

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 (20th Dec.) in addition to the control date (20th 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, 2mM and 3mM) 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 (0% 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 (β -Phenyl- α -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 0.0, 1.0 and 1.0 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 campestris*, *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.*; 2012). Haroun *et al.* (2012) conducted a field experiment to determine the effect of two planting dates; early (20th Oct.) and late (10th Dec.) in addition to the control planted at 20th Nov. on phenology, 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 phenological 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 (10th 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, 0mM and 10mM) and / or two different concentrations of urea fertilizer (0% and 100%) of the recommended dose during a different planting late date (20 Dec)

and control one (20 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 (1 mM, 2 mM and 3 mM) and two levels of urea fertilizer were chosen [20% urea (0.5 g/pot) and 100% (1.5 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, 20 and 120 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 (1962) and Handel (1968). The method used for estimation of polysaccharides in the present study was that of Thayumanavan and Sadasivam (1982).

Estimation of nitrogenous constituents:

The method used in this investigation was essentially that adopted by Yemm and Willis (1966). 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 quantitatively transferred to a boiling tube, brought quickly to water bath maintained at 100°C for 10 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 $P \leq 0.05$ was carried out by using CoHort/ CoStat statistical software version display ANOVA (998 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, 0mM and 10mM cinnamic acid, but it increased with 0% and 10% urea fertilizer. Also the interaction between cinnamic acid (0mM and 10mM) and urea (0% and 10%) 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 0% and 10% 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 (cm² plant⁻¹) of *Pimpinella anisum* plants during vegetative stage.

Date	Treatment	Shoot Length	Root Length	Leaf area	Fresh mass	Dry mass
Control	Control	11.70*	3.71*	2.234	4.273	0.778
	1mM CA	11.00*	3.30*	2.212	3.921*	0.670*
	0mM CA	9.00*	2.90*	1.970*	3.412*	0.533*
	10mM CA	8.40*	2.70*	1.922*	3.032*	0.490*
	Urea 0%	11.70*	3.70*	2.42*	4.330	0.77*
	Urea 0% + 1mM CA	11.10*	3.40*	2.278	3.927*	0.648*
	Urea 0% + 0mM CA	9.40*	3.00*	2.182	3.048*	0.487*
	Urea 0% + 10mM CA	8.80*	2.90*	2.004	3.129*	0.393*
	Urea 10%	11.10*	3.20*	2.071*	4.427	0.782
	Urea 10% + 1mM CA	11.30*	3.10*	2.172	4.304	0.740
	Urea 10% + 0mM CA	9.70*	3.00*	2.142	4.172	0.697
	Urea 10% + 10mM CA	9.01*	2.80*	2.128	3.780*	0.577*
L.S.D	0.817	0.417	0.247	0.318	0.103	
Late	Control	9.00*	3.20*	2.472	3.812	0.747
	1mM CA	9.10*	2.90*	2.284*	3.042	0.537*
	0mM CA	8.80*	2.80*	2.082*	3.072*	0.47*
	10mM CA	8.10*	2.20*	1.97*	3.071*	0.402*
	Urea 0%	11.10*	3.00*	2.072	4.012	0.719*
	Urea 0% + 1mM CA	9.70*	3.10*	2.428	3.974	0.770
	Urea 0% + 0mM CA	9.10*	2.90*	2.272*	3.702	0.712
	Urea 0% + 10mM CA	8.90*	2.90*	2.107*	3.218*	0.582*
	Urea 10%	11.70*	3.70*	2.718*	4.32*	0.732*
	Urea 10% + 1mM CA	11.00*	3.00*	2.041	4.190*	0.781
	Urea 10% + 0mM CA	9.40*	3.10*	2.430	3.882	0.709
	Urea 10% + 10mM CA	9.20*	2.80*	2.322	3.277*	0.590
L.S.D	0.73	0.217	0.149	0.288	0.073	

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 0% 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 0% 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 (cm² plant⁻¹) of *Pimpinella anisum* plants during flowering stage.

Date	Treatment	Shoot Length	Root Length	Leaf area	Fresh mass	Dry mass
Control	Control	34,10*	0,450	4,271	7,247	1,040
	1mM CA	32,100*	4,900*	4,180	7,117	1,306*
	0mM CA	31,700*	4,400*	4,210	7,171	1,373
	10mM CA	29,900*	4,000*	3,947*	4,770*	0,880*
	Urea 0%	34,300	0,100	4,307	7,424	1,092
	Urea 0% + 1mM CA	34,000	4,900	4,090	7,328	1,487
	Urea 0% + 0mM CA	33,400*	4,900*	4,273	7,134	1,020
	Urea 0% + 10mM CA	32,200	4,800*	4,044	7,018	1,190*
	Urea 100%	30,300*	0,700	4,327	7,771	2,017*
	Urea 100% + 1mM CA	32,900	0,200	4,282	7,480	1,824*
	Urea 100% + 0mM CA	32,900*	0,200	4,223	7,300	1,370
	Urea 100% + 10mM CA	32,700*	4,700*	3,270*	0,984	1,290*
L.S.D	1,149	0,049	0,281	0,018	0,187	
Late	Control	27,000	4,900	4,090	0,342	1,984
	1mM CA	20,000*	4,000*	3,847*	0,024*	1,073*
	0mM CA	20,100*	4,200*	3,042*	0,013*	1,308*
	10mM CA	22,000*	3,700*	3,437*	4,408*	1,012*
	Urea 0%	27,900	0,100	4,214	0,721*	2,112*
	Urea 0% + 1mM CA	27,200	4,000*	4,127	0,024	2,014
	Urea 0% + 0mM CA	27,000	4,700	4,002	0,283	1,800*
	Urea 0% + 10mM CA	20,000*	4,000*	3,874*	4,972*	1,044*
	Urea 100%	28,400	0,100	4,339*	7,248*	2,227*
	Urea 100% + 1mM CA	27,900	0,100	4,273*	7,043*	2,197*
	Urea 100% + 0mM CA	27,400	4,800	4,010	0,422	2,047
	Urea 100% + 10mM CA	20,000*	4,700	3,903	0,117	1,942
L.S.D	1,007	0,228	0,107	0,312	0,117	

Leaf area of anise plant decreased on treating with 1mM, 0mM and 10mM CA but increased with 0% 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 0mM and 10mM CA. Fresh mass, decreased in case of 1mM, 0mM and 10mM CA concentration. Meanwhile 0% urea fertilizer either alone or in combination with 1mM CA

caused increase in fresh mass, while its combination with 0mM and 10mM CA led to decrease in fresh mass. Also 100% urea fertilizer either alone or combined with 1mM and 0mM 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 0% urea-fed plants showed increase in dry mass, whereas 0% 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 0mM 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 (0.00, 20 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.*, 2000) 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, 0mM and 10mM CA. In 0% 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 0% 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 0mM CA and 0% urea caused decrease in chlorophyll a, chlorophyll b, total chlorophylls (chl a+ chl b), carotenoids and total pigments. Also 10mM CA mixed with 0% 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	0,880	3,490	9,370	3,160	12,030
	1mM CA	0,700	3,120	8,770	2,880	11,700
	0mM CA	0,770	3,070	8,740	2,720	11,460
	10mM CA	4,100*	2,860*	7,010*	2,180*	9,190*
	Urea 0%	0,970	3,080	9,040	3,300	12,890
	Urea 0% + 1mM CA	0,780	3,010	9,290	3,220	12,480
	Urea 0% + 0mM CA	0,720	3,440	9,160	3,070	12,230
	Urea 0% + 10mM CA	4,830*	3,160	7,990*	2,920	10,910
	Urea 100%	0,920	4,210*	10,130	3,770	13,800
	Urea 100% + 1mM CA	0,730	4,130*	9,860	3,420	13,280
	Urea 100% + 0mM CA	0,740	3,880	9,020	3,100	12,170
	Urea 100% + 10mM CA	0,070	3,100	8,220	2,940	11,160
	L.S.D	0,884	0,700	1,330	0,090	1,727
Late	Control	4,930	2,310	7,240	2,230	9,470
	1mM CA	4,470	2,100	6,570	2,240	8,800
	0mM CA	3,900*	2,130*	6,030*	2,100	8,130
	10mM CA	3,380*	2,070*	0,400*	1,880*	7,330*
	Urea 0%	4,970	2,420	7,380	2,270	9,650
	Urea 0% + 1mM CA	4,090	2,170	6,260	2,310	8,570
	Urea 0% + 0mM CA	4,070*	2,000*	6,070*	2,020	8,090
	Urea 0% + 10mM CA	3,380*	1,900*	0,330*	1,890*	7,220*
	Urea 100%	0,090	2,470	7,000	2,200	9,760
	Urea 100% + 1mM CA	4,710	2,280	6,990	2,170	9,160
	Urea 100% + 0mM CA	3,820*	2,030*	0,800*	2,010	7,860*
	Urea 100% + 10mM CA	3,000*	1,900*	0,000*	1,970	7,470*
	L.S.D	0,042	0,168	0,703	0,202	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 0mM 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, 0mM and 10mM CA. The application of 0% urea fertilizer caused increase in chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments. 0% 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 0mM CA as well as with 10mM CA and 0% 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, 0mM 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	2,100	6,560	2,240	8,800
	0mM CA	3,900*	2,130*	6,030*	2,100	8,130*
	10mM CA	3,380*	2,070*	5,450*	1,880*	7,330*
	Urea 0%	4,960	2,420	7,380	2,260	9,640
	Urea 0% + 1mM CA	4,090	2,170	6,260	2,310	8,570
	Urea 0% + 0mM CA	4,070*	2,000*	6,070*	2,020	8,090
	Urea 0% + 10mM CA	3,380*	1,900*	5,280*	1,890*	7,170*
	Urea 100%	5,090	2,460	7,550	2,200	9,750
	Urea 100% + 1mM CA	4,710	2,280	6,990	2,170	9,160
	Urea 100% + 0mM CA	3,820*	2,030*	5,850*	2,010	7,860*
	Urea 100% + 10mM CA	3,000*	1,900*	4,900*	1,960	6,860*
	L.S.D	0,042	0,168	0,170	0,302	0,192
Late	Control	2,700	1,930	4,630	1,900	6,530
	1mM CA	2,490	1,800	4,290	1,860	6,150
	0mM CA	2,430	1,820	4,250	1,760*	6,010
	10mM CA	1,010*	1,710*	2,720*	1,700*	4,420*
	Urea 0%	2,760	1,980	4,740	1,990	6,730
	Urea 0% + 1mM CA	2,010	1,890	3,900	1,870	5,770
	Urea 0% + 0mM CA	2,400	1,830	4,230	1,800	6,030
	Urea 0% + 10mM CA	1,930*	1,760	3,690*	1,740*	5,430*
	Urea 100%	2,780	2,070	4,850	2,000	6,850
	Urea 100% + 1mM CA	2,020	1,910	3,930	1,910	5,840
	Urea 100% + 0mM CA	2,440	1,830	4,270	1,820	6,090
	Urea 100% + 10mM CA	2,070*	1,700	3,770*	1,800	5,570*
	L.S.D	0,019	0,207	0,297	0,108	0,139

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, 2mM and 10mM CA. But increased by 5% or 10% urea or their combination with different CA concentrations. Also different concentrations of CA led to decrease in these parameters. Meanwhile applying 5% urea to anise plants either alone or in combination with 1mM CA increased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates (Table 2).

On the other hand, 2mM CA+ 5% urea decreased glucose and sucrose and increased total soluble sugars, polysaccharides and total carbohydrates, whereas 10mM CA with 5% urea decreased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates. Fertilization by 10% urea caused increase in all the determined carbohydrates content. Also 10% urea and 1mM CA increased glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates, whereas 10% urea+ 2mM CA increased all the determined carbohydrates

content except for sucrose content which decreased. Regarding the interaction of 1.0% urea and 1.0mM CA a decrease in all the determined carbohydrates was detected.

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 vegetative stage.

Date	Treatment	Glucose	Sucrose	TSS	Polysaccharides	Total carbohydrates
Control	Control	21,77.	32,22.	76,80.	40,12.	116,92.
	1mM CA	21,28.	29,87.*	76,34.	40,33.	116,77.
	0mM CA	20,87.	29,04.*	78,00.*	38,77.	106,77.*
	1.0mM CA	19,10.*	28,18.*	72,27.*	30,89.*	98,16.*
	Urea 0.0%	23,28.	38,12.*	82,40.*	42,89.*	120,29.*
	Urea 0.0% + 1mM CA	23,03.	30,83.*	82,24.*	42,70.*	124,94.*
	Urea 0.0% + 0mM CA	22,10.	30,90.*	80,10.*	42,02.*	122,12.*
	Urea 0.0% + 1.0mM CA	22,00.	34,42.*	78,30.	41,00.*	119,30.*
	Urea 1.0%	20,77.*	40,77.*	94,20.*	48,00.*	142,20.*
	Urea 1.0% + 1mM CA	20,02.*	40,47.*	94,10.*	47,72.*	141,77.*
	Urea 1.0% + 0mM CA	20,10.*	38,27.*	93,81.*	47,13.*	140,94.*
	Urea 1.0% + 1.0mM CA	24,29.*	38,08.*	92,10.*	47,33.*	138,48.*
L.S.D	2,03	1,933	2,737	2,02	3,796	
Late	Control	20,04.	22,08.	73,22.	49,82.	113,04.
	1mM CA	22,41.*	22,12.	72,04.	48,70.	111,19.
	0mM CA	22,79.*	20,47.*	72,24.	48,21.*	110,00.*
	1.0mM CA	20,70.*	20,17.*	70,17.*	48,17.*	108,24.*
	Urea 0.0%	27,24.	23,06.	70,29.	00,07.	110,77.*
	Urea 0.0% + 1mM CA	20,82.	23,72.	70,11.	00,42.	110,03.*
	Urea 0.0% + 0mM CA	24,72.	22,47.	70,07.	00,18.	110,20.
	Urea 0.0% + 1.0mM CA	23,47.*	21,00.	74,21.	48,37.*	112,07.
	Urea 1.0%	27,49.*	23,89.*	79,34.*	02,47.*	121,81.*
	Urea 1.0% + 1mM CA	27,17.	23,04.	77,42.*	02,12.*	119,04.*
	Urea 1.0% + 0mM CA	20,00.	22,14.	70,22.	01,34.*	116,06.*
	Urea 1.0% + 1.0mM CA	20,42.	21,31.	73,14.	49,31.	112,40.
L.S.D	1,783	1,240	3,018	1,402	2,371	

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 0.0% urea fertilizer caused increase in glucose, sucrose, polysaccharides and total carbohydrates content but decreased total soluble sugars content. Whereas the interaction of 0.0% urea with 1mM CA decreased sucrose, total soluble sugars and total carbohydrates content but increased glucose and polysaccharides contents. Also 0.0% urea + 0mM CA increased glucose, total soluble sugars, total carbohydrates and polysaccharides, and

decreased sucrose content. While 0% urea with 1 mM 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 1 mM and decreased with 0 mM and 1 mM CA mixed with 100% urea.

Table 1: 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	40,340	144,120
	1 mM CA	31,740	44,210	97,240*	47,210	144,460
	0 mM CA	31,070	41,470	90,730*	40,000	141,730
	1 mM CA	30,730	40,770*	90,220*	40,810	141,020
	Urea 0%	33,090	40,280	98,320	47,230	145,000
	Urea 0% + 1 mM CA	33,420	44,230	97,730	47,220	144,900
	Urea 0% + 0 mM CA	33,000	42,330	97,200*	40,300	141,000
	Urea 0% + 1 mM CA	31,210	43,020	90,260*	44,720	139,880*
	Urea 100%	40,090*	50,230*	110,470*	50,720*	171,190*
	Urea 100% + 1 mM CA	38,130*	48,000*	99,720	48,210*	147,830
	Urea 100% + 0 mM CA	37,730*	40,060	102,040*	47,230	149,770*
	Urea 100% + 1 mM CA	33,420*	44,800	98,300	40,210	144,060
	L.S.D	2,073	2,177	2,333	2,408	4,450
Late	Control	47,180	39,260	87,320	49,820	137,140
	1 mM CA	44,200	38,420	87,240	47,360*	134,700
	0 mM CA	43,870*	37,040	80,770	47,200*	132,920*
	1 mM CA	42,730*	37,160	84,210*	47,220*	131,430*
	Urea 0%	50,470*	43,010*	88,470*	50,270	138,740
	Urea 0% + 1 mM CA	50,210*	40,720	87,940	49,970	137,910
	Urea 0% + 0 mM CA	48,760*	39,020	87,240	48,770	135,910
	Urea 0% + 1 mM CA	48,220*	38,140	80,230	47,090*	132,320*
	Urea 100%	53,740*	42,070*	89,760*	52,470*	142,230*
	Urea 100% + 1 mM CA	51,080*	41,780	88,340	52,140*	140,480*
	Urea 100% + 0 mM CA	50,420*	39,780	87,410	50,890	138,300
	Urea 100% + 1 mM CA	50,040*	38,740	87,080	50,120	137,200
	L.S.D	1,973	2,746	2,077	1,349	3,102

Also at the late date of cultivation during the flowering stage, results in table ٦ show that, comparing to control levels, ١mM, ٥mM and ١٠mM CA decreased all the determined carbohydrates fractions. On the other hand ٥٠% urea increased the determined carbohydrate contents. Meanwhile the interaction of ٥٠% urea with the three concentrations of CA increased glucose content significantly, whereas ٥٠% urea+ ١mM CA decreased sucrose, total soluble sugars, polysaccharides and total carbohydrates. Also ٥٠% urea+ ٥mM led to increase in sucrose and decrease in total soluble sugars, polysaccharides and total carbohydrates content, while the combination of ٥٠% urea and ١٠mM CA decreased sucrose, total soluble sugars polysaccharides and total carbohydrates. Regarding ١٠٠% urea either alone or combined with ١mM CA, an increase in all the determined carbohydrates content was detected. Interaction of ٥mM and ١٠mM CA with ١٠٠% urea led to increase in glucose, polysaccharides and total carbohydrates content and increased sucrose and total soluble sugars with ٥mM CA combined with ١٠٠% urea and decreased in response to ١٠mM CA + ١٠٠% 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, ١٩٩٧; Nielsen *et al.*, ١٩٩٨; Paul and Foyer, ٢٠٠١). N plays an important role in synthesis of the plant constituents through the action of different enzymes activity and protein synthesis (Jones *et al.*, ١٩٩١) 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, ٢٠٠٠). Increase the N fertilizer caused a significant increase in the seed yield of *Mentha arvensis* L and *Anethum graveolens* L. (Munsi, ١٩٩٢; Randhawa *et al.*, ١٩٩٦ respectively). N fertilization increased the vegetative growth, essential oil, fixed oil, total carbohydrates, soluble sugars and NPK content of *Nigella sativa* L. plants (Khalid, ٢٠٠١). Zheljzkov and Margina (١٩٩٦) 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 (٢٠٠٤) 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 ٧ 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 ١mM 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 ٥٠% urea either alone or when mixed with the three concentrations of CA. However, ١٠٠% urea either alone or combined with different CA concentrations increased both ammonia- and amino-N. Amide- N increased with ٥٠% urea either alone or in combination with ١mM CA, and this

parameter decreased with 0mM and 1mM CA mixed with 0% urea. Meanwhile, 100% urea either alone or in addition to 1mM and 0mM CA increased amide- N content, while 100% urea+1mM CA caused in amide- N content.

Table 5: 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	4.33.	3.62.	1.88.	11.37.	23.46.	22.19.
	1mM CA	4.20.	3.74.	1.82.	11.96.	23.19.*	21.80.
	0mM CA	4.07.	3.40.	1.79.	11.23.*	21.86.*	21.63.
	1mM CA	3.92.*	3.18.	1.74.	11.07.*	21.42.*	21.30.
	Urea 0%	4.63.*	3.94.*	1.99.	12.48.	25.62.*	23.14.
	Urea 0% + 1mM CA	4.61.*	3.82.*	1.89.	12.12.	24.10.	23.03.
	Urea 0% + 0mM CA	4.00.	3.01.	1.80.	11.84.	23.80.	23.01.
	Urea 0% + 1mM CA	4.07.	3.12.	1.79.	11.03.	23.26.	22.73.
	Urea 100%	4.14.*	4.26.*	1.98.	12.93.*	28.72.*	26.79.*
	Urea 100% + 1mM CA	4.06.*	4.17.*	1.96.	12.44.	27.40.*	24.01.
	Urea 100% + 0mM CA	4.81.*	4.13.*	1.97.	11.96.	27.29.*	24.33.
	Urea 100% + 1mM CA	4.72.*	3.91.*	1.87.	11.70.	24.12.	23.47.
	L.S.D	1.270	1.200	1.311	1.494	1.079	1.237
Late	Control	4.60.	4.00.	1.79.	12.48.	24.71.	22.23.
	1mM CA	4.23.*	3.92.	1.70.	11.87.	23.16.	23.29.*
	0mM CA	4.12.*	3.73.*	1.79.*	11.03.	23.76.*	22.23.
	1mM CA	3.80.*	3.04.*	1.76.*	11.37.*	23.12.*	21.50.
	Urea 0%	4.79.	4.27.*	1.96.*	14.42.	27.67.	22.24.
	Urea 0% + 1mM CA	4.63.	4.12.	1.94.*	12.69.	24.90.	22.26.
	Urea 0% + 0mM CA	4.49.	4.07.	1.91.*	12.44.	24.80.	23.01.
	Urea 0% + 1mM CA	4.36.*	3.87.	1.87.*	12.29.	24.76.	23.47.*
	Urea 100%	4.37.*	4.07.*	1.14.*	10.73.*	28.97.*	23.24.*
	Urea 100% + 1mM CA	4.21.*	4.33.*	1.88.*	12.74.	27.40.*	23.81.*
	Urea 100% + 0mM CA	4.44.*	4.21.	1.91.*	12.70.	27.40.	23.70.
	Urea 100% + 1mM CA	4.80.	3.94.	1.83.	11.88.*	24.82.	24.74.*
	L.S.D	1.187	1.193	1.074	1.480	1.077	1.184

Total soluble- and protein- N increased as a result of treating anise plants with 0% urea either alone or in combination with three different CA concentrations. On the other hand total- N increased in plants supplied with 0% 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 6 show that, all the determined nitrogenous constituents decreased with the three concentrations of CA. Fertilization with 0% 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 ^Λ: 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	0,43*	4,32*	1,47*	13,40*	47,31*	32,91*
	1mM CA	0,37*	4,18*	1,30*	12,87*	47,16*	32,29*
	0mM CA	4,82*	3,80*	1,33*	12,02*	44,82*	32,20*
	1.0mM CA	4,01*	3,47*	1,19*	11,82*	43,98*	32,16*
	Urea 0.0%	0,98*	4,79*	1,82*	14,07*	50,82*	35,26*
	Urea 0.0% + 1mM CA	0,93*	4,46*	1,72*	14,13*	49,73*	35,20*
	Urea 0.0% + 0mM CA	0,47*	4,01*	1,73*	13,86*	48,21*	34,30*
	Urea 0.0% + 1.0mM CA	0,12*	4,44*	1,07*	13,42*	48,11*	34,79*
	Urea 1.0%	7,78*	0,23*	1,97*	10,44*	52,96*	37,02*
	Urea 1.0% + 1mM CA	7,03*	4,87*	1,84*	14,83*	52,79*	37,97*
	Urea 1.0% + 0mM CA	7,07*	4,72*	1,82*	14,04*	51,84*	37,30*
	Urea 1.0% + 1.0mM CA	0,92*	4,42*	1,79*	13,92*	50,91*	37,99*
	L.S.D	0,221	0,422	0,107	1,217	1,087	1,308
Late	Control	7,24*	0,02*	1,32*	10,28*	47,89*	31,21*
	1mM CA	7,00*	0,11*	1,28*	14,84*	40,27*	30,43*
	0mM CA	0,87*	4,88*	1,76*	14,00*	40,31*	30,81*
	1.0mM CA	0,29*	4,39*	1,09*	13,77*	44,47*	30,70*
	Urea 0.0%	7,94*	0,79*	1,07*	17,72*	49,04*	31,22*
	Urea 0.0% + 1mM CA	7,93*	0,76*	1,02*	17,43*	47,78*	31,20*
	Urea 0.0% + 0mM CA	7,47*	0,08*	1,47*	17,77*	47,22*	30,77*
	Urea 0.0% + 1.0mM CA	7,12*	0,04*	1,37*	10,48*	47,94*	31,47*
	Urea 1.0%	7,30*	7,27*	1,89*	17,93*	51,28*	32,30*
	Urea 1.0% + 1mM CA	7,86*	7,07*	1,77*	17,09*	51,19*	32,70*
	Urea 1.0% + 0mM CA	7,39*	0,93*	1,74*	17,82*	50,79*	32,97*
	Urea 1.0% + 1.0mM CA	7,12*	0,71*	1,71*	10,80*	50,00*	32,10*
	L.S.D	0,207	0,381	0,093	1,718	2,422	1,067

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 النيتروجيني على نمو وأيض نبات الينسون

عمر عبد السميع الشهابي، سامية على هارون، سامي أبو القاسم أبو حامد و ريهام عبد الله شمس الدين
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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 الصبغي) أيضا حدث اختزال في محتوى الكربوهيدرات (الجلوكوز، السكروز، السكريات العديدة والمحتوى الكلي للكربوهيدرات) كما أدى إلى نقص في محتوى المركبات النيتروجينية (الأمونيا، الامينو، الاميد، المحتى النيتروجيني الذائب والبروتين والمحتوى النيتروجيني الكلي) عند مقارنتها بالعينات الضابطة وذلك خلال مواعي الزراعة المختلفين. بينما أدت معاملات تركيزي اليوريا المستخدم (٥٠% - ١٠٠%) إلى زيادة كل دالات النمو، المكونات النسبية لأصباغ البناء الضوئي، محتوى الكربوهيدرات و المحتوى النيتروجيني 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 مواعده عن زراعته في الموعد المتأخر.