IMPROVING PETAL, SEED AND OIL YIELDS OF his arti SAFFLOWER USING MELAGROW, GIBBERELLIC ACID AND CYTOKININ Hamza, M. Agronomy Department, Fac. Agric., Cairo Univ., Giza, Egypt Corresponding author email: mohamedhamza@agr.cu.edu.eg



ABSTRACT

Safflower seed yield is generally lower than that of other oil-seed crops. So for this reason, it is necessary to increase safflower yield. Information regarding the effect of growth regulators and its suitable concentration on growth and yield of safflower is very limited. Therefore, two field experiments were conducted at the Desert Experimental Station in Wadi El-Natroon, Fac. Agric., Cairo Univ., during 2012/2013 and 2013/2014 seasons. Three growth regulators i.e., Melagrow, gibberellic acid, cytokinin were applied at three different concentrations viz., 20, 40 and 60 ppm, as well as, the control treatment of distilled water. The results revealed that all safflower traits were significantly affected by the tested growth regulators, except seed index and seed-oil %. Foliar application of the growth promoter Melagrow gave higher values for all studied traits compare with GA₃ and Cytokinin, except plant height and linoleic%. Increase concentration of growth regulators from 0 up to 60 ppm significantly increased petal yield plant⁻¹ by 48.3% and 37.1%, seed yield fed⁻¹ by 26.4% and 21.3%, as well as, oil yield fed⁻¹ by 29.4% and 27.5% in the first and second seasons, respectively. Quality traits, in terms of carthamin%, carthamidin%, oleic%, and oleic/linoleic ratio were significantly increased by increasing levels of growth regulators in both seasons. The highest values of such traits were obtained at 60 ppm level. The interaction effect between growth regulators and concentration on number of capitula plant⁻¹, seed weight plant⁻¹, petal yield plant⁻¹, seed and oil yields fed⁻¹ was significant in both seasons. Spraying Giza-1 cultivar with Melagrow at 40 or 60 ppm four times was recommended for improving yields and its quality.

Keywords: Carthamus tinctorius L.; Growth regulators; Oleic; Linoleic; Oil-seed; Carthamin; Carthamidin.

NTRODUCTION

Safflower has been grown commercially as one of the oldest oil-seed crops. Safflower is mainly used for edible oil (rich in linoleic and/or oleic acids), and for natural dyes (carthamin and carthamidin) as a source of yellow and red dyes for food and clothing (Weiss, 2000). Also, safflower oil is use for cosmetic, soaps, varnishes and paints (Hameed, 2002). Safflower is more drought and salt tolerant than some other oil-seed crops. So it is suitable for the newly reclaimed soils where other oil-seed crops are difficult to grow (Hamza, 2014).

Gupta and Gupta (2005) cleared that phytohormones are organic substances produced naturally in higher plants and control growth or other physiological functions such as the gibberellins; that regulate protein synthesis and stem elongation and cytokinin; that control organ differentiation. Synthetic compounds that act like natural phytohormones are

called plant growth regulators. The application of growth regulators in low concentration regulates growth, differentiation and development of safflower, either by promotion or inhibition (Dholekar *et al.*, 2001), allows physiological processes to occur at their normal rate in soya (Gulluoglu, 2004) and effect on growth in mustard (Akter *et al.*, 2007). Major plant growth regulators significantly improved floral buds in Jojoba (Prat *et al.*, 2008).

 GA_3 is a very potent hormone and it has many effects regulating various physiological processes including seed germination, shoot growth, flowering, floral development and fruit set. Khan *et al.* (1998) reported that foliar application of gibberellic acid at the pre-flowering stage of mustard plants caused 35.5 % increase in leaf area, which apparently enhances dry matter. Baydar (2002) cleared that treating safflower plants by GA_3 had less oil content than the non- GA_3 treated plants. Khandagale *et al.* (2009) found that foliar application of GA_3 at 200 ppm recorded higher number of capitula plant⁻¹ and 100-seed weight of safflower.

Cytokinin stimulates leaf expansion, development of reproductive organs and delays senescence (Mok, 1994). Cytokinins have been used for increasing yield and oil contents of oil seed crops (Rijavec and Dermastia, 2010). Ullah and Bano (2011) used kinetin at rate of 10⁻⁵M during safflower flowering (140 day after sowing) as foliar application. They found that kinetin was highly effective in increasing achen yield, 100-achen weight. Kinetin also was highly effective in increasing oleic acid (C18:1) but decreasing the content of linoleic acid (C18:2).

Melagrow is natural growth promoters extracted from pollen grain of cabbage flowers. It promotes growth, increase yield, improves quality, increase percentage of fruit setting, promotes formation of flower buds and resist flower and fruit dropping. Melagrow is combined effect of auxins, gibberellins, cytokinin, ethylene and hydrogen cyanamid. Its chemical composition is 20% phosphorus, 10% potassium, 3% boron and 0.2% brassinoliods (Attia *et al.*, 2011 and Seadh *et al.*, 2012). Foliar application with Melagrow at rate of 50 ppm twice after 30 and 70 days from sowing significantly recorded the highest values of safflower plant height, number of branches plant⁻¹ and petal yield fed⁻¹ (Attia *et al.*, 2011). Seadh *et al.* (2012) found that the highest values of safflower number of capitula plant⁻¹, 100-seed weight and seed yield fed⁻¹ were obtained also by application of Melagrow at rate of 50 ppm.

The objective of this study was to determine the effect of growth regulators *viz.*, Melagrow, GA_3 and cytokinin with different concentrations as foliar application on petal, seed and oil yields of Giza-1 cv. grown under newly reclaimed sandy soil conditions.

MATERIALS AND METHODS

Two field experiments were conducted at Wadi El-Natroon, Desert Experimental Station, Fac. Agric., Cairo Univ. under drip irrigation system in 2012/13 and 2013/14 seasons. This station is located between 30°32'30" and 30°33'0" N and between 29°57'15" and 29°58'15" E with an altitude of 45

meters. Safflower was preceded by sesame and sunflower in the first and second seasons, respectively. Soil properties of the experimental field are presented in Table (1). This analysis in Table (1) indicates that soil was sandy, saline and poor in nutrients, as well as, organic matter.

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	2012/ 2013	2013/ 2014
Physical properties		
Sand %	93.15	94.85
Silt %	3.85	3.00
Clay %	3.00	2.15
Soil texture	Sandy	Sandy
Chemical properties		
рН	7.80	7.95
Ec (dS/m)	5.33	5.25
Na (mq/l)	36.23	34.24
CI (mq/l)	32.44	32.24
Organic matter (%)	0.30	0.28
Total CaCO ₃ %	3.10	2.55
N (mg kg ⁻¹)	9.63	8.78
P (mg kg ⁻¹)	4.5	3.6
$K (mg kg^{-1})$	80	70

Table 1. Soil properties of experimental field during 2012/13 and 2013/14 seasons.

Each experiment included 12 treatments which were the combination of 3 growth regulators (Melagrow, gibberellic acid and cytokinin) and 3 concentrations (20, 40 and 60 ppm) of each one, as well as, the control treatment with distilled water. The growth regulators were repeted four times at 60, 90, 120 and 150 days after sowing as a foliar application. The commercial growth regulator gibberellic acid 10% $(C_{19}H_{22}O_6)$ was obtained by Elahlia Company. Cytokinin (kinetin, $C_{10}H_9N_{50}$) was obtained by Sigma Company. Melagrow was imported by Green India Export Company. The experimental design was a split plot with three replications. Growth regulators to sub-plots. Each sub-plot consisted of five rows 4.0 m in length, 0.60 m in width, and its area was 12 m². Seeds were sown in hills 10 cm apart on 15th October in both seasons. After 3 weeks from planting, plants were thinned to one plant/hill.

Nitrogen was added at rate of 60 kg N/fed in form of ammonium nitrates (33.5% N) in 5 equal splits, the first was applied after thinning at 21 days from sowing and the rest splits were added at a 14-day interval. Phosphorous in form of super phosphate (15.5% P_2O_5) at rate of 30 Kg P_2O_5 /fed was added before sowing and during soil preparation. Potassium in form of potassium sulfate (48% K₂O) was added at rate of 48 Kg K₂O/fed in five equal splits with the doses of N. Mixture of micronutrients was also sprayed, four times, as a foliar application after thinning at 21-day intervals. Other cultural practice procedures were done as recommended.

At harvest, ten plants from each sub-plot were taken at random where the following characters were recorded: plant height (cm), number of branches plant⁻¹, number of capitula plant⁻¹, seed yield plant⁻¹ (g), seed index (100-seed weight, g) except, petal yield plant⁻¹ (g) was collected after fertilization. Seed yield fed⁻¹ (kg) was weighed from the whole area of each sub-plot and adjusted to yield per feddan (4200 m²). Oil percentage was determined in seeds of each treatment according to AOAC (2000) using Soxthelt apparatus. Oil yield fed⁻¹ (kg) was calculated by multiplying seed-oil percentage by seed yield fed⁻¹. Oleic and linoleic fatty acids were separated according to Vogel (1975) and identified by Gas Liquids Chromatography, Trace GC Ultra, Thermo Scientific (GLC) apparatus according to Farag *et al.* (1981). The water-soluble yellow dye, carthamidin, and the water-insoluble red dye, carthamin, which is readily soluble in alkali, can be obtained from safflower petal according to method of assay by FAO (1997).

Data were statistically analyzed according to procedures described by Steel *et al.* (1997) using MSTAT-C computer package (Freed *et al.* 1989). The treatment means were compared by least significant difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Effect of growth regulators:

An apparent association seems to exist between growth regulators and safflower traits. Plant height, number of branches and capitula plant⁻¹, petal and seed yield plant⁻¹, as well as, ultimately enhanced seed and oil yields fed⁻¹ were significantly affected by growth regulators in both seasons (Table 2). These results, in general, it may be explain by "source to sink" theory which was promoted by growth regulators. The tallest plants were obtained by GA₃ application. This result may be due to the ability of GA₃ to elongate the stem especially certain dwarf and rosette types. These results are in accordance with Ullah and Bano (2011).

Spraying Melagrow surpassed GA_3 and cytokinin and resulted in increasing values of number of branches and capitula plant⁻¹, petal and seed yield plant⁻¹, as well as, seed and oil yields fed⁻¹. The increased branching led to the increase of capitula number plant⁻¹. The increase in number of capitula plant⁻¹ may be resulted in the increase of petal yield plant⁻¹ and seed yield plant⁻¹. On the other hand, Khandagale *et al.* (2009) found that foliar application of GA_3 at 200 ppm recorded higher number of capitula plant⁻¹ and 100-seed weight. Such increase in seed yield fad⁻¹ may be attributed to the considerable increase in number of branches plant⁻¹ and number of capitula plant⁻¹.

Considerable increase of oil yield fed⁻¹ may be due to increases in seed yield fed⁻¹. Attia *et al.* (2011) and Seadh *et al.* (2012) explain the increase of safflower plant height, number of branches plant⁻¹ and petal yield plant⁻¹ by foliar application with Melagrow twice because it contains P, K, B and brassinolide may be due to the role of macro and micronutrients in activating physiological processes.

Also, carthamin%, carthamidin%, oleic%, linoleic%, and oleic/linoleic ratio were significantly affected by growth regulators in both seasons (Table 2). The application of Melagrow was significantly recorded higher values of carthamin%, carthamidin%, oleic% and oleic/linoleic ratio. But the highest percentage of linoleic acid was recorded by GA_3 and the lowest percentage was obtained by cytokinin application. The same trend was observed by Ullah and Bano (2011) who reported that kinetin was highly effective in increasing oleic acid but decreased the content of linoleic acid.

Effect of concentration of growth regulators:

Results in Table (3) show that safflower yields and all of its attributes except, number of branches plant⁻¹ and seed-oil% were significantly affected by concentration of growth regulators in both seasons. A gradual increase in plant height, number of capitula plant⁻¹, petal yield plant⁻¹, seed yield plant⁻¹, seed index, as well as, seed and oil yields fed⁻¹ as concentration of growth regulators increased up to 60 ppm. Increase concentration of growth regulators from 0 up to 60 ppm significantly increased petal yield plant⁻¹ by 48.3% and 37.1%, seed yield fed⁻¹ by 26.4% and 21.3%, as well as, oil yield fed⁻¹ by 29.4% and 27.5% in the first and second seasons, respectively. Such increase in seed yield fed⁻¹ and seed index. Also, oil yield fed⁻¹ may be attributed to increases in seed yield fed⁻¹.

Among various doses of growth regulators the dose of 60 ppm was found to be the most effective dose in increasing safflower quality traits, in terms of carthamin%, carthamidin%, oleic%, and oleic/linoleic ratio in both seasons (Table 3). Conversely, the control treatment recorded the highest percentage of linoleic. Baydar (2000) reported that oil synthesis increases with increasing dose of GA_3 in safflower. From the nutritional point of view, safflower oil is in the top of stable vegetable oils. This view may be due to its higher ratio of oleic/linoleic which resulted by concentrations of 40 and 60 ppm.

Effect of the interaction between growth regulator and concentration:

Results in Table (4) indicate that, number of capitula plant⁻¹, petal yield plant⁻¹, seed yield plant⁻¹, seed and oil yields fed⁻¹ were significantly affected by the interaction between growth regulators and its concentrations in both seasons.

during $2012/2013$ and $2013/2014$ seasons.										
Characters	2012/2013	2013/2014								
Plant height	ns	ns								
No. of branches plant ⁻¹	ns	ns								
No. of capitula plant ⁻¹	*	*								
Petal yield plant ¹	*	*								
Seed index	ns	ns								
Seed yield plant ⁻¹	*	*								
Seed yield fed ⁻¹	*	*								
Seed-oil %	ns	ns								
Oil yield fed ⁻¹	*	*								
Carthamin %	ns	ns								
Carthamidin %	ns	ns								
Oleic %	ns	ns								
Linoleic %	ns	ns								
Oleic/linoleic ratio	ns	ns								

Table	4.	Significance	e of some	safflowe	er character	s as	affected	by the
		interaction	between	growth	regulators	and	concent	rations
		during 2012	/2013 and	2013/201	14 seasons.			

Results in Figure (1) reveal that a gradual increases in number of capitula plant⁻¹ via increasing concentration level of Melagrow, GA_3 and Cytokinin. That it may be explain by the role of these growth regulators in floral development and fruit setting which reflected on number of capitula plant⁻¹. Spraying Melagrow at 60 ppm recorded the highest number of capitula plant⁻¹ (26.4 and 25.0) in both seasons, respectively.



Fig. 1. Number of capitula/plant of safflower as affected by the interaction between growth regulators and its concentrations during 2012/13 and 2013/14 seasons.

The observed increase in petal yield $plant^{-1}$ by the interaction between growth regulators and concentration (Fig. 2) may be due to the increase in number of capitula $plant^{-1}$. The best petal yield $plant^{-1}$ (0.79 and 0.87 g) was obtained from Melagrow × 60 ppm interaction in both seasons, respectively.

ns = not significant and * = significant at probability of 0.05.



Fig. 2. Petal yield/plant (g) of safflower as affected by the interaction between growth regulators and its concentrations during 2012/13 and 2013/14 seasons.

Also, seed yield plant⁻¹ was affected significantly by the interaction between growth regulators and its concentration (Fig. 3). All of growth regulators \times concentration interaction recorded heavier seed weight plant⁻¹ than the control application but the heaviest seed weight plant⁻¹ was obtained by Melagrow \times 60 ppm followed by Melagrow \times 40 ppm interactions in both seasons. The observed increase in seed yield plant⁻¹ may be attributed to the enhanced translocation of assimilates from leaves to reproductive parts and to increases in number of capitula plant⁻¹.



Fig. 3. Seed yield/plant (g) of safflower as affected by the interaction between growth regulators and its concentrations during 2012/13 and 2013/14 seasons.

Figure (4) show that the highest seed yield fed⁻¹ was recorded via Melagrow \times 60 ppm interaction (1390.5 and 1305.2 kg) in both seasons, respectively with insignificant effect between Melagrow \times 60 ppm and Melagrow \times 40 ppm. Also, insignificant effect was observed between gibberellic acid \times 40 and gibberellic acid \times 60 ppm in the second season. Meanwhile, clear decline was observed in seed yield fed⁻¹ with increasing

concentration of cytokinin from 40 to 60 ppm in both seasons. Considerable increases in number of capitula plant⁻¹ and seed yield plant⁻¹ were reflected to seed yield fed⁻¹. These results are in agreement with those obtained by Roitsch and Ehness (2000), as well as, Ullah and Bano (2011).



Fig. 4. Seed yield/feddan (kg) of safflower as affected by the interaction between growth regulators and its concentrations during 2012/13 and 2013/14 seasons.

Figure (5) clear that a gradual increases in oil yield fed⁻¹ via increasing concentration from 0 up to 60 ppm of Melagrow and GA₃ except, Cytokinin which increase oil yield up to 40 ppm. The highest oil yield fed⁻¹ was recorded via Melagrow × 60 ppm interaction (433.8 and 421.6 kg) in both seasons, respectively. An insignificant effect of the interactions among Melagrow × 40 and 60 ppm, GA₃ × 40 and 60 ppm, as well as, cytokinin × 40 and 60 ppm in both seasons. Oil yield fed⁻¹ may be attributed to increases in seed yield fed⁻¹.





CONCLUSION

The present research clearly demonstrated that three growth regulators and four concentrations have a major effect on petal, seed and oil yields, as well as, quality characters in safflower. It was concluded that growth regulators could be successfully employed for enhancement of safflower seed yield, directly or indirectly, through its components. Based on the findings, it is recommended the use of Melagrow four times, at 60 or 40 ppm for higher petal, seed and oil yields, as well as, carthamin%, carthamidin%, oleic acid% and oleic/linoleic ratio.

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تحسين محصول البتلات, البدرة والزيت في القرطم باستخدام الميلاجرو, حامض الجبر ايلليك والسيتوكينين محمد حمزة قسم المحاصيل- كلية الزراعة- جامعة القاهرة

يعتبر محصول البذرة من القرطم بشكل عام قليل مقارنة بمحاصيل البذور الزيتية الأخرى. ولهذا السبب من الضروري العمل على زيادة المحصول. هناك ندرة في المعلومات المتاحة عن تأثير رش منظمات النمو وتركيز ها الأمثل على نمو ومحصول القرطم. لذلك أجريت تجربتان حقليتان بمحطة التجارب الصحراوية بوادي النطرون التابعة لكلية الزراعة, جامعة القاهرة، خلال الموسيمين ٢٠١٣/٢٠١٢ و٢٠١٤/٢٠١٣ لدراسة تحسين محصول القرطم ومكوناته للرش بثلاثة منظمات للنمو هي الميلاجرو, حامض الجبريلليك والسيتوكينين وكذلك تحديد التركيز المناسب لكل منهم. حيث تم استخدام ثلاثة تركيزات مختلفة من كل منظم نمو هي ٢٠, ٤٠ و ٦٠ جزء في المليون بالاضافة الي معاملة الكنترول الرش بالماء المقطر. استخدام تصميم القطع المنشقة حيث وضىعت منظمات النمو في القطع الرئيسية وتركيز اتها في القطع المنشقة. اظهرت النتائج تأثر جميع الصفات تحت الدر اسة معنوياً بمنظمات النمو فيما عدا دليل البذور ونسبة الزيت بالبذرة. وقد أعطت معاملة الرش بمحفز النمو ميلاجرو أعلي قيم لكل الصفات المدروسة مقارنة بحامض الجبريلليك والسيتوكينين فيما عدا طول النبات ونسبة الحامض الدهني لينوليك. حققت زيادة تركيز منظمات النمو من المستوى صفر إلى ٦٠ جزء في المليون زيادة معنوية في وزن بتلات النبات بنسبة ٤٨.٣٪ و ٢٠.١١٪، محصول البذرة/فيدان بنسبة ٢٦.٤٪ و٢٠.٢٧٪ ومحصول الزيت/فيدان بنسبة ٢٩.٤٪ و٢٠.٧٪ في الموسم الأول والثاني على التوالي. زادت صفات الجودة بشكل معنوي من حيث: نسبة القارطامين، نسبة القارطاميدين, نسبة الاوليك ونسبة الاوليك الي اللينوليك وذلك من خلال زيادة مستويات منظمات النمو في كلا الموسيمين. كما تم الحصول على أعلى القيم من هذه الصفات عند تركيز ٦٠ جزء في المليون. كان التفاعل معنوياً بين منظمات النمو وتركيز اتها لكل من عدد الرؤس الزهرية, وزن بتلات النبات، وزن بذور النبات, محصولي البذرة والزيت للفدان في كلا الموسمين. توصبي هذه الدراسة برش صنف القرطم جيزة-١ بمنشط النمو ميلاجرو بتركيز ٤٠ أو ٦٠ جزء في المليون ٤ مرات للحصول على أعلى عائد من المحصول وصفات الجودة.

Table 2. Means of some	safflower	characters a	as	affected	by	three	growth	regulators	during	2012/2013	and
2013/2014 seasons	5.										

Growth regulators (G)	Plant height (cm)	Branches plant ⁻¹ (no)	Capitula plant ⁻¹ (no)	Petal yield plant ⁻¹ (g)	Seed index (g)	Seed yield plant ⁻¹ (g)	Seed yield fed ⁻¹ (kg)	Seed oil (%)	Oil yield fed ^{⁻1} (kg)	Carth- amin (%)	Carth- amidin (%)	Oleic (%)	Linoleic (%)	Oleic/ Linoleic ratio
							2012/13	season						
Melagrow	130.3b	9.5a	24.6a	0.78a	5.6a	36.9a	1007.8a	30.8a	310.4a	0.020a	0.32a	20.8a	28.4b	0.73a
GA ₃	134.0a	6.5b	20.3b	0.69b	5.3a	25.4b	900.7b	30.7a	276.4b	0.014b	0.29b	15.9b	30.2a	0.53b
Cytokinin	126.4c	4.5c	15.0c	0.56c	5.2a	18.6c	799.2c	30.7a	245.4c	0.011c	0.24c	14.0b	26.2b	0.53b
-							2013/14	season						
Melagrow	129.0 b	9.4a	20.3a	0.70a	4.2a	30.0a	960.2a	31.8a	305.3a	0.020a	0.37a	22.1a	28.9b	0.76a
GA ₃	132.6a	7.0b	16.2b	0.63b	4.1a	18.7b	880.9b	31.7a	279.2b	0.016b	0.32b	18.9b	31.6a	0.60b
Cytokinin	126.1c	5.4c	10.0c	0.52c	4.0a	16.3c	823.4c	31.2a	256.9c	0.013c	0.30c	18.7b	26.0b	0.72a
							41 1100		0.05				1.00	

Means followed by the same letter within columns are not significantly different at p = 0.05 according to least significant difference test.

Table 3. Means of some safflower characters as affected by four concentrations of growth regulators during 2012/2013 and 2013/2014 seasons.

Concent- ration, ppm (C)	Plant height (cm)	Branches plant ⁻¹ (no)	Capitula plant ⁻¹ (no)	Petal yield plant ⁻¹ (g)	Seed index (g)	Seed yield plant ⁻¹ (g)	Seed yield fed ⁻ ¹ (kg)	Seed oil (%)	Oil yield fed⁻¹ (kg)	Carthamin (%)	Carthamidin (%)	Oleic (%)	Linoleic (%)	Oleic/ Linoleic ratio
						20	12/13 seas	on						
Control	100.2d	9.0a	11.8c	0.60d	5.0bc	14.2d	870.3c	29.5a	256.7d	0.012c	0.17d	10.6c	28.7a	0.37c
20	134.5c	9.4a	15.9b	0.70c	5.2b	28.8c	920.5b	29.8a	274.3c	0.013bc	0.19c	15.8b	25.9b	0.61c
40	140.0b	9.4a	20.6a	0.78b	5.4b	32.8b	1056.1b	30.1a	317.9b	0.014b	0.21b	20.4a	18.8c	1.09b
60	144.6a	9.5a	21.9a	0.89a	5.9a	36.2a	1100.0a	30.2a	332.2a	0.016a	0.24a	22.6a	14.9d	1.52a
						20	13/14 seas	on						
Control	90.9d	6.9a	10.4c	0.62d	4.1b	16.6d	750.3d	31.3a	234.8d	0.011cd	0.28d	14.5d	30.1a	0.48c
20	130.3c	7.1a	14.1b	0.67c	4.1b	20.3c	834.2c	31.9a	266.1c	0.012c	0.30c	18.6c	25.9b	0.72c
40	145.4b	7.1a	16.6b	0.81b	4.1b	24.5b	870.9b	32.2a	280.4b	0.014b	0.32b	22.3b	20.6c	1.08b
60	150.5a	7.2a	18.9a	0.85a	4.7a	27.9a	910.1a	32.9a	299.4a	0.016a	0.37a	25.8a	16.5d	1.56a

Means followed by the same letter within columns are not significantly different at p = 0.05 according to least significant difference test.