 0.2	2.4 ± 0.2	2.4 ± 0.1
	200	5.6 ± 0.1	2.4 ± 0.2	2.9 ± 0.1	7.9 ± 0.2	2.4 ± 0.2	2.8 ± 0.2
Benzoic acid	5	5.9 ± 0.3	2.4 ± 0.3	3.7 ± 0.0	8.2 ± 0.1	2.5 ± 0.5	2.2 ± 0.0
	10	9.4 ± 1.0	3.2 ± 0.8	3.9 ± 0.1	12.6 ± 0.3	2.8 ± 0.9	2.8 ± 0.2
	20	9.96 ± 1.2	3.5 ± 0.7	4.5 ± 0.1	13.4 ± 0.7	3.0 ± 1.0	3.0 ± 0.2
Tarns-cinnamic acid	5	3.6 ± 0.1	1.7 ± 0.2	2.1 ± 0.1	5.3 ± 0.2	2.1 ± 0.2	2.5 ± 0.2
	10	6.1 ± 0.1	2.5 ± 0.3	3.1 ± 0.0	8.6 ± 0.2	2.5 ± 0.3	2.8 ± 0.0
	20	8.5 ± 0.1	3.1 ± 0.2	3.8 ± 0.0	11.6 ± 0.3	2.8 ± 0.1	3.1 ± 0.1
Salicylic acid	5	3.8 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	5.6 ± 0.1	2.1 ± 0.2	2.7 ± 0.1
	10	4.8 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	6.7 ± 0.2	2.4 ± 0.1	2.7 ± 0.2
	20	6.7 ± 0.1	2.7 ± 0.1	2.9 ± 0.0	9.4 ± 0.1	2.5 ± 0.2	3.2 ± 0.0
LSD at							
0.05		0.55	0.44	0.13	0.38	0.61	0.31
0.01		0.78	0.62	0.18	0.54	0.86	0.43

chlorophylls and carotenoids is recorded in leaves treated with 20 mg L^{-1} benzoic acid. The increase in pigment level was attributed to the promotion in its synthesis and/or retardation of pigment degradation. These results are similar to those obtained by Sharma et al. (1995) who found that excised leaves of *A. majus*, treated with t-cinnamic acid, retained more chlorophyll (60% higher at 10^{-3} M) compared to control. Moreover, the potent effects of particularly salicylic acid might be ascribed firstly to the reduction in chlorophyll loss due to its ability to increase the antioxidant capacity of the plants (Kuorzer et al., 1999) or inducing the synthesis of stabilizing substances (Németh et al., 2008). Salicylic acid caused significant increases in photosynthetic pigments (Table 9). These results corroborate with those of Khodary (2004) on maize, El-Tayeb (2005) on barley, Gunes et al. (2005) on maize plant and Dawood et al. (2012) on sunflower.

The enhancing effects of SA on photosynthetic capacity could be attributed to its stimulatory effects on Rubisco activity and pigment contents (Khodary, 2004) as well as increased CO2 assimilation, photosynthetic rate and increased mineral uptake by the plant (Szepesi et al., 2005). In addition, Arfan et al. (2007), pointed that application of salicylic acid

Table 10 Changes in the percentage of total, soluble and insoluble carbohydrates of *Ammi visnaga* L. plants (%) treated with different concentrations of amino acids and phenolic compounds during the vegetative stage, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Total carbohydrates	Soluble sugar	Insoluble sugar
Control	0	724.9 ± 95.9	120.8 ± 16.0	604.1 ± 79.9
Tyrosine	50	891.3 ± 41.8	148.6 ± 7.0	742.8 ± 34.8
	100	1028.4 ± 66.3	171.4 ± 11.1	857.0 ± 55.3
	200	1091.7 ± 19.3	181.9 ± 3.2	909.8 ± 16.1
Phenylalanine	50	1059.3 ± 33.2	176.6 ± 5.5	882.8 ± 27.6
	100	1094.7 ± 123.9	182.4 ± 20.6	912.2 ± 103.2
	200	1152.1 ± 215.0	192.0 ± 35.8	960.1 ± 179.2
Benzoic acid	5	1339.2 ± 34.5	223.2 ± 5.8	1116.0 ± 28.8
	10	1446.8 ± 96.3	241.1 ± 16.0	1205.6 ± 80.2
	20	1572.0 ± 155.7	262.0 ± 25.9	1310.0 ± 129.7
Tarns- cinnamic acid	5	1177.2 ± 6.8	196.2 ± 1.1	981.0 ± 5.6
	10	1292.1 ± 75.2	215.3 ± 12.5	1076.7 ± 62.7
	20	1374.6 ± 64.2	229.1 ± 10.7	1145.5 ± 53.5
Salicylic acid	5	1156.5 ± 86.9	192.8 ± 14.5	963.8 ± 72.4
	10	1256.7 ± 83.4	209.5 ± 13.9	1047.3 ± 69.5
	20	1292.1 ± 10.2	215.3 ± 1.7	1076.7 ± 8.5
LSD at				
0.05		124.5	20.8	103.8
0.01		176.1	29.3	146.7

Table 11 Changes in the values of phytohormone contents of *Ammi visnaga* L. plants (μg g^{-1}) treated with different concentrations of amino acids and phenolic compounds during the vegetative stage.

Treatments (mg L^{-1})		GA3	IAA	ABA	Z	ZR	Cytokinins
Control	0	32.52	12.97	324.25	60.65	9.49	70.14
Tyrosine	50	78.90	13.41	44.93	62.01	11.36	73.37
	100	89.65	14.52	27.80	80.59	20.08	100.67
	200	134.45	15.76	19.59	149.43	27.10	176.53
Phenylalanine	50	84.68	14.78	42.52	70.18	17.87	88.05
	100	131.63	15.16	19.76	109.88	26.98	136.86
	200	135.68	17.50	10.77	165.85	34.38	200.23
Benzoic acid	5	554.31	30.67	5.62	333.74	50.33	384.07
	10	568.08	39.39	3.32	428.50	102.79	531.29
	20	657.61	46.92	2.57	603.04	104.15	707.19
Tarns- cinnamic acid	5	154.55	20.91	7.22	210.59	46.14	256.73
	10	190.68	23.99	5.15	227.79	59.42	287.21
	20	516.47	34.06	4.39	404.88	99.32	504.2
Salicylic acid	5	151.01	20.06	12.76	168.01	45.82	213.83
	10	167.69	21.26	9.70	291.90	55.98	347.88
	20	210.82	27.50	5.03	368.22	70.38	438.6

improved the photosynthetic capacity and retained pigment content through increasing IAA and Cytokinins therefore inhibiting their senescence. Similar results were obtained by Hassanein (2003) on *Foeniculum vulgare* L. plants and Abou Dahab and Abd El-Aziz (2006) on *Philodendron erubescens* plant. They reported that foliar application of amino acid (Tryptophan) caused an increase in photosynthetic pigment contents.

The increases of the photosynthetic pigments in the treated *Ammi* leaves were concomitant with a gradual increase in total, soluble and insoluble carbohydrates (Table 10. The maximum increases in soluble and insoluble carbohydrates were measured in the plants exposed to foliar application of phenolic compounds compared to those treated with amino acids. Moreover, such increments in the levels of total, soluble and insoluble carbohydrates were recorded in leaves exposed to 20 mg L^{-1} benzoic acid. These results are in agreement with those obtained by Goss (1973), who indicated that amino acids can serve as a source of carbon and energy when carbohydrates become deficient in the plant; amino acids are determinate, releasing the ammonia and organic acid from which the amino acid was originally formed. The organic acids then enter Kreb's cycle, to be broken down to release energy through respiration. These results could also, be explained by the findings obtained by Gamal El-Din et al. (1997) found also, that treatment of lemon grass plants with 100 ppm phenylalanine in the first cut and ornithine in the second cut recorded the highest level of carbohydrate percentage compared with control. Refaat and Naguib (1998) reported that application of all amino acids (alanine, cytosine, guanine, thiamine and L-tyrosine) increased the total carbohydrate percentage in peppermint leaves. The effect of the amino acids on the total

Table 12 Changes in the values of fruit yield, oil percentage and oil yield (ml plant^{-1}) of *Ammi visnaga* L. plants treated with different concentrations of amino acids and phenolic compounds, each value is mean of ten replicates ± SD.

Treatments (mg L^{-1})		Fruit yield	Oil%	Oil yield (ml plant^{-1})
Control	0	3.9 ± 0.1	1.0 ± 0.1	0.04 ± 0.01
Tyrosine	50	4.1 ± 0.1	1.02 ± 0.07	0.04 ± 0.01
	100	4.6 ± 0.1	1.11 ± 0.03	0.05 ± 0.01
	200	5.8 ± 0.2	1.43 ± 0.07	0.08 ± 0.01
Phenylalanine	50	4.4 ± 0.2	1.23 ± 0.07	0.08 ± 0.001
	100	5.9 ± 0.1	1.35 ± 0.08	0.12 ± 0.01
	200	6.9 ± 0.1	1.47 ± 0.03	0.14 ± 0.01
Benzoic acid	5	6.9 ± 0.1	1.34 ± 0.06	0.06 ± 0.01
	10	8.7 ± 0.1	1.36 ± 0.06	0.08 ± 0.01
	20	9.8 ± 0.2	1.45 ± 0.07	0.1 ± 0.02
Tarns- cinnamic acid	5	5.8 ± 0.1	1.2 ± 0.04	0.07 ± 0.01
	10	6.7 ± 0.2	1.31 ± 0.08	0.09 ± 0.01
	20	7.5 ± 0.1	1.4 ± 0.07	0.11 ± 0.01
Salicylic acid	5	4.8 ± 0.2	1.12 ± 0.04	0.05 ± 0.01
	10	5.0 ± 0.06	1.34 ± 0.05	0.07 ± 0.01
	20	6.9 ± 0.2	1.45 ± 0.05	0.1 ± 0.05
LSD at				
0.05		0.19	0.09	0.01
0.01		0.27	0.12	0.02

carbohydrate content may be due to their important role in the biosynthesis of chlorophyll molecules which in turn affected carbohydrate metabolism. In this respect, Talaat and Balbaa (2010) reported that chemical analysis of the leaves of sweet basil indicated that the contents of total soluble and total carbohydrates were significantly increased as a result of foliar application of trans-cinnamic acid. Tari et al. (2002) and Dawood et al. (2012) reported that salicylic acid application resulted in a significant increase in total soluble carbohydrate content in leaves of tomato and sunflower, thus maintaining the carbohydrate pool in the chloroplasts at a high level.

Plant hormones play an important role in development processes; some of them have an important role in most plant mechanisms. Data represented in Table 11 showed increments in gibberellins (GA3), indole acetic acid (IAA) and cytokinins (Z & ZR) in plants treated with amino acids and phenolic compounds. High concentrations of gibberellins (GA3), indole acetic acid (IAA) and cytokinins (Z & ZR) were measured in *Ammi* leaves treated with phenolic compounds compared with amino acids. The highest values of GA3, IAA and Cytokinins were recorded in plants exposed to 20 mg L^{-1} benzoic acid. A reduction in abscisic acid (ABA) level was concomitant with such increments in growth promoters estimated in plants exposed to either phenolic compounds or amino acids. The increases in the levels of endogenous growth promoters could be attributed to the increase in their biosynthesis and/or decrease in their degradation and conjugation. On the other hand, the reduction in ABA level could be due to the shift of the common precursor isopentenyl pyrophosphate to biosynthesis of cytokinins and/or gibberellins instead of ABA (Hopkins and Huner, 2004). These results are in accordance with those obtained by Shehata et al. (2000, 2001) and Zaghlool (2002). The increases in IAA and GA3 in shoot tissues of sunflower plant concurrently with the increase in growth rate due to the role of these endogenous hormones in stimulating cell division and/or the cell enlargement and subsequently growth (Taiz and Zeiger, 1998). It is well known that salicylic acid induces flowering, increases flower life, retard senescence and increases cell metabolic rate. In addition, salicylic acid may be a prerequisite for the synthesis of auxin and/or cytokinin. (Metwally et al., 2003 and Gharib, 2006). Furthermore, these increments in growth regulating substances might be a prerequisite for acceleration of growth resumption of sunflower plant. In addition, salicylic acid effects on abscisic acid (Senaratna et al., 2000), gibberellins (Traw and Bergelson, 2003) regulate many physiological processes and plant growth. Moreover, Dawood et al. (2012) reported that SA caused marked increments in IAA, GA3, zeatin and zeatin riboside, in the meantime decrease in ABA content comparing with untreated controls.

Table 12 indicated that the fruit yield, oil yield percentage and oil yield (ml plant^{-1}) increased in plants treated with phenolic compounds and amino acids. The maximum levels of oil yield percentage (ml plant^{-1}) were recorded in seeds exposed to 20 mg L^{-1} benzoic acid. The increment in oil% and protein% might be due to the increase in vegetative growth and nutrient uptake. Similar results were reported by Gharib (2006) and Çag et al. (2009). In addition, Noreen and Ashraf (2010) mentioned that high doses of salicylic acid caused marked increases in sunflower achene oil content as well as some key fatty acids and significant decrease in stearic acid.

Table 13 represents the compounds of essential oil obtained from *A. visnaga* as detected by GC–MS. The relative levels of various constituents of oil yield were increased, decreased or disappeared in *A. visnaga* fruits under plants treated with amino acids and phenolic compounds as compared with untreated control plants. 2,2-Dimethylbutanoic acid, isobutyl isobutyrate, linalool, thymol and croweacin are the major constituents of *A. visnaga* fruits. These results are similar to those obtained by Khalfallah et al. (2011) who found that the major components of essential oil in *A. visnaga* L. are 2, 2-dimethylbutanoic acid, isobutyl isobutyrate, croweacin, linalool and thymol. The effect of different treatments on essential oil and its constituents may be due to its effect on enzyme activity and metabolism of essential oil production (Burbott and Loomis, 1969).

Table 13 The constituents of essential oil of *Ammi visnaga* L. plants.

No.	Components (%)	KI	Treatments (ppm)															
			0	Tyrosine			Phenylalanine			Benzoic acid			Tarns-cinnamic acid			Salicylic acid		
				50	100	200	50	100	200	5	10	20	5	10	20	5	10	20
1	α-Thujene	931	–	2.5	1.3	1.0	1.2	1.9	–	1.1	–	3.9	2.2	0.4	0.9	1.5	1.2	3.9
2	Myrcene	991	–	2.0	0.4	8.0	3.6	3.6	–	1.2	–	3.7	1.9	0.4	1.6	2.1	1.4	4.9
3	Isobutyl isobutyrate	1004	22.9	20.6	35.3	15.9	18.9	18.6	24.1	14.8	24.3	9.9	11.4	24.4	22.6	6.4	16.5	15.6
4	Linalool	1029	5.7	2.9	0.6	1.3	3.3	1.3	–	0.8	–	4.5	2.1	0.3	1.1	1.1	2.5	2.6
5	2,2-Dimethylbutanoic acid	1108	28.9	35.4	55.4	30.4	20.6	38.8	50.5	35.0	25.9	21.1	27.4	36.5	34.6	59.0	34.4	38.2
6	α-Isophorone	1121	13.4	17.9	0.9	3.0	2.7	1.2	9.2	11.9	16.7	9.6	13.8	19.3	21.1	6.4	11.3	13.8
7	Fenchyl acetate	1220	6.3	3.8	0.3	2.5	7.8	5.0	–	1.0	–	4.8	7.0	0.2	3.2	3.7	4.7	3.5
8	Bornyl acetate	1289	–	1.7	0.4	7.8	2.6	5.1	–	0.8	–	4.3	5.3	0.5	2.3	0.9	0.8	2.0
9	Thymol	1290	13.2	8.5	1.8	13.1	9.3	2.8	–	2.1	15.2	7.0	8.0	0.8	1.7	6.7	3.7	5.7
10	Geranyl acetate	1381	–	–	0.3	1.4	4.9	2.6	9.1	11.5	–	5.2	3.8	11.2	2.7	0.9	6.9	4.5
11	Lavandulyl acetate	1439	–	–	0.2	0.7	7.6	3.0	–	1.4	–	3.7	2.7	0.7	1.1	2.2	0.9	–
12	Citronellyl propionate	1446	–	–	0.6	5.6	7.9	3.3	–	1.0	–	5.3	1.6	–	1.2	3.1	2.4	–
13	Croweacin	1460	9.6	4.7	1.5	6.7	8.1	11.0	7.1	10.4	15.0	5.9	7.2	2.8	3.3	6.0	8.7	5.3
14	α-Damascone	1689	–	–	0.4	1.5	2.1	1.0	–	3.2	2.9	5.7	2.7	2.4	0.9	–	2.2	–
15	(Z,E)-farnesal	1701	–	–	0.6	1.1	1.4	0.8	–	3.8	–	5.4	2.9	0.1	1.7	–	2.4	–
Total identified			100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Monoterpene compounds			100	100	99.4	98.9	98.6	99.2	100	96.2	100	94.6	97.1	99.9	98.3	100	97.6	100
Sesquiterpene compounds			–	–	0.6	1.1	1.4	0.8	–	3.8	–	5.4	2.9	0.1	1.7	–	2.4	–

SA has a role in controlling gene expression (He et al., 2005) that most of the genes regulated by SA are defense related genes and many of them participate in plant responses to biotic and abiotic stresses. Therefore SA may change secondary metabolites and its pathway by effects on plastid, chlorophyll level and tolerate condition stress. The SA like stress manipulated quality and quantity of essential oil of salvia macrosiphon. The yield of essential oil was increased. The useful component such as Linalool was increased. Seventeen components were identified in SA-treated plants (Rowshan et al., 2010).

4. Conclusion

It may be concluded that the foliar application of either amino acids or phenolics significantly promoted the growth parameters in terms of shoot height, fresh and dry biomass, number of branches and number of umbels per plant. All treatments led to significant increments in yield seeds and oil content. On the other hand the chemical composition (endogenous hormones, photosynthetic pigments, total and soluble carbohydrate) of *A. Visnaga* was affected by adding amino acids or phenolics. Moreover, Gas Liquid Chromatographic analysis revealed that the main identified components of essential oil were 2,2-dimethyl butanoic acid, isobutyl isobutyrate, α-isophorone, thymol, fenchyl acetate and linalool. Phenolics and amino acid treatments resulted in qualitative differences in these components of essential oil. Moreover, the greatest increase in the growth parameters and chemical constituents was obtained at 20 mg L^{-1} of benzoic acid.

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