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Effect of harvest time of spring safflower (*Carthamus tinctorius* L.) florets on the production of red and yellow pigments

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RESEARCH ARTICLE

Abstract

A factorial experiment in a randomised complete block design with three replications was conducted to study the effect of harvest time of florets of spring safflower varieties on the amount of red (carthamin) and yellow (carthamidin) pigments in the petals. The first factor was the variety of safflower (MEC88, MEC59, Goldasht, Zendehrood, Sina) and the second factor was harvest time of florets (onset of flowering, after pollination at onset of petal wilting). The results showed that, at the onset of flowering, spring safflower varieties showed the highest yield of florets and MEC59, MEC88, Zendehrood and Sina showed good yield of florets. Harvest time affected the amount of carthamin and carthamidin; florets produced more carthamidin at the beginning of flowering and the amount of carthamidin decreased during flower development, while the amount of carthamin increased. The effect of cultivars was significant. To get the highest level of carthamidin, Zendehrood should be harvested at the onset of flowering. If carthamin is needed, Zendehrood should be harvested immediately after pollination, at the onset of petal wilting.

Keywords: carthamidin, carthamin, harvest time, safflower

1. Introduction

The worldwide demand for natural dyes is nowadays of great interest due to the increased awareness on therapeutic properties of natural dyes in public. Natural dyes are derived from naturally occurring sources such as plants, insects, animals and minerals without any chemical treatment (Chengaiah *et al.*, 2010). Among the all-natural dyes, plant-based pigments have wide range of medicinal values. Although known for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognised only in the recent past. Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity (Hussein *et al.*, 1997). Singh *et al.* (2005) studied the antimicrobial activity of some natural dyes. Optimised natural dye powders of *Acacia catechu*, *Kerria lacca*, *Rubia cordifolia* and *Rumex maritimus* were obtained from commercial industries and they showed antimicrobial activities (Singh *et al.*, 2005). This is clear evidence that some natural dyes by themselves have medicinal properties.

Safflower (*Carthamus tinctorius* L.), belonging to the family Asteraceae, is a multipurpose oilseed crop grown mainly for its high quality edible oil and bird seed. Safflower is also grown for its flowers which are used as cut flowers, in colouring and flavouring food, in textile dyes, as livestock forage, as a vegetable, in herbal teas, and for medicinal purposes (Emongor, 2010). Crops of safflower are cultivated in India, Mexico, Argentina, Australia, Canada, China, Spain, Italy, Turkey, Iraq, Iran, Egypt, and Ethiopia, and the safflower is an alternative oil crop for the dry lands of these countries (Basalma *et al.*, 2008). It is resistant to cold, drought, and salinity stress (Soliman *et al.*, 2012). Therefore, it can be grown successfully on dry land and in surrounding regions which have insufficient precipitation (Basalma *et al.*, 2008; Lovelli *et al.*, 2007; Mahasi *et al.*, 2006). Safflower oil is preferred over other seed oils because of its high degree of poly-unsaturation and elevated levels of α-tocopherol (Talat and Anwar, 2009; Velasco *et al.*,

2005). Vegetable oil is one of the fundamental components in foods that have important functions in human health and nutritional physiology. Consumers have demanded healthier oils that are naturally low in saturated fats. From this perspective, safflower has received a lot of attention as a source of vegetable oil. Safflower seeds contain 35-50% oil, 15-20% protein, and 35-45% hull fraction (Rahamatalla *et al.*, 2001). Safflower is also used as a source of alternative fuel (biodiesel) (Nosheen *et al.*, 2011).

Before cheaper aniline dyes became available; safflower was mainly grown for dye. The water-soluble yellow dye, carthamidin, and a water-insoluble red dye, carthamin, which is readily soluble in alkali can be obtained from safflower florets (Bernard *et al.*, 2011; Chavan *et al.*, 2011; Cho *et al.*, 2000; Gao *et al.*, 2000; Kizil *et al.*, 2008; Machewad *et al.*, 2012). These compounds have been classified into the quinochalcone family of flavonoids which have a unique structure with a C-glycosylated cyclohexanonedienol moiety that occurs only in *C. tinctorius* (Kazuma *et al.*, 2000; Meselhy *et al.*, 1993). Carthamin is extracted from its flowers and it is used for treatment in the form of infusion for circulatory system related diseases (Carapetian and Zarei, 2005). In addition to the colouring properties (Carvalho *et al.*, 2006), safflower petals are used for curing several chronic diseases such as hypertension, coronary heart ailments, rheumatism, male and female fertility problems (Bernard *et al.*, 2011). Furthermore, in China, safflower is also used for the treatment of many cardiovascular, cerebrovascular and gynaecological diseases (Tian *et al.*, 2010). Currently, there are many scientific evidences about safflower biological activities, for example, anti-coagulant effect, anti-hypertensive property and lipid lowering activity (Fan *et al.*, 2009). Carthamidin has been used as a natural food colourant for a long time, mainly in coloured juice, jelly and candy because of its water solubility (Sato *et al.*, 2005). Besides their colour additive uses, yellow pigments are of pharmaceutical interest (Yu and Xu, 1997).

Natural colourants often are very sensitive to external factors and sooner or later, deteriorate from their original colouration. Temperature, UV light, pH, gas phase, metal ions and certain chemicals are known to all be decisive instigators for facilitating colour bleaching (Kanehira *et al.*, 1990). Carthamidin is more stable than carthamin at temperature and pH, but carthamin is more stable than carthamidin at light irradiation (Fatahi *et al.*, 2009). Carthamidin is relatively stable to temperature at acidic pH and preservation of pigments on basic pH is less than acidic pH (Yoon *et al.*, 2003). Under both acid and basic conditions, carthamin readily lost its normal red colouration (Kanehira *et al.*, 1990). This study was initiated to determine whether or not harvest times of florets significantly alter the yield of florets, petal colour, percentage of carthamin and carthamidin and yield of carthamin and carthamidin of five safflower cultivars.

2. Materials and methods

Growth conditions

This study was conducted at the Agricultural Research Farm of Zanjan University, Zanjan, Iran (36° 41' N, 48° 29' E) in the crop year of 2012-2013. Each plot had a length of 5 m with 4 planting lines 50 cm apart; plant density was 40 plants per m^2. Fertiliser consisting of 40 kg/ha N (ammonium nitrate, 33%) and 40 kg/ha of phosphorus (triple super phosphate, 46% phosphorus) was applied at the time of sowing. When the plants were 10 cm high, 40 kg/ha nitrogen dose was side dressed. Weeds were controlled by hand, when needed.

Experimental design and treatments

The experiment was factorial in a randomised complete block design with three replications. The first factor was the cultivars of spring safflower at five levels and the second factor was harvest time of florets at two levels. Cultivars included: MEC88, MEC59, Goldasht, Zendehrood and Sina which were selected based on the different agronomical characteristics (Table 1). These cultivars have suitable seed yield and are cultivated for oil production. Cultivar Sina has improved tolerance to rain. Florets were harvested twice: at the beginning of the flowering stage (immediately after the flowers had opened), and after pollination (at the beginning of petal wilting).

Table 1. Characteristics of safflower cultivars used in this study.

Cultivar	Height	Spine	Head size	Colour of florets
MEC88	medium	spiny	medium	yellow
MEC59	medium	spiny	large	light red
Goldasht	short	spineless	large	red
Zendehrood	tall	spineless	medium	red
Sina	medium	spiny	small	yellow

Yield and colour of the florets

After harvest, florets were placed in an oven at 40 °C for 12 hours and their yield was calculated. A colour code was developed to quantify the colour characteristics of the petals. Colours ranged from pale yellow (code 1) and increased in intensity of red to dark red (code 10). Calculation of the percentage of yellow pigment (carthamidin) and red pigment (carthamin) was based on recommendations by the FAO (1998) with slight modifications.

Extraction of carthamin

Dry petal (10 mg) was weighed accurately and yellow pigments were extracted with 100 ml of citric acid/disodium hydrogen phosphate buffer solution (pH 5) for overnight and then filtered. After that, residual petals were soaked in 100 ml of distilled water for 1 hour (it was repeated 3 times). The residual petals were air dried; then soaked in 15 ml dimethylformamide for 3 hours and filtered. Carthamin was determined at 525-535 nm using a spectrophotometer (Lambada-25; Perkinelmer, Waltham, MA, USA). The percentage of colouring matter (P) was calculated using the following equation:

$$P = \frac{A}{992} \times \frac{200}{W} \quad (1)$$

Where W = weight of the sample in gram; A = the maximum absorbance of the sample in the range of 525-535 nm; 992 = the specific absorbance of carthamin.

The carthamin yield was calculated by multiplying the percentage of carthamin in the yield of florets.

Extraction of carthamidin

Dry petal (20 mg) was weighed accurately and yellow pigments were extracted with 200 ml of citric acid/disodium hydrogen phosphate buffer solution (pH=5) for overnight and then filtered. Carthamidin was determined at 400-408 nm using spectrophotometer. The percentage of colouring matter (P) was calculated using the following equation:

$$P = \frac{A}{487} \times \frac{200}{W} \quad (2)$$

Where W = weight of the sample in gram; A = the maximum absorbance of the sample in the range of 400-408 nm; 487= the specific absorbance of carthamidin.

The carthamidin yield was calculated by multiplying the percentage of carthamidin in the yield of florets.

Data analysis

Data was analysed using SAS software (version 9.1; SAS Institute, Cary, NC, USA). The means were compared using Duncan's multiple range test ($P \leq 0.05$) and the graphs were executed in Excel 2007 (Microsoft, Redmond, WA, USA).

3. Results and discussion

Yield of florets

The results of ANOVA showed a significant effect for cultivar at the 5% probability level and a significant effect for harvest time at the 1% probability level (Table 2). The comparison of means showed that MEC59 cultivar (mean = 647.53 kg/ha) had the highest yield of florets (Table 3). The lowest yield of florets was for Goldasht (mean =

Table 2. Analysis of variance for different traits including yield of florets, petal colour, carthamidin percentage, carthamin percentage, carthamidin yield and carthamin yield of safflower cultivars.[1]

		Mean squares					
Source of variation	DF	Yield of florets	Petal colour	Carthamidin percentage	Carthamin percentage	Carthamidin yield	Carthamin yield
Replication	2	51,583.54**	0.03ns	1.10*	0.0004**	349,146,539.8**	30,585.63**
Cultivars	4	11,401.27*	64.72**	1.73**	0.0007**	150,459,473.1**	25,018.66**
Harvest times	1	48,510.15**	2.13**	7.15**	0.0002*	841,872,559.1**	369.43ns
Cultivars×harvest times	4	886.93ns	0.22ns	1.05*	0.0001**	67,026,211.2ns	4,546.48ns
Error	18	3,518.30	0.11	0.28	0.00002	28,091,596.0	1,870.38
CV (%)		9.80	6.47	10.43	16.61	16.92	25.11

[1] *, ** significantly different at the 0.05 and 0.01 levels of probability, respectively; ns not significant at the 0.05 level of probability.
DF = degrees of freedom; CV = coefficient of variation.

543.47 kg/ha) (Table 3). We grouped the cultivars from an agricultural point of view into high yield (MEC59, MEC88 and Zendehrood) and acceptable yield (Sina and Goldasht). Red flowered spineless cultivars should be preferred for ornamental or dye purposes (Pascual-Villalobos and Alburquerque, 1995). Little differences were seen in the yield of florets, except when harvesting was done at the onset of flowering compared to the onset of petal wilting. Harvest time had an effect on yield of florets; safflower plants produced the best yield at onset of flowering, but this decreased during flower development (Table 3). If the goal of cultivation of safflower is to use its florets, harvesting them at the onset of flowering results in the highest yield of florets. The decrease in yield of florets was a result of the loss of florets after pollination because they gradually withered and some were lost. Petal harvest period is very important for yield of florets. This could affect the quality of petal regarding dye content. For this reason, quality and yield of florets are correlated. The petal collection and seed harvesting can take place at the same time without loss in seed production. Kizil *et al.* (2008) stated that petal yield of spiny cultivars was lower compared to the yield from spineless cultivars, which indicated that the farmers could earn more profit from petals by cultivating spineless cultivars without having adverse effects on yield components like seed yield and oil content (Kizil *et al.*, 2008).

Petal colour

The results of ANOVA showed a significant effect on the colour of the petals for cultivars at the 1% probability level and a significant effect for harvest time at the 1% probability level (Table 2). A comparison of means showed that the cultivars were significantly different for petal colour, which is indicative a genetic component for this trait over harvest time. Goldasht cultivar had the highest rating for redness in petals and MEC88 cultivar had the highest rating for yellow pigment in petals (Table 3). The colour of the petals is the result of the combination of the two pigments; the results indicated that there was a higher percentage of red pigment in Goldasht cultivar. Harvest time also had a significant on the colour of the petals. Harvesting florets after pollination at the onset of petal wilting resulted in petals of more intense red colour. If the goal of cultivation of safflower is to harvest red pigment, harvesting the florets at the onset of petal wilting is appropriate. If the goal is to harvest yellow pigment, harvesting florets at the onset of flowering produced the highest values.

Percentage of carthamidin and carthamin

The results of ANOVA showed the significance of the cultivar at the 1% level of probability, harvest time at the 1% level of probability, and the interaction of cultivar and harvest time at the 5% level of probability for percentage of carthamidin (Table 2). The comparison of means showed a significant difference between cultivars for carthamidin. Zendehrood (mean = 5.933%) had the highest percentage of carthamidin, but this was not significantly different from the percentage of carthamidin for MEC59 (mean = 5.313%) (Table 3). The lowest percentage of carthamidin was recorded for Sina (mean = 4.618%) (Table 3). The stage at which the florets were harvested affected the percentage of carthamidin. Florets had a greater percentage of carthamidin when harvested at the onset of flowering; for those harvested after pollination at the onset of petal wilting, the percentage of carthamidin decreased (Table 3).

Table 3. Effects of cultivars and harvest times of florets on different traits of safflower including yield of florets, petal colour, carthamidin percentage, carthamin percentage, carthamidin yield and carthamin yield.[1]

Treatments	Yield of florets (kg/ha)	Petal colour	Carthamidin percentage (%)	Carthamin percentage (%)	Carthamidin yield (g/ha)	Carthamin yield (g/ha)
Cultivars						
MEC88	629.89^{a}	1.500^{e}	4.758^{b}	0.018^{b}	30,867^{bc}	113.86^{c}
MEC59	647.53^{a}	4.333^{c}	5.313^{ab}	0.022^{b}	34,845^{ab}	143.56^{c}
Goldasht	543.47^{b}	9.667^{a}	4.840^{b}	0.038^{a}	26,328^{c}	210.53^{b}
Zendehrood	628.83^{a}	7.000^{b}	5.933^{a}	0.041^{a}	37,811^{a}	266.57^{a}
Sina	576.40^{ab}	2.833^{d}	4.618^{b}	0.022^{b}	26,779^{c}	126.59^{c}
Harvest times of florets						
Onset of flowering	645.44^{a}	4.800^{b}	5.581^{a}	0.026^{b}	36,623^{a}	168.71^{a}
After pollination at onset of petal wilting	565.01^{b}	5.333^{a}	4.604^{b}	0.031^{a}	26,028^{b}	175.73^{a}

[1] Mean values sharing similar superscript letter(s) in a parameter are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests.

The interaction between cultivar and harvest time was significant and was caused by the reactions of the cultivars to the stage at harvest. Zendehrood florets harvested at the onset of flowering showed the highest percentage of carthamidin (Figure 1). MEC88 florets harvested after pollination at the onset of petal wilting showed the lowest percentage of carthamidin (Figure 1). Zendehrood, MEC59, and MEC88 cultivars showed significantly different percentages of carthamidin between harvest times (onset of flowering and after pollination at the onset of petal wilting) (Figure 1). Goldasht and Sina cultivars did not show a significant difference for percentage of carthamidin by harvest time (Figure 1).

The results of ANOVA showed a significant effect on the percentage of carthamin for cultivars at the 1% level of probability, harvest time at the 5% level of probability, and the interaction of cultivars and harvest times at the 1% level of probability (Table 2). A comparison of means showed that there was a significant difference for percentage of carthamin by cultivar. The Zendehrood cultivar (mean = 0.041%) had the highest percentage of carthamin, but this not significantly different from the percentage of carthamin for Goldasht (mean = 0.038%) (Table 3). The lowest percentage of carthamin was recorded for MEC88 cultivar (mean = 0.018%) (Table 3). Harvest time also had an effect on the percentage of carthamin. Florets showed a lower percentage of carthamin at onset of flowering; the percentage increased after pollination at the onset of petal wilting (Table 3). The significance of the interaction between cultivars and harvest times was a result of the different reactions of the cultivars to harvest time. Zendehrood cultivar had the highest percentage of carthamin for harvest after pollination at onset of petal wilting (Figure 2). Sina cultivar had the lowest percentage of carthamin for harvest at the onset of flowering (Figure 2). Zendehrood, Goldasht, Sina, and MEC59 showed significant differences between times for the percentage of carthamin; MEC88 did not show a significant difference between harvest times (Figure 2).

The colour of the petals varied by cultivar and also in one cultivar at different stages of flower development (bud, bloom, wilting). Overall, florets were white, light yellow, yellow, orange, reddish orange, or dark red. When the florets were freshly picked, the petals appeared to be yellow, whether they were orange flowers or yellow ones, however, the yellow flowers tended toward brownish yellow at wilting, while the orange flowers looked red-orange at wilting. The results showed that florets harvested after pollination at the onset of petal wilting became darker in colour and the percentage of carthamin increased. The flower colour of safflower is yellow just after flowering and gradually changes from yellow to red, which is due to the synthesis of carthamin from the yellow precursors. Precarthamin was thought to be a direct precursor of carthamin (Kazuma *et al.*, 1995; Kumazawa *et al.*, 1994). Precarthamin was converted to a red pigment by a homogeneously purified enzyme from the yellow petals of safflower in 50 mM citrate

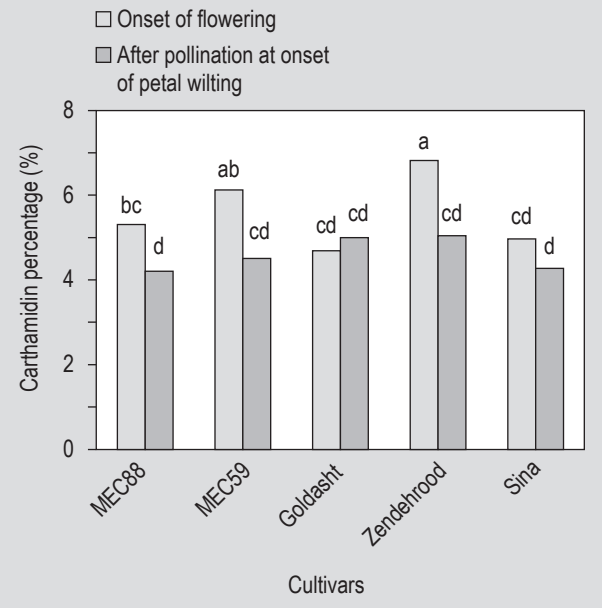

Figure 1. Combined effects of safflower cultivars and harvest time on carthamidin (yellow pigment) in florets. Mean values sharing similar letter(s) are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests.

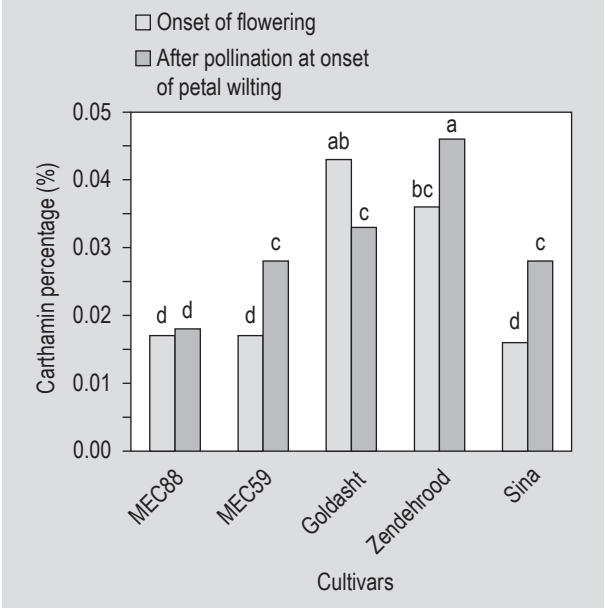

Figure 2. Combined effects of safflower cultivars and harvest time on carthamin (red pigment) in florets. Mean values sharing similar letter(s) are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests

buffer (pH=5.0), and the red pigment was identified as carthamin (Cho *et al.*, 2000). The trait for flower colour is genetic. Narkhede and Deokar (1986) found four dominant genes for colour (R-O-C-Y). They found that C genes and combinations of C+O, C+R and C+O+R create greyish-white flowers, Y+C creates red flowers, Y+C+O and Y+C+O+R are responsible for yellowish-brown flowers (Narkhede and Deokar, 1986). Zendehrood and MEC59 cultivars had the highest percentages of carthamidin. Zendehrood and Goldasht cultivars had the highest percentages of carthamin. Zendehrood cultivar showed high potential for production of carthamin and carthamidin. If safflower is cultivated to produce carthamidin, Zendehrood florets harvested at the onset of flowering is recommended. If safflower is cultivated for carthamin, Zendehrood florets harvested after pollination at the onset of petal wilting is recommended. MEC59 cultivar is also suitable for carthamidin and Goldasht is also suitable for carthamin.

Yield of carthamidin and carthamin

The results of ANOVA showed that both cultivar and harvest time had significant effects on carthamidin yield at the 1% level of probability (Table 2). A comparison of means showed that cultivars differed significantly for yield of carthamidin. Zendehrood cultivar (mean = 37,811 g/ha) had the highest yield of carthamidin which was not significantly different from the yield of the MEC59 cultivar for carthamidin (mean = 34,845 g/ha) (Table 3). The lowest yield for carthamidin was for Goldasht (mean = 26,328 g/ha) (Table 3). Harvest time also influenced yield of carthamidin. Florets produced a greater amount of carthamidin at the onset of flowering, but the amount decreased during flower development (Table 3). Carthamidin yield was calculated by multiplying the percentage of carthamidin by the yield of florets. The correlation coefficients indicated that carthamidin yield had the highest correlation with the percentage of carthamidin (r=0.89) and then yield of florets (r=0.88) (Table 4).

The results of ANOVA showed that cultivars had a significant effect on the yield of carthamin at the 1% level of probability (Table 2). A comparison of means showed that cultivars had a significant difference on carthamin yield. Zendehrood cultivar (mean = 266.57 g/ha) had the highest yield for carthamin and MEC88 (mean = 113.86 g/ha) had the lowest yield for carthamin (Table 3). Florets had lower yield for carthamin at the onset of flowering and this increased during flower development, although this was not statistically significant (Table 3). The carthamin yield was obtained by multiplying the percentage of carthamin by the yield of florets. The correlation coefficients indicated that carthamin yield had the highest correlation with the percentage of carthamin (r=0.94; Table 4).

When choosing which variety to cultivate, the characteristics of the cultivars and the production goal should be considered. Zendehrood and MEC59 cultivars produced the highest yield of carthamidin; Goldasht, MEC88, and Sina produced the lowest yield of carthamidin. Zendehrood cultivar produced the highest yield of carthamin; MEC88, MEC59, and Sina produced the lowest yield of carthamin.

4. Conclusions

The observed data indicated that if the goal of cultivation is to produce carthamidin, Zendehrood and MEC59 are recommended. If the goal of planting is to produce carthamin, Zendehrood is recommended. Furthermore, if carthamidin is desired, florets should be harvested at the onset of flowering because a higher rate of carthamidin is produced at this time. If carthamin is desired, florets should be harvested after pollination at the onset of floret wilting to obtain the highest yield of carthamin. For conclusion, if safflower is cultivated for the production of carthamin, it is recommended to harvest after the pollination, at the onset of petal wilting. On the contrary, if high levels of carthamidin (yellow pigment) are needed, it is preferable to harvest safflower plants at the onset of flowering.

Table 4. Correlation coefficients of different traits for safflower cultivars.[1]

Characteristics	Yield of florets	Petal colour	Carthamidin percentage	Carthamin percentage	Carthamidin yield
Petal colour	-0.25^{ns}				
Carthamidin percentage	0.58**	0.15^{ns}			
Carthamin percentage	0.04^{ns}	0.69**	0.11^{ns}		
Carthamidin yield	0.88**	-0.05^{ns}	0.89**	0.09^{ns}	
Carthamin yield	0.35^{ns}	0.55**	0.29^{ns}	0.94**	0.36^{ns}

[1] *, ** significantly different at the 0.05 and 0.01 levels of probability, respectively; ns not significant at the 0.05 level of probability.

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