J. Plant Production, Mansoura Univ., Vol. 5 (6): 1021 - 1038, 2014

PLANTING DATE, CINNAMIC ACID AND N FERTILIZER AFFECT GROWTH AND METABOLITES OF *Pimpinella anisum*

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ABSTRACT

Two field experiments were conducted, to determine the effect of planting anise plant on late date (25th Dec.) in addition to the control date (25th Oct.) on growth parameters, photosynthetic pigments, carbohydrates and nitrogen content of *Pimpinella anisum* L. treated with cinnamic acid (CA) and/ or urea. In general the obtained data showed that, treating anise plant with different concentrations of CA (1mM, 5mM and 10mM) decreased all the growth parameters, photosynthetic pigments, carbohydrate and nitrogen contents during the two dates of cultivation. On the other hand the two used levels of urea fertilizer (50% and 100%) especially 100%, increased all the growth parameters, photosynthetic pigments, carbohydrate and nitrogen contents during the two dates of cultivation. In general, the results obtained by CA and urea; either alone or together in case of control date of cultivation were much better than those obtained on the late date of cultivation.
Keywords: Planting date, cinnamic acid, urea, *Pimpinella anisum* L, growth.

INTRODUCTION

Anise, *Pimpinella anisum* L., a herbaceous annual native to the Mediterranean region and Egypt, is cultivated in Europe, the Middle East, Mexico, North Africa, India and Russia chiefly for its fruits, called aniseed, the flavour of which resembles that of licorice. Anise was well known to the ancient Egyptians and Romans. Anise is used for the treatment of a carminative, antiseptic, antispasmodic, expectorant, stimulant, and stomachic. In addition, it has been used to promote lactation in nursing mothers and as a medicine against bronchitis and indigestion Muller-Schwarze, Dietland (2006).

Cinnamic acid (3-Pheynl-2-propenoic acid) is a phenolic compound found naturally in many spices (cinnamon and cloves), cranberries, and prunes, and provides a natural protection against pathogenic organisms. At low concentration (30 mg/L) cinnamic acid has been reported to have a flowery or cinnamon type flavor (Chambel *et al.,* 1999; Anslow and Stradford, 2000; Eblen *et al.,* 2004). Cinnamic acid was shown to inhibit the uptake of phosphate and potassium ions by barley roots (Glass, 1974). Some studies point to cinnamic acid as being an uncoupler of oxidative phosphorylation (Van Sumere *et al.,* 1972), while others have shown that cinnamic acid is probably a weak uncoupler of oxidative phosphorylation but the effect could not totally account for the strong inhibitory effect observed (Tillberg, 1970). Glass and Dunlop (1974) demonstrated that cinnamic acid caused a rapid, strong depolarization of the membranes of barley roots, and they suggested that this depolarization would account for inhibition of uptake of inorganic ions. Cinnamic acid was found to inhibit seed germination and seedling growth of tomato (Yao, 2007). Singh *et al,* (2013) reported that at 0.5, 1.0 and 1.5 mM concentrations CA has shown inhibitory effects on shoot and root length, fresh and dry weight of cabbage seedlings. Also CA significantly decreased the photosynthetic pigments and protein content.

Urea is a low cost nitrogen fertilizer form. This is because of its high nitrogen composition and consequent low transport and storage costs. It converts to ammonium bicarbonate within about 48 hours after field application. Nitrogen in this form will tend to volatilize to the air as ammonia gas. This lost fertilizer investment risk can be minimized or eliminated by assuring that the urea gets into the soil and does not merely remain on the surface of the soil or crop foliage (James, 2010). Urea was reported to increase the length, fresh mass and dry mass of the shoots and roots of wheat plant (Gharakand *et al.;* 2012). Similar results were obtained on studying the effect of urea application as a common fertilizer on some vegetables; *Brassica compestris*, *Trigonella foenum graecum* and *Anethum graveolens*, as urea was found to increase the percentage of seed germination, root length, shoot length (root /shoot ratio) and seedling height (Ramteke and Shirgave, 2012). Younis *et al,* 2008 studied the effect of treating lettuce plants with increasing concentrations of urea fertilizer and found that urea led to significant increases in nitrate-, ammonia-, amide-, urea-, protein- and total-N contents, led to positive changes in the protein content of the treated lettuce plants.

Global climate change is resulting in increases in the daily, seasonal, and annual mean temperatures experienced by plants. Moreover, climate change will increase the intensity, frequency, and duration of abnormally low and high temperatures (Christensen *et al.;* 2007). Temperature limits plant growth and is also a major determining factor in the distribution of plants across different environments (Mittler 2006). Since photosynthesis has long been recognized as one of the most temperature-sensitive processes in plants, understanding the physiological processes that underlie the temperature response of photosynthesis and its acclimation is important to both agriculture and the environment (Yamori *et al.;* 2013). Haroun *et al.* (2012) conducted a field experiment to determine the effect of two planting dates; early (25th Oct.) and late (15th Dec.) in addition to the control planted at 20th Nov. on phonology, growth, yield (yield components and yield quality) of four bread wheat (*Triticum aestivum* L.). In general, the obtained data showed that, the requirement of days and Growing degree days (GDD) to attain different phonological stages (seedling, booting, heading, anthesis and maturity) decreased with delay in sowing date. Also, planting on the control date (20th Nov) surpassed the other sowing dates in all yield studied parameters and flour quality. However, late sowing date (15th Dec.) caused an increase in most technological properties (protein, wet and dry mass) of the yielded grains.

Accordingly the objective of this study was to evaluate the effect of the foliar application of different concentrations of cinnamic acid (1mM, 5mM and 10mM) and / or two different concentrations of urea fertilizer (50% and 100%) of the recommended dose during a different planting late date (25

Dec) and control one (25 Oct) on growth parameters, some metabolites of *Pimpinella anisum* L. plant.

MATERIALS AND METHODS

Plants used and growth conditions:

Pure strains of *Pimpinella anisum* L. (anise) were purchased from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Two pot experiments were conducted at the Faculty of Science, Mansoura University during two different seasons control date (October 2011) and late date (December 2012). The experiments were carried in clay: sandy soil (2:1) mixed with phosphate and nitrate fertilizers as common practice. The pots were kept in the greenhouse under a normal day/night conditions and irrigated as usual practice with equal amounts of tap water when required.

The experiments were carried out to study the effect of foliar application of different concentrations of cinnamic acid and urea fertilizer; either each alone or together on growth parameters, photosynthetic pigments, different carbohydrate fractions, total protein content and different nitrogen constituents. According to a preliminary experiment three different concentrations of cinnamic acid (1mM, 5mM and 10mM) and two levels of urea fertilizer were chosen [50% urea (0.7 g/pot) and 100% (1.4 g/pot)] according to the recommended dose for anise crop which is 200 Kg/fed; according to the announcement of Ministry of Agriculture of Egypt.

In the two experiments, 45 and 125 days old samples were collected as a vegetative and flowering stages referring to stage I and II respectively.

Analytical methods:

Growth parameters:

Length of root, length of shoot, fresh mass, dry mass and leaf area were estimated.

Estimation of photosynthetic pigments:

The plant photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were determined at different stages of plant growth using the spectrophotometric method as recommended by Arnon (1949) for chlorophylls and Horvath *et al.,* (1972) for carotenoids as adopted by Kissimon (1999).

Estimation of carbohydrates:

The method of extraction of different carbohydrate fractions (glucose, sucrose and total soluble sugars), used in this investigation was essentially those adopted by Yemm and Willis (1954) and Handel (1968). The method used for estimation of polysaccharides in the present study was that of Thayumanavan and Sadasivam (1984).

Estimation of nitrogenous constituents:

The method used in this investigation was essentially that adopted by Yemm and Willis (1956). The dried tissue samples were ground to a fine powder, then a known weight of this powder was extracted in distilled water by grinding the samples for 30 minutes, at room temperature, in a glass mortar. The mixture was then quantitively transferred to a boiling tube, brought quickly to water bath maintained at 80oC for 15 minutes. The insoluble residue was removed by filtration and the filtrate was made up to volume and used for estimation of ammonia-, amino-, amide- and total soluble-nitrogen fractions. Whereas, total nitrogen was determined directly using the dried powder tissue.

The full data of different treatments were statistically analyzed and comparison among means at P ≤ 0.05 was carried out by using CoHort/ CoStat statistical software version display ANOVA (798 lighthouse Ave. PMB 320, Monterey, CA, 93940, USA) (McCrae and Costa, 2004).

RESULTS AND DISCUSSION

Changes in growth parameters:

During the control date of cultivation and at vegetative stage, the shoot length of anise plants decreased in case of 1mM, 5mM and 10mM cinnamic acid, but it increased with 50% and 100% urea fertilizer. Also the interaction between cinnamic acid (5mM and 10mM) and urea (50% and 100%) caused decrease in shoot length of anise plants. The pattern of changes in root length, in response to the different treatments was more or less similar to that of shoot length (Table 1). Anise leaf area and fresh and dry masses in general, decreased with the three concentrations of CA. Whereas 50% and 100% urea fertilizer increased the leaf area, fresh and dry masses. Moreover urea interaction with the different concentrations of CA caused decrease in the leaf area of anise plants.

Table 1: The effect of two planting dates , different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on growth parameters; length of root (cm plant^{-1}), length of shoot (cm plant^{-1}), fresh mass (g plant^{-1}), dry mass (g plant^{-1}) and leaf area (cm^{2} plant $^{-1}$) of *Pimpinella anisum* plants during vegetative stage.

Date	Treatment	Shoot Length	Root Length	Leaf area	Fresh mass	Dry mass
Control	Control	10.650	3.600	2.234	4.273	0.668
	1mM CA	10.000	3.350	2.212	3.921*	0.465*
	5mM CA	9.500*	2.900*	1.965*	3.412*	0.437*
	10mM CA	8.400*	2.750*	1.927*	3.032*	0.415*
	Urea 50%	10.700	3.750	2.420	4.335	0.670
	Urea 50% + 1mM CA	10.150	3.450	2.278	3.927*	0.548*
	Urea 50% + 5mM CA	9.450*	3.550	2.183	3.548*	0.487*
	Urea 50% + 10mM CA	8.850*	2.950*	2.054	3.129*	0.393*
	Urea 100%	11.100	4.250*	2.561*	4.426	0.682
	Urea 100% + 1mM CA	10.300	4.100*	2.163	4.354	0.645
	Urea 100% + 5mM CA	9.650*	3.550	2.142	4.162	0.596
	Urea 100% + 10mM CA	9.510*	3.400	2.128	3.685*	0.477*
	L.S.D	0.816	0.417	0.246	0.318	0.103
Late	Control	9.550	3.250	2.463	3.812	0.647
	1mM CA	9.150	2.950*	2.284*	3.542	0.537*
	5mM CA	8.850*	2.800*	2.083*	3.076*	0.470*
	10mM CA	8.150*	2.250*	1.970*	3.061*	0.452*
	Urea 50%	10.150	3.550*	2.563	4.012	0.719*
	Urea 50% + 1mM CA	9.750	3.150	2.428	3.964	0.660
	Urea 50% + 5mM CA	9.150	3.000*	2.263*	3.653	0.612
	Urea 50% + 10mM CA	8.900	2.900*	2.107*	3.218*	0.583*
	Urea 100%	10.750*	3.750*	2.718*	4.320*	0.734*
	Urea 100% + 1mM CA	10.000	3.500*	2.541	4.195*	0.681
	Urea 100% + 5mM CA	9.450	3.150	2.435	3.882	0.609
	Urea 100% + 10mM CA	9.200	2.850*	2.322	3.267*	0.595
	L.S.D	0.063	0.217	0.149	0.288	0.063

At flowering stage the results in table 2 show that, all the different concentrations of cinnamic acid decreased the shoot length of anise plants. Meanwhile 50% urea fertilizer increased shoot length while its combination with different concentrations of CA decreased the shoot length of anise plants. The shoot length of plants supplied with 100% urea increased, whereas its combination with different concentrations of CA decreased the shoot length. Root length of anise plants decreased on treating with the three concentrations of CA. Also 50 % urea fertilizer either alone or combined with the three CA concentrations decreased root length and increased with 100% urea fertilizer, whereas its combination with the different concentrations of CA decreased root length, as compared to control values.

Table 2: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on growth parameters; length of root (cm plant^{-1}), length of shoot (cm plant^{-1}), fresh mass (g plant^{-1}), dry mass (g plant^{-1}) and leaf area (cm^2 plant $^{-1}$) of *Pimpinella anisum* plants during flowering stage.

Date	Treatment	Shoot Length	Root Length	Leaf area	Fresh mass	Dry mass
Control	Control	34.150	5.450	4.271	6.247	1.540
	1mM CA	32.150*	4.900*	4.180	6.116	1.356*
	5mM CA	31.750*	4.450*	4.215	6.171	1.373
	10mM CA	29.900*	4.550*	3.946*	4.660*	0.885*
	Urea 50%	34.350	5.100	4.307	6.424	1.592
	Urea 50% + 1mM CA	34.050	4.950	4.090	6.328	1.486
	Urea 50% + 5mM CA	33.400*	4.900*	4.263	6.134	1.520
	Urea 50% + 10mM CA	33.250	4.800*	4.034	6.018	1.195*
	Urea 100%	35.300*	5.650	4.326	6.761	2.013*
	Urea 100% + 1mM CA	33.950	5.250	4.282	6.480	1.824*
	Urea 100% + 5mM CA	32.900*	5.350	4.223	6.355	1.370
	Urea 100% + 10mM CA	32.650*	4.700*	3.260*	5.984	1.295*
	L.S.D	1.149	0.549	0.281	0.518	0.186
Late	Control	27.500	4.900	4.095	5.342	1.984
	1mM CA	25.550*	4.500*	3.846*	5.024*	1.563*
	5mM CA	25.150*	4.250*	3.542*	5.013*	1.308*
	10mM CA	23.000*	3.750*	3.436*	4.458*	1.012*
	Urea 50%	27.900	5.100	4.214	5.721*	2.112*
	Urea 50% + 1mM CA	27.250	4.550*	4.126	5.524	2.014
	Urea 50% + 5mM CA	26.500	4.650	4.052	5.283	1.850*
	Urea 50% + 10mM CA	25.500*	4.500*	3.864*	4.972*	1.544*
	Urea 100%	28.400	5.150	4.339*	6.248*	2.226*
	Urea 100% + 1mM CA	27.900	5.100	4.263*	6.043*	2.196*
	Urea 100% + 5mM CA	26.450	4.800	4.015	5.422	2.047
	Urea 100% + 10mM CA	25.500*	4.600	3.953	5.116	1.942
	L.S.D	1.057	0.328	0.157	0.312	0.116

Leaf area of anise plant decreased on treating with 1mM, 5mM and 10mM CA but increased with 50% urea, whereas its interaction with different concentrations of CA decreased leaf area. On the other hand, 100% urea either alone or in combination with 1mM CA caused increase in leaf area while its combination with CA decreased leaf area at 5mM and 10mM CA. Fresh mass, decreased in case of 1mM, 5mM and 10mM CA concentration. Meanwhile 50% urea fertilizer either alone or in combination with 1mM CA caused increase in fresh mass, while its combination with 5mM and 10mM CA led to decrease in fresh mass. Also 100% urea fertilizer either alone or combined with 1mM and 5mM CA increased fresh mass while 10mM CA decreased it. The dry mass of plants treated with different concentrations of CA decreased with the three concentrations of CA. The 50% urea-fed plants showed increase in dry mass, whereas 50% urea combined with all CA concentrations decreased this parameter. Treatment with 100% urea alone or in combination with 1mM CA increased dry mass, while its combination with 5mM and 10mM CA decreased this parameter.

Perusal of the data, at both vegetative and flowering stage during the late date of cultivation, shows nearly the same pattern of change occurred in the control date. It's worthy to mention that results obtained by CA and urea; independent or together in case of control date of cultivation were much better than those obtained on the late date of cultivation.

In this connection, under CA stress, the reduction in plant growth (root length, shoot length) and biomass was observed in *Lactuca sativa* (Hussain *et al.,* 2010). Similar results were observed by Ye *et al.,* 2004 on *Cucumis sativus* L. The CA (0.5-0.25 mM) has been shown to cause oxidative stress in cucumber roots (Ding *et al.,* 2007). Altered root morphology in *Pisum sativum* (Vaughan and Ord, 1991). Trans- cinnamic acid inhibited the root elongation of *Lactuca sativa* (Fujita and Kabo, 2003). Likewise, Ding *et al.* (2007) reported that CA significantly inhibited the growth of cucumber.

In other study, urea was reported to increase the length, fresh mass and dry mass of the shoots and roots of wheat plant (Gharakand *et al.;* 2012). Similar results were obtained on studying the effect of urea application as a common fertilizer on some vegetables; *Brassica compestris*, *Trigonella foenum graecum* and *Anethum graveolens*, as urea was found to increase the percentage of seed germination, root length, shoot length, root /shoot ratio and seedling height (Ramteke and Shirgave, 2012). In support of the present observations, Puttanna *et al.,* (2001) demonstrated that foliar application of urea fertilizer significantly enhanced the growth and yield of citronella plants and increased most of the growth parameters (plant height, leaf area, fresh and dry masses.

The improvement of vegetative characteristics of anise plant (height, fresh mass and dry mass) with increase in nitrogen fertilizer rate could be attributed to increased uptake of nitrogen and its associated role in chlorophyll synthesis and hence the process of photosynthesis and carbon dioxide assimilation (Jasso-chaverria *et al.,* 2005) leading to enhance growth. In addition, nitrogen stimulates vegetative growth resulting in large stems and leaves. The significant response of leaf area by urea fertilizer may be an indication that nitrogen was taken up by the plant and subsequently utilized in cell multiplication, amino acid synthesis and energy formation that acts as structural compound of the chloroplast which carries out photosynthesis as nitrogen fertilizer has been reported to be a constituent of chlorophyll (Lawlor, 2002). In support, nitrogen insufficiencies have been reported to reduce the individual leaf area, leaf area index, and total leaf area resulting to reduced surface light interception for photosynthesis (Cechin and Fumis, 2004).

Changes in photosynthetic pigments:

As compared to control values, the different concentrations of CA at vegetative stage caused decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and consequently total pigments in case of 1mM, 5mM and 10mM CA. In 50% urea-fed plants there was an increase in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments. The combination of 50% urea fertilizer and 1mM CA caused decrease in chlorophyll a, total chlorophylls (chl a+ chl b) and total pigments, but increased chl b and carotenoids. Meanwhile the interaction between 5mM CA and 50% urea caused decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments. Also 10mM CA mixed with 50% urea caused decrease in chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments content during the control date of cultivation (Table 3).

Table 3: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on photosynthetic pigments (mg g^{-1} dry weight) of *Pimpinella anisum* plants during the vegetative stage.

Date	Treatment	Chl a	Chl b	Chl a + b	Cars	Total pigments
Control	Control	5.880	3.490	9.370	3.160	12.530
	1mM CA	5.650	3.120	8.770	2.880	11.650
	5mM CA	5.670	3.070	8.740	2.720	11.460
	10mM CA	4.150*	2.860*	7.010*	2.180*	9.190*
	Urea 50%	5.960	3.580	9.540	3.350	12.890
	Urea 50% + 1mM CA	5.780	3.510	9.290	3.220	12.480
	Urea 50% + 5mM CA	5.720	3.440	9.160	3.070	12.230
	Urea 50% + 10mM CA	4.830*	3.160	7.990*	2.920	10.910
	Urea 100%	5.920	4.210*	10.130	3.670	13.800
	Urea 100% + 1mM CA	5.730	4.130*	9.860	3.420	13.280
	Urea 100% + 5mM CA	5.640	3.880	9.520	3.150	12.670
	Urea 100% + 10mM CA	5.070	3.150	8.220	2.940	11.160
	L.S.D	0.884	0.605	1.335	0.590	1.726
Late	Control	4.930	2.310	7.240	2.230	9.470
	1mM CA	4.460	2.150	6.610	2.240	8.850
	5mM CA	3.900*	2.130*	6.030*	2.150	8.180
	10mM CA	3.380*	2.070*	5.450*	1.880*	7.330*
	Urea 50%	4.960	2.420	7.380	2.260	9.640
	Urea 50% + 1mM CA	4.590	2.170	6.760	2.310	9.070
	Urea 50% + 5mM CA	4.070*	2.000*	6.070*	2.020	8.090
	Urea 50% + 10mM CA	3.380*	1.950*	5.330*	1.890*	7.220*
	Urea 100%	5.090	2.460	7.550	2.250	9.800
	Urea 100% + 1mM CA	4.710	2.280	6.990	2.170	9.160
	Urea 100% + 5mM CA	3.820*	2.030*	5.850*	2.010	7.860*
	Urea 100% + 10mM CA	3.550*	1.950*	5.500*	1.960	7.460*
	L.S.D	0.542	0.168	0.703	0.302	1.792

Plants supplied with 100% urea fertilizer at the control date of cultivation showed increase in chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments. The interaction of 100% urea with 1mM CA caused decrease in chlorophyll a and increase in chlorophyll b, total chlorophylls, carotenoids and total pigments. The application of 5mM CA with 100% urea decreased chlorophyll a and carotenoids, but increased chlorophyll b, total chlorophylls and total pigments content. Meanwhile, combination of 100% urea and 10mM CA decreased chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments.

At flowering stage during the control date of cultivation, showed decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments in case of 1mM, 5mM and 10mM CA. The application of 50% urea fertilizer caused increase in chlorophyll a, chlorophyll b, total chlorophylls, carotenoinds and total pigments. 50% urea + 1mM CA caused decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), and total pigments and increase in carotenoids. Whereas, interaction of 5mM CA as well as with 10mM CA and 50% urea decreased chlorophyll a, chlorophyll b, carotenoids, total chlorophylls and total pigments. 100% urea fertilizer caused increase in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments. Combination of 100% urea with 1mM, 5mM or 10mM CA resulted in decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments (Table 4).

Table 4: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on photosynthetic pigments (mg g $^{-1}$ dry weight) of *Pimpinella anisum* plants during the flowering stage.

Date	Treatment	Chl a	Chl b	Chl a + b	Cars	Total pigments
Control	Control	4.930	2.310	7.240	2.230	9.470
	1mM CA	4.460	2.150	6.610	2.240	8.850
	5mM CA	3.900*	2.130*	6.030*	2.150	8.180
	10mM CA	3.380*	2.070*	5.450*	1.880*	7.330*
	Urea 50%	4.960	2.420	7.380	2.260	9.640
	Urea 50% + 1mM CA	4.590	2.170	6.760	2.310	9.070
	Urea 50% + 5mM CA	4.070*	2.000*	6.070*	2.020	8.090
	Urea 50% + 10mM CA	3.380*	1.950*	5.330*	1.890*	7.220*
	Urea 100%	5.090	2.460	7.550	2.250	9.800
	Urea 100% + 1mM CA	4.710	2.280	6.990	2.170	9.160
	Urea 100% + 5mM CA	3.820*	2.030*	5.850*	2.010	7.860*
	Urea 100% + 10mM CA	3.550*	1.950*	5.500*	1.960	7.460*
	L.S.D	0.542	0.168	0.703	0.302	1.792
Late	Control	2.650	1.930	4.580	1.950	6.530
	1mM CA	2.490	1.850	4.340	1.860	6.200
	5mM CA	2.430	1.820	4.250*	1.760*	6.010
	10mM CA	1.510*	1.710*	3.220*	1.750*	4.970*
	Urea 50%	2.760	1.980	4.740	1.990	6.730
	Urea 50% + 1mM CA	2.510	1.890	4.400	1.870	6.270
	Urea 50% + 5mM CA	2.450	1.830	4.280*	1.800	6.080
	Urea 50% + 10mM CA	1.930*	1.760	3.690*	1.640*	5.330*
	Urea 100%	2.680	2.070	4.750	2.050	6.800
	Urea 100% + 1mM CA	2.520	1.910	4.430	1.910	6.340
	Urea 100% + 5mM CA	2.440	1.830	4.270*	1.820	6.090
	Urea 100% + 10mM CA	2.070*	1.750	3.820*	1.800	5.620*
	L.S.D	0.519	0.207	0.297	0.158	0.639

Data in tables 3 and 4 shows that at both vegetative and flowering stages during the late date of cultivation, the pattern of change didn't differ so much from the control date. But as mentioned before, results obtained by CA and urea; independent or together in case of control date of cultivation were much better than those obtained on the late date of cultivation.

In this respect, chlorophylls are the core component of pigment protein complexes embedded in the photosynthetic membranes and play a major role in the photosynthesis. Any changes in chlorophyll content are expected to bring about change in photosynthesis (Reigosa *et al.,* 2006). The influence of N on plant growth and development is often connected with the process of photosynthesis, because the quantity of N, in the highest degree, determines the formation and the functional state of assimilation apparatus of plants including the content of photosynthetic pigments, the synthesis of the enzymes taking part in the carbon reduction and the formation of the membrane system of chloroplasts (Stanev, 1984; Ivanova and Vassilev, 2003).

Also the positive effects of N fertilization may be due to the important physiological role of N in molecule structure as porphyrin. The porphyrin structure is found in such metabolically important compounds as the chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. Accordingly, nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes activities and protein synthesis (Jones *et al,.* 1991) that reflected in the increase in growth parameters of plants such as anise, coriander and sweet fennel plants. Also, these results are in accordance with those obtained by Khalid (1996, 2001) on some Apiaceae and *Nigella sativa* L. plants; Ashraf *et al.* (2006) on cumin; Akbarinia *et al.* (2007) on coriander; Hellal *et al.* (2011) on dill (*Anethum graveolens* L.), all of them reported that N fertilizer treatments were superior to the control treatment and significantly improved the vegetative growth characters of family Apiaceae.

Changes in carbohydrate contents:

Regarding the control date of cultivation at vegetative stage, glucose content, sucrose, total soluble sugars, polysaccharides and total carbohydrates of anise plant were decreased by treating with 1mM, 5mM and 10mM CA. But increased by 50% or 100% urea or their combination with different CA concentrations. Also different concentrations of CA led to decrease in these parameters. Meanwhile applying 50% urea to anise plants either alone or in combination with 1mM CA increased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates (Table 5).

On the other hand, 5mM CA+ 50% urea decreased glucose and sucrose and increased total soluble sugars, polysaccharides and total carbohydrates, whereas 10mM CA with 50% urea decreased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates. Fertilization by 100% urea caused increase in all the determined carbohydrates content. Also 100% urea and 1mM CA increased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates, whereas 100% urea+ 5mM CA increased all the determined carbohydrates content except for sucrose content which decreased. Regarding the interaction of 100% urea and 10mM CA a decrease in all the determined carbohydrates was detected.

Table 5: The effect of two plantimg dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on carbohydrates content (mg g $^{-1}$ dry weight) of *Pimpinella anisum* plants during the vegetative stage.

Date	Treatment	Glucose	Sucrose	TSS	Polysaccharides	Total carbohydrates
Control	Control	21.670	32.220	76.800	40.120	116.920
	1mM CA	21.280	29.870*	76.340	40.330	116.670
	5mM CA	20.870	29.040*	68.000*	38.670	106.670*
	10mM CA	19.150*	28.180*	62.270*	35.890*	98.160*
	Urea 50%	23.280	38.120*	82.400*	42.890*	125.290*
	Urea 50% + 1mM CA	23.030	35.830*	82.240*	42.700*	124.940*
	Urea 50% + 5mM CA	22.150	35.900*	80.100*	42.020*	122.120*
	Urea 50% + 10mM CA	22.000	34.420*	78.300	41.000*	119.300*
	Urea 100%	25.670*	40.670*	94.200*	48.000*	142.200*
	Urea 100% + 1mM CA	25.520*	40.470*	94.150*	47.620*	141.770*
	Urea 100% + 5mM CA	25.150*	38.270*	93.810*	47.130*	140.940*
	Urea 100% + 10mM CA	24.290*	38.080*	92.150*	46.330*	138.480*
	L.S.D	2.503	1.933	2.737	2.052	3.696
Late	Control	25.540	22.580	63.220	49.820	113.040
	1mM CA	23.410*	22.120	62.540	48.650	111.190
	5mM CA	22.690*	20.470*	62.240	48.260*	110.500*
	10mM CA	20.750*	20.160*	60.170*	48.170*	108.340*
	Urea 50%	26.340	23.560	65.290	50.570	115.760*
	Urea 50% + 1mM CA	25.820	23.720	65.110	50.420	115.530*
	Urea 50% + 5mM CA	24.730	22.460	65.070	50.180	115.250
	Urea 50% + 10mM CA	23.460*	21.550	64.210	48.360*	112.570
	Urea 100%	27.490*	23.890*	69.340*	52.470*	121.810*
	Urea 100% + 1mM CA	26.170	23.540	67.420*	52.120*	119.540*
	Urea 100% + 5mM CA	25.550	22.140	65.220	51.340*	116.560*
	Urea 100% + 10mM CA	25.420	21.310	63.140	49.310	112.450
	L.S.D	1.783	1.245	3.018	1.402	2.361

In table 6 the results show that, at flowering stage at the control date of cultivation, the different concentrations of CA decreased glucose, sucrose, polysaccharides, total soluble sugars and total carbohydrates content. The application of 50% urea fertilizer caused increase in glucose, sucrose, polysaccharides and total carbohydrates content but decreased total soluble sugars content. Whereas the interaction of 50% urea with 1mM CA decreased sucrose, total soluble sugars and total carbohydrates content but increased glucose and polysaccharides contents. Also 50% urea + 5mM CA increased glucose, total soluble sugars, total carbohydrates and polysaccharides, and decreased sucrose content. While 50% urea with 10mM CA decreased these metabolites.

Treatment with 100% urea fertilizer increased all determined carbohydrates fractions. Glucose, sucrose and polysaccharides content also increased in response to 100% urea combined with the three different concentrations of CA. Total soluble sugars and total carbohydrates also increased with 1mM and decreased with 5mM and 10mM CA mixed with 100% urea.

Table 6: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on carbohydrates content (mg g $^{-1}$ dry weight) of *Pimpinella anisum* plants during the flowering stage.

Date	Treatment	Glucose	Sucrose	TSS	Polysaccharides	Total carbohydrates
Control	Control	32.480	44.400	98.780	45.340	144.120
	1mM CA	31.640	44.210	96.240*	46.210	142.450
	5mM CA	31.070	41.470	95.630*	45.000	140.630
	10mM CA	30.630	40.670*	95.220*	45.810	141.030
	Urea 50%	33.590	45.280	98.320	47.230	145.550
	Urea 50% + 1mM CA	33.420	44.230	97.730	46.220	143.950
	Urea 50% + 5mM CA	33.000	42.330	96.200*	45.350	141.550
	Urea 50% + 10mM CA	31.210	43.520	95.260*	44.620	139.880*
	Urea 100%	40.090*	50.230*	110.470*	50.720*	161.190*
	Urea 100% + 1mM CA	38.130*	48.000*	99.620	48.210*	147.830
	Urea 100% + 5mM CA	36.730*	45.560	102.540*	47.230	149.770*
	Urea 100% + 10mM CA	33.420*	44.850	98.350	45.210	143.560
	L.S.D	2.563	2.177	2.333	2.408	4.450
Late	Control	46.180	39.260	86.320	49.820	136.140
	1mM CA	44.250	38.420	86.240	47.360*	133.600
	5mM CA	43.870*	37.540	85.670	47.250*	132.920*
	10mM CA	42.730*	37.160	84.210*	46.220*	130.430*
	Urea 50%	50.470*	43.510*	88.470*	50.270	138.740
	Urea 50% + 1mM CA	50.210*	40.720	86.940	49.970	136.910
	Urea 50% + 5mM CA	48.760*	39.520	86.240	48.670	134.910
	Urea 50% + 10mM CA	48.220*	38.140	85.230	47.090*	132.320*
	Urea 100%	53.740*	42.570*	89.760*	52.470*	142.230*
	Urea 100% + 1mM CA	51.080*	41.780	88.340	52.140*	140.480*
	Urea 100% + 5mM CA	50.420*	39.780	87.410	50.890	138.300
	Urea 100% + 10mM CA	50.040*	38.640	86.080	50.120	136.200
	L.S.D	1.963	2.746	2.077	1.349	3.152

Also at the late date of cultivation during the flowering stage, results in table 6 show that, comparing to control levels, 1mM, 5mM and 10mM CA decreased all the determined carbohydrates fractions. On the other hand 50% urea increased the determined carbohydrate contents. Meanwhile the interaction of 50% urea with the three concentrations of CA increased glucose content significantly, whereas 50% urea+ 1mM CA decreased sucrose, total soluble sugars, polysaccharides and total carbohydrates. Also 50% urea+ 5mM led to increase in sucrose and decrease in total soluble sugars, polysaccharides and total carbohydrates content, while the combination of 50% urea and 10mM CA decreased sucrose, total soluble sugars polysaccharides and total carbohydrates. Regarding 100% urea either alone or combined with 1mM CA, an increase in all the determined carbohydrates content was detected. Interaction of 5mM and 10mM CA with 100% urea led to increase in glucose, polysaccharides and total carbohydrates content and increased sucrose and total soluble sugars with 5mM CA combined with 100% urea and decreased in response to 10mM CA + 100% urea.

There has been increase in research on the role of the demand for photo-assimilates in regulating photosynthesis through changes in carbohydrate partitioning and accumulation under stress condition (Paul and Driscoll, 1997; Nielsen *et al.,* 1998; Paul and Foyer, 2001). N plays an important role in synthesis of the plant constituents through the action of different enzymes activity and protein synthesis (Jones *et al.,* 1991) that reflected on an increase in growth parameters and chemical constituents of anise, coriander and sweet fennel plants. The obtained results are in accordance with those obtained by previous literature. N is a necessary component of several vitamins. N improves the quality and quantity of dry matter in leafy plants and protein in grain crops (Silva and Uchida, 2000). Increase the N fertilizer caused a significant increase in the seed yield of *Mentha arvensis* L and *Anethum graveolens* L. (Munsi, 1992; Randhawa *et al.,* 1996 respectively). N fertilization increased the vegetative growth, essential oil, fixed oil, total carbohydrates, soluble sugars and NPK content of *Nigella sativa* L. plants (Khalid, 2001). Zheljazkov and Margina (1996) established that vegetative growth and essential oil (yield and constituents) of *Mentha piperita* and *Mentha arvensis* were increased as N fertilizer increase. Arabaci and Bayram (2004) found that N fertilizer increased the amount of green herb yield, drug herb yield, drug leaves, essential oil (% & yield) of basil (*Ocimum basilicum* L.).

Changes in nitrogenous fractions:

Results presented in table 7 show that, at the vegetative stage, the different concentrations of CA decreased ammonia-N content with the three CA concentrations. Amino- N content increased with 1mM CA and decreased with the two other concentrations. Whereas amide-, total soluble- N, total- N and protein- N they decreased with the different concentrations of CA during the control date of cultivation. Ammonia- and amino- N increased with 50% urea either alone or when mixed with the three concentrations of CA. However, 100% urea either alone or combined with different CA concentrations increased both ammonia- and amino-N. Amide- N increased with 50% urea either alone or in combination with 1mM CA, and this parameter decreased with 5mM and 10mM CA mixed with 50% urea. Meanwhile, 100% urea either alone or in addition to 1mM and 5mM CA increased amide- N content, while 100% urea+10mM CA caused in amide- N content.

Table 7: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on nitrogenous constituents (mg g -1 dry weight) of *Pimpinella anisum* plants during the vegetative stage.

Date	Treatment	Ammonia N	Amino N.	Amide N.	Total soluble N.	Total nitrogen	Protein nitrogen
Control	Control	4.330	3.620	0.880	11.370	33.460	22.090
	1mM CA	4.250	3.740	0.820	10.960	32.190*	21.850
	5mM CA	4.070	3.450	0.790	10.230*	31.860*	21.630
	10mM CA	3.920*	3.180	0.740	10.070*	31.420*	21.350
	Urea 50%	4.630*	3.940*	0.920	12.480	35.620*	23.140
	Urea 50% + 1mM CA	4.610*	3.820*	0.890	12.120	35.150	23.030
	Urea 50% + 5mM CA	4.500	3.510	0.850	11.840	34.850	23.010
	Urea 50% + 10mM CA	4.570	3.120	0.790	11.530	34.260	22.730
	Urea 100%	5.140*	4.260*	0.980	12.930*	38.720*	25.790*
	Urea 100% + 1mM CA	5.060*	4.170*	0.960	12.440	36.450*	24.010*
	Urea 100% + 5mM CA	4.810*	4.130*	0.970	11.960	36.290*	24.330*
	Urea 100% + 10mM CA	4.720*	3.910*	0.870	11.650	35.120	23.470
	L.S.D	0.275	0.205	0.311	1.494	1.079	1.236
Late	Control	4.650	4.050	0.790	13.480	35.710	22.230
	1mM CA	4.230*	3.920	0.750	11.870	35.160	23.290*
	5mM CA	4.120*	3.730*	0.690*	11.530	33.760*	22.230
	10mM CA	3.080*	3.540*	0.660*	11.370*	33.120*	21.750
	Urea 50%	4.790	4.270*	0.960*	14.420	36.660	22.240
	Urea 50% + 1mM CA	4.630	4.130	0.940*	13.690	35.950	22.260
	Urea 50% + 5mM CA	4.490	4.070	0.910*	12.840	35.850	23.010
	Urea 50% + 10mM CA	4.360*	3.870	0.870*	12.290	35.760	23.470*
	Urea 100%	5.370*	4.570*	1.140*	15.730*	38.970*	23.240*
	Urea 100% + 1mM CA	5.210*	4.330*	1.080*	13.640	37.450*	23.810*
	Urea 100% + 5mM CA	4.940*	4.210	0.910*	12.700	36.450	23.750*
	Urea 100% + 10mM CA	4.800	3.940	0.830	11.080*	35.820	24.740*
	L.S.D	0.187	0.193	0.074	1.985	1.567	0.984

Total soluble- and protein- N increased as a result of treating anise plants with 50% urea either alone or in combination with three different CA concentrations. On the other hand total- N increased in plants supplied with 50% urea alone or combined with the different concentrations of CA. Fertilization of anise plant with 100% urea alone or combined with the different CA concentrations increased total soluble-, total- and protein- N.

During flowering stage results illustrated in table 8 show that, all the determined nitrogenous constituents decreased with the three concentrations of CA. Fertilization with 50% urea either alone or combined with the CA used concentrations increased all the determined nitrogenous constituents. The highest concentration of urea (100%) when used either alone or in combination with CA different concentrations caused significant increase in all the determined nitrogenous fractions.

Table 8: The effect of two planting dates, different concentrations of cinnamic acid (CA) and urea, either alone or in combination, on nitrogenous constituents (mg g -1 dry weight) of *Pimpinella anisum* plants during the flowering stage.

Date	Treatment	Ammonia N	Amino N.	Amide N.	Total soluble N.	Total nitrogen	Protein nitrogen
Control	Control	5.430	4.320	1.470	13.400	46.310	32.910
	1mM CA	5.370	4.180	1.350*	12.870	46.160	32.290
	5mM CA	4.820*	3.850*	1.330*	12.520	44.820*	32.300
	10mM CA	4.510*	3.470*	1.190*	11.820*	43.980*	32.160
	Urea 50%	5.980	4.690	1.830*	14.560*	50.820*	36.260*
	Urea 50% + 1mM CA	5.930	4.460	1.620*	14.130	49.730*	35.600*
	Urea 50% + 5mM CA	5.470	4.510	1.630*	13.860	48.210*	34.350*
	Urea 50% + 10mM CA	5.120	4.440	1.560	13.420	48.110*	34.690*
	Urea 100%	6.680*	5.230*	1.970*	15.440*	52.960*	37.520*
	Urea 100% + 1mM CA	6.530*	4.870*	1.840*	14.830*	52.790*	37.960*
	Urea 100% + 5mM CA	6.070*	4.730	1.820*	14.540	51.840*	37.300*
	Urea 100% + 10mM CA	5.920	4.420	1.790*	13.920	50.910*	36.990*
	L.S.D	0.621	0.422	0.107	1.217	1.087	1.308
Late	Control	6.240	5.520	1.330	15.680	46.890	31.210
	1mM CA	6.050	5.110*	1.280	14.840	45.270	30.430
	5mM CA	5.870*	4.880*	1.160*	14.500	45.310	30.810
	10mM CA	5.290*	4.390*	1.090*	13.770*	44.470	30.700
	Urea 50%	6.940*	5.790	1.560*	17.720*	49.540*	31.820
	Urea 50% + 1mM CA	6.930*	5.760	1.520*	16.430	47.680	31.250
	Urea 50% + 5mM CA	6.460*	5.580	1.470*	16.660	47.320	30.660
	Urea 50% + 10mM CA	6.120	5.540	1.360	15.480	46.940	31.460
	Urea 100%	7.350*	6.270*	1.890*	17.930*	51.280*	33.350*
	Urea 100% + 1mM CA	6.860*	6.070*	1.770*	17.590*	51.190*	33.600*
	Urea 100% + 5mM CA	6.390	5.930*	1.740*	16.820	50.790*	33.970*
	Urea 100% + 10mM CA	6.120	5.610	1.610*	15.850	50.000*	34.15*
	L.S.D	0.207	0.381	0.093	1.618	2.462	1.056

Similar pattern of change in general was obtained at the two stages of growth during the late date of cultivation. Again, results obtained by CA and urea; independent or together in case of control date of cultivation were much better than those obtained on the late date of cultivation.

Based on the mentioned results CA as an allelochemical was found to decrease the nitrogenous fractions and according to the allelopathy definition, it is so evident that allelochemicals could affect all phases of nitrogen cycle that involve plant or microorganisms. When plants take up nitrate, they must use energy to convert it to ammonium form before it can be used. Thus, growth reduction due to missing of energy could be an argument for nitrogen reduction in seedlings which treated by allelochemicals, also loosing of nitrogen content in some seedling, could be occurred by limiting or reducing some key factors in nitrogen metabolism such as nitrate reductase and glutamine synthetase (Reigosa *et al.,* 2006).

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تأثير تاريخ الزراعة، حمض السيناميك و السماد النيتروجينى على نمو وأيض نبات الينسون

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تمت زراعة الينسون في تجربتين منفصلتين، أحدهما في الموعد المعتاد لزراعته والأخرى في موعد متأخر. و أدت معاملة نبات الينسون بالتركيزات المختلفة من حمض السيناميك خلال مرحلتي النمو إلى نقص في كل دالات النمو المختلفة للنبات (طول الجذر- طول الساق – مساحة الورقة – الوزن الطازج و الوزن الجاف) بالمقارنة بالعينات الضابطة، كذلك لوحظ نقصان معنوي في المكونات النسبية لأصباغ البناء الضوئي (كلوروفيل أ ، كلوروفيل ب ، كلوروفيل أ+ب ، الكاروتينات، و المحتوى الصبغى) أيضا حدث اختزال في محتوى الكربوهيدرات (الجلوكوز، السكروز، السكريات العديدة والمحتوى الكلى للكربوهيدرات) كما أدى إلى نقص في محتوى المركبات النيتروجينيه (الامونيا، الامينو، الاميد، المحتى النيتروجينى الذائب والبروتين والمحتوى النيتروجينى الكلى) عند مقارنتها بالعينات الضابطة وذلك خلال موعدي الزراعة المختلفين. بينما أدت معاملات تركيزي اليوريا المستخدمين (٥٠% - ١٠٠%) إلى زيادة كل دالات النمو، المكونات النسبية لأصباغ البناء الضوئي، محتوى الكربوهيدرات و المحتوى النيتروجينى وذلك عند استخدام اليوريا منفردة أو مع التركيزات المختلفة من حمض السيناميك، مع الأخذ في الاعتبار أن اليوريا منفردة كانت أفضل. ومما تجدر الإشارة إليه أن جميع النتائج كانت أفضل في حالة زراعة الينسون في موعده عن زراعته في الموعد المتأخر.