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EFFECT OF SINK REMOVAL, KINETIN AND CPPU ON MONOCARPIC SENESCENCE OF SUNFLOWER PLANT

Zaghlool, Sanaa, A. M.

Dept. Agric. Bot., Fac. Agric., Ain Shams Univ., Shoubra El-Kheima. Cairo, Egypt

ABSTRACT

Pot experiments were carried out in the green house, Faculty of Agric. Ain Shams University during the two successive seasons of 2005 and 2006. Treatments included head buds removal at 45 days after sowing. As well as, foliar spry of intact plants with kinetin (Kin) at 50, 100 ppm, CPPU (N-(2- chloro-4-pyridyl)-N- phenylurea) at 10, 20 ppm and distilled water as control. Plants received 3 foliar sprays started at 4 weeks after sowing with one week interval. Head removal treatment induced significant reduction in plant height and an increase in leaf area in both seasons. Furthermore, head removal showed an increase in shoot dry weight, this effect reached the levels of significant in the 2nd season.

Application of Kin at 50 and 100 ppm showed marked increase in shoot dry weight. Insignificant differences were found between both Kin concentrations on leaf area and shoot dry weight. Pronounced increase in heads weight was recorded by CPPU at 10 ppm followed by Kin treatments, while, CPPU at 20 ppm showed insignificant effect. Application of CPPU at 10 ppm improved seeds weight/head and 100 seeds weight in both seasons. Head removal treatment developmed lateral heads with few large seeds on the upper 5-6 nodes. Meanwhile, CPPU at 20 ppm resulted in visible inflorescence buds or tiny heads in leaf axils.

All treatments showed an increase in total soluble proteins, chlorophylls and carbohydrates. The highest values were recorded by Kin at 50 ppm. The alternation in sunflower senescence by head

removal and cytokinin treatments and correlated changes in biochemical constituents was discussed.

Key words: Sunflower- Monocarpic senescence- Head removal- Kinetin- CPPU- Growth- plant yield.

INTRODUCTION

Sunlower (*Helanthus annus* L.) became a major international oil seed crop due primarily to the introduction of short stemmed, high yielding hybrid cultivars. Emphasis on the advantage of polyunsaturated fats in human diet spread the use of sunflower oil in edible fats and processed foods (Weiss 2000). Sunflower plants provide an excellent model for studying the mechanism of monocarpic senescence where the sink is situated solely in the top. The onset of monocarpic senescence in sunflower plants occurs at early seed development stage (Zaghlool 1996).

On the other hand, both endogenous cytokinin and auxin signals control monocarpic senescence in sunflower plant. Cytokinins are involved in slowing many of processes that contributed to plant senescence (Mok and Mok 1994). High cytokinins levels in the xylem sap of sunflower characterized the period of vegetative growth reaching their maximum 28 days after planting. Therefore, a little decline occurred in cytokinins level before flowering then dramatically fells as flowering began (Zaghlool 1996), whereas, the pattern of auxin concentration showed an opposite trend to cytokinins (Bangerth *et al.*, 2000).

Applications of synthetic cytokinins are typically more effective in delaying senescence probably due to their stability. It appears that exogenously applied cytokinins delay senescence by correcting an internal deficiency of cytokinins which occur during the senescence process (Singh *et al.*, 1992 a,b). Delaying senescence by exogenous cytokinin application depends on the time of application. Zaghlool (1996) found that application of kinten (kin) during vegetative growth improved plant growth whilst application during flowering period had no effect.

Also, delaying senescence could be achieved by sink (pods) removal in soybean plant resulted in retention of leaves color (Nooden 1984). This also was true with sunflower, Purohit (1982) reported that flower removal slowed the degradation of leaf chlorophyll and consequently delayed leaf senescence.

Thus both sink removal and application of exogenous cytokinins could be delayed senescence. Therefore, the present study aimed to compare both effects; sink removal and cytokinins application on sunflower senescence. Furthermore, the effects of two different synthetic cytokinins; kinetin (kin) and CPPU on plant growth and yield were investigated.

MATERIALS AND METHODS

Pot experiments were carried out in the green house, Faculty of Agric. Ain Shams University during the two successive seasons of 2005 and 2006. Sunflower (*Helianthus anuus L.*) seeds cv. Vedoc kindly obtained from agriculture research center were sown on 2nd of July in both seasons in 35 cm diameter plastic pots filled with clay loamy soil (Table 1).

Table (1): a) Mechanical and b) Chemical properties of the soil.

a) Mechanical analysis

Sand%	Silt%	Clay%
17	27	56

b) Chemical analysis

pH	Organic matter %	Cations (meq/l)				Anions (meq/l)				Total soluble salts (m mhos/cm)
		Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	So_4^{--}	Co_3^{--}	CHO_3^-	
7.9	1.45	3.40	0.63	6.00	3.00	2.60	3.00	---	2.4	0.80

Plants were thinned to 2plants/pot after two weeks from sowing. Pots were arranged in complete block design with 3 replicates. Fertilization was applied with irrigation as Hogland nutrient solution (Gauch, 1972) one strength twice a week starting with thinning till 75 days after sowing. Treatments included head buds removal at 45 days after sowing. As well as, foliar spry of intact plants with kinetin (Kin) at 50, 100 ppm, CPPU (N-(2- chloro-4-pyridyl)-N- phenylurea) at 10, 20 ppm and distilled water as control. Plants received 3 foliar sprays

started at 4 weeks after sowing with one week interval. Tween 20 at 0.05 ml/l was added as a wetting agent.

Two samples were taken at 70 and 95 days from sowing. Growth parameters, plant height, leaf area (7-8 from the top) and shoot dry weight were recorded in th first sample. Plants were oven dried at 70°c /48 hr. for dry weight determination. Leaf area measured by mechanical polar planimeter (Lasico).

Furthermore, chemical analysis was conducted on the upper leaves for determination of total soluble proteins, total chlorophylls and total soluble carbohydrates in the two successive seasons. Endogenous phytohormones, Auxin (IAA) and cytokinin (Zeatin) was determined in leaves of second season. The second sample was taken at harvest, 90 days after sowing where heads weight, seeds weight/head and 100 seeds weight was estimated. The number of lateral buds developed into lateral heads as a result of head removal was recoded.

1. Chemical analysis

1.1. Determination of total soluble proteins.

One gram sample was dried and mixed with 5 ml of extraction buffer (0.125M tris borate, pH 8.9) then shaked for one hour and filtered. The supernatant were contained the soluble protein. A colorimetric determination of soluble proteins was carried out by using the method of (Bradford, 1976).

1.2. Determination of total chlorophyll.

0.1g fresh weight from leaves was homogenized with 10 ml of 80% acetone and the extraction was obtained by filtration of the solvent in Buchner funnel was made up to 20 ml by acetone (80%). Total chlorophylls were determined spectrophotometerically at 663 and 645 nm (Spectronic 21) using the method of Arnon (1949).

1.3. Determination of total soluble carbohydrates.

For extraction 1.0g leaves fresh weight was hydrolyzed with 30 ml HCl 2N. The tubes were placed in a boiling water bath for 6 hr. After cooling, resulting solution was filtered (wt.No.1). The sample was transferred into a calibrated flask (100 ml). Total carbohydrates were estimated by the alkine potassium ferricyanide method (Shales and Schales, 1945).

1.4. Determination of endogenous phytohormones.

Endogenous phytohormones IAA and Zeatin was determined in leaves, two gram fresh weight of sample was extracted overnight in dark at 4°C with 80% redistilled methanol. The method for phytohormones extraction was followed as described by Knegt and Bruinsma (1973). Sep-pak cartridge (C_{18}) was used to separate different groups of phytohormones by using different concentrations of water and methanol with 0.1 N acetic acid as described by Lee *et al* (1989).

1.4.1. cytokinins

Bioassay technique of Fletcher and Mccullagh, (1971) was followed for cytokinin (Zeatin) determination.

1.4.2. Auxin

Indole acetic acid (IAA) determination was carried out according to Larsen *et al* (1962). One ml of the extraction was added to 4 ml PDAB 1% (Paradimethyl aminobenzaldehyde), shaken 3 min and allowed to stand for 60 min. The optical density was determined at 530 nm using spectrophotometer (Spectronic 21). Amount of auxin was calculated according to standerd curve of IAA.

Statistical analysis

The statistical analyses of all data were done by (SAS 1999). Tukey test for separation between means using the following model.

$Y_{ij}= \mu+T_i+e_{ij.}$

RESULTS AND DISCUSSION

A- The results:

Growth parameters:

Data in Table (2) showed significant reduction in plant height induced by head removal treatment in both seasons. Kin and CPPU treatments did not significantly affect this parameter in the first season whereas significant reduction in plant height was noticed in the 2nd season by Kin at 50 ppm and CPPU at 10 ppm.

An increase in leaf area was obtained by head removal and Kin at 50 and 100 ppm treatments in both seasons. The highest values was recorded by Kin at 50 ppm which recorded 123.37 and 132.49 cm^2 comparing with its control plants 62.10 and 66.00 cm^2 in the two

successive seasons respectively. Application of CPPU showed insignificant effects on this parameter.

Furthermore, head removal showed an increase in shoot dry weight, this effect reached the levels of significant in the 2nd season. Application of Kin at 50 and 100 ppm showed marked increase in shoot dry weight. Insignificant differences were found between both Kin concentrations on leaf area and shoot dry weight. CPPU treatments gave insignificant effect on shoot dry weight.

Table (2) Effect of head removal, Kin and CPPU on some growth parameters of sunflower plants at 70 days after sowing during the seasons of 2005 and 2006.

Treatments	2005			2006		
	plant height (cm)	leaf area (cm^2)	shoot dry weight (g)	plant height (cm)	leaf area (cm^2)	shoot dry weight (g)
Control	69.30a	61.50c	13.00b	73.20a	65.80c	13.34c
Head removal	59.22b	85.63b	15.02b	58.33c	89.57b	16.27b
Control*	69.12a	62.10c	12.57b	72.00a	66.00c	13.33c
Kin at 50 ppm	69.32a	123.37a	18.55a	67.67b	132.49a	19.37a
Kin at 100 ppm	69.67a	118.90a	18.67a	71.67a	124.37a	19.71a
Cppu at 10 ppm	66.24a b	64.80c	13.53b	67.67b	67.77c	14.00c
Cppu at 20 ppm	68.23a	66.57c	12.63b	72.33a	69.00c	14.33c
MSD	**7.62**	**13.56**	**2.99**	**3.82**	**9.75**	**1.85**

Control*: Plants foliar sprayed with water

Plant yield parameters:

Pronounced increase in heads weight **(Table 3)** was recorded by CPPU at 10 ppm (172,175.57g) comparing with control plants (74.27, 77.1g) in both seasons respectively followed by Kin treatments. Whereas, CPPU at 20 ppm showed insignificant effect.

Application of Kin at 50 ppm and CPPU at 10 ppm improved seeds weight/head and 100 seeds weight. This effect reached the levels of significance by CPPU at 10 ppm in both seasons, and Kin at 50 ppm on 100 seeds weight in the second season.

Head removal treatment led to the development of 5-6 lateral heads with few large seeds on the upper 5-6 nodes. While, CPPU at 20 ppm resulted in visible inflorescence buds or tiny heads in leaf axils.

Table (3) Effect of kinetin and CPPU on yield parameters/plant at 95 days after sowing during the seasons of 2005 and 2006.

Treatments	2005			2006		
	Head weight (g)	Seeds weight/head (g)	100 seeds weight (g)	Head weight (g)	Seeds weight/head (g)	100 seeds weight (g)
Control*	74.27c	22.87b	4.27b	77.10c	28.30bc	4.70c
kin50	119.73b	29.57ab	4.77ab	126.72b	30.93ab	5.37b
kin100	119.13b	27.43ab	4.67ab	114.77b	26.90bc	4.37c
cppu10	172.00a	32.33a	5.43a	175.57a	33.93a	6.10a
cppu20	90.40c	27.7ab	4.10b	95.00c	25.23c	4.47c
MSD	**23.97**	**8.29**	**1.07**	**27.68**	**4.61**	**0.61**

Control*: Plants foliar sprayed with water

Chemical analysis

Total soluble proteins

Significant increase in total soluble proteins was observed by all treatments (Table 4). The highest value was recorded by Kin at 50 ppm followed by Kin at 100 ppm then CPPU at 10 ppm in both seasons.

Total chlorophyll

An enhancement in total chlorophylls was noticed by head removal treatment (Table 4) but this effect did not reach the levels of significance. Kin and CPPU treatments showed significant increase in chlorophyll concentration. The best results were obtained by Kin at 50 ppm which recorded 3.11 and 3.20 mg/g comparing with control plants which recorded 2.14 and 2.19 mg/g in both seasons respectively.

Total carbohydrates:

Significant increase in total carbohydrates was recorded by all treatments (Table 4) the highest values were recorded by Kin at 50 ppm. Both head removal and CPPU at 10 ppm showed similar trend.

Table (4) Effect of head removal, kin and CPPU on some biochemical constituents at 70 days after planting during the seasons of 2005 and 2006.

Treatments	2005			2006		
	Total soluble proteins (mg/g)	Total chlorophyll (mg/g)	Total soluble carbohydrates (mg/g)	Total soluble proteins (mg/g)	Total chlorophyll (mg/g)	Total soluble carbohydrates (mg/g)
Control	1.52 d	2.12 c	16.32 d	1.66 c	2.15 c	17.00 d
head removal	2.72 c	2.32 c	18.43 c	2.77 b	2.37 c	19.00 c
Control*.	1.53 d	2.14 c	16.56 d	1.70 c	2.19 c	17.01d
kin50	3.57 a	3.11a	23.32 a	3.83 a	3.20 a	25.57 a
kin100	3.28 ab	2.86 ab	20.27 b	3.77 a	3.09 ab	22.40 b
cppu10	2.89 bc	2.77 b	18.38 c	2.93 b	2.83 b	19.77 c
cppu20	2.68 c	2.62 b	19.68 b	2.75 b	2.93 b	20.73b c
MSD	0.52	0.26	0.81	0.34	0.33	1.96

Control*: Plants foliar sprayed with water

Indole acetic acid:

As shown in Fig (1) all treatments reduced IAA concentration comparing with control plants. The lowest values were obtained by head removal treatment followed by CPPU then Kinetin treatments.

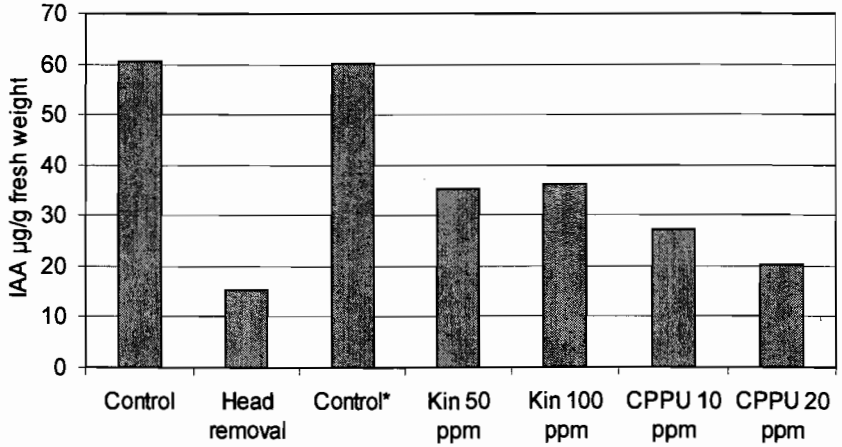

Fig (1): Effect of head removal, Kin and CPPU on IAA concentration in sunflower leaf extracts at 70 days after sowing during the season of 2006 (control*:plants foliar sprayed with water).

Cytokinins:

As shown in Fig (2), endogenous cytokinins showed opposite trend to IAA where, promotion in cytokinins level was observed by all treatments, the highest levels of cytokinins were obtained by head removal treatment followed by CPPU at 20 ppm.

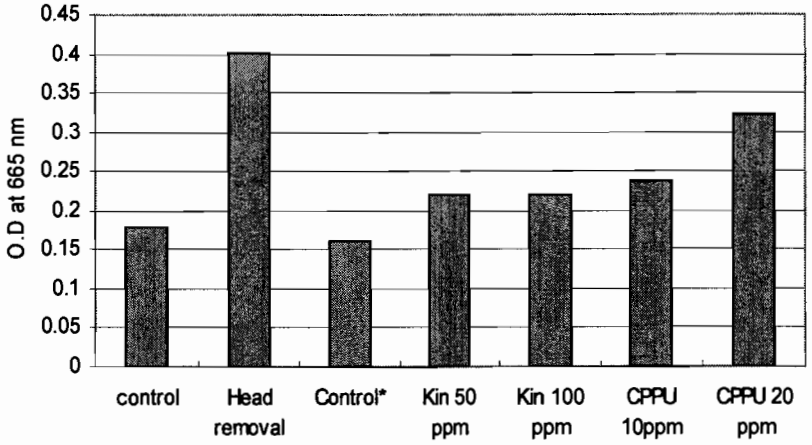

Fig (2): Effect of Head removal, Kin and cppu on cytokinins level of sunflower leaf extracts at 70 days after sowing during the season of 2006 (control*:plants foliar sprayed with water).

B- The discussion:

Head removal was done at the productive stage R1, when the inflorescence surrounded by immature bracts becomes visible. Sunflower stem shows further elongation in the next stages (R2, R3) in the internodes below the inflorescence (Schneiter and Miller 1981). Head (inflorescence) removal blocked shoot elongation in the present study. This reduction in stem elongation referred to shortage in auxin supply by removing the head (Artica, 1996). On the other hand, an increase in leaf area and shoot dry weight was observed at the 1st sample (70 days after sowing) simultaneous with the onset of senescence as previously described by Zaghlool (1996). Meantime, head removal treatment showed an increase in total soluble proteins and carbohydrates in leaves. Purohit (1982) reported that, head removal slowed the degradation of leaf chlorophyll and consequently delayed leaf senescence. However, head removal showed insignificant increase in total chlorophyll, comparing with control plants. It seemed that sink removal did not affect plant senescence as close as Kin did,

and the increase in dry mater could be due to both leaves and stems serves as alternate sink for dry mater in the absence of developing seed heads (Ho and Below, 1989).

Kinetin treatment showed the highest leaf area, dry weight, total soluble proteins and total chlorophylls in the presence of the mean head, elevating the concentration of Kin from 50 to 100 ppm did not show any additive stimulation. The role of cytokinins in improving photosynthetic activity, leaf dry matter and chlorophylls concentration has been mentioned by many workers (Seidel 1996; Elagina and Yakushina, 1997; Benbella and Paulsen 1998).

As mentioned previously, both Kin and CPPU are synthetic cytokinins. The initial bioassays indicated that CPPU had greater biological activity on plants than natural or endogenous cytokinins such as Zeatin (Takahashi 1978). So, CPPU usually used in low concentrations comparing with other cytokinins.

However, CPPU treatments showed senescence delaying effects as indicated by increasing total chlorophylls, soluble proteins and carbohydrates in the presence of the main sink (head).

Head removal in the present study led to the development of lateral heads included few large seeds on the upper 5-6 nodes. Bangerth (1989) mentioned that, polar IAA export of the earlier developed sink inhibits IAA export of later developed sinks. It was suggested that IAA exported by sunflower heads depress the development of lateral buds by preventing IAA formed within these buds to move out, causing "autoinhibition" effect. Eliminating IAA main source by head removing liberated the arrested buds.

Endogenous cytokinins assay showed an opposite trend to auxins. The high levels of cytokinins were concomitant with bud outbreaking and lateral buds development in head removal treatment. These results confirmed by Bangerth (1994), he found that pronouced increase in cytokinins levels in xylem exudates following decapitation of bean (*Phaseolus vulgaris*) plants.

CPPU at 20 ppm showed lower IAA concentration and higher cytokinins level comparing with CPPU at 10 ppm and Kin treatments. Anyhow, this hormonal interaction (low IAA + High cytokinin) led to release inhibitory lateral buds and the development of lateral inflorescence buds into tiny heads.

Regarding seeds weight/head, the highest values was obtained by CPPU at 10 ppm. Arteca (1996), mentioned that, synthetic

cytokinins have been shown to be effective in increasing fruit set, it has been suggested that the ability of cytokinins to mobilize assimilates to the area of application is responsible for increasing fruit set. Kinetin treatments gave also significant increase in seeds weight/head. The developed lateral buds by CPPU at 20 ppm probably competed with the main head and consequently seeds weight was decreased.

It could be concluded in the present study that cytokinin treatments were more involved in delaying senescence than sink removal. Kin treatments acted better than CPPU in this regard. The effects of CPPU were concentration dependent; at low concentration improved seeds yield/plant, while, the high concentration led to release inhibitory lateral buds and did not significantly affect seeds yield/plant.

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تأثير ازالة الرأس والكينتين وCPPU على الشيخوخة وحيدة الأثمار فى نبات عباد الشمس

سناء عبد الرحمن مصطفى زغلول

قسم النبات الزراعى، كلية الزراعه،جامعة عين شمس،شبرا الخيمه-القاهره- مصر

تمت هذه الدراسة فى الصوبة الخاصة بقسم النبات الزراعى ، كلية الزراعة ، جامعة عين شمس لدراسة تأثير كلا من إزالة الرأس ، الكينتين وCPPU على الشيخوخة وحيدة الإثمار فى نبات عباد الشمس صنف فيدوك. شملت المعاملات إزالة النورة (الرأس) وذلك بأزالة البرعم الزهرى الطرفى عند عمر 45 يوم ، كما تم رش النباتات الكاملة بالكينتين بتركيزى 50 و 100 جزئ فى المليون و CPPU بتركيزي 10 و20 جزئ فى المليون ثلاث مرات خلال مرحلة النمو الخضرى عند عمر 4 ، 5 ، 6 أسابيع من الزراعة.

تم أخذ عينتين ، العينة الأولى عند عمر 70 يوم من الزراعة حيث تم أخذ قياسات الطول ومساحة الورقة (عند العقدة 7 – 8 من أعلى) والوزن الجاف للمجموع الخضرى. وتم عمل التحليلات الكيمائية فى الأوراق لتقدير البروتين الكلى الذائب ، الكربوهيدرات الكلية الذائبة ، الكلوروفيل الكلى والهرمونات النباتية ، السيتوكينين (الزياتين) والأوكسين (أندول حمض الخليك). أخذت العينة الثانية عند عمر 95 يوم وتم فيها قياس وزن الرأس ، وزن البذور/الرأس ، ووزن 100 بذرة.

وتتلخص أهم النتائج فيما يلى:

– أدت معاملة إزالة الرأس إلى نقص معنوى فى طول النبات وزيادة فى الوزن الجاف.

– أدت معاملات الكينتين بكلا التركيزين إلى زيادة واضحة فى الوزن الجاف ومساحة الورقة.

– أعطت معاملة CPPU أعلى زيادة فى وزن الرأس يليها معاملة الكينتين بتركيز 50 جزئ فى المليون فى حين أن معاملة CPPU بتركيز 20 جزئ فى المليون لم تؤثر معنويا على هذا القياس.

– كما أدت معاملة CPPU بتركيز 10 جزئ فى المليون إلى زيادة وزن البذور/الرأس ووزن 100 بذرة.

– أدت معاملة إزالة الرأس إلى ظهور رؤوس جانبية تحتوى على عدد قليل من البذور على العقد العلوية (5–6 من أعلى). كما أدت معاملة CPPU بتركيز 20 جزئ فى المليون إلى ظهور نورات غير متفتحه فى إباط الأوراق.

– أدت جميع المعاملات إلى حدوث زيادة فى البروتين الكلى الذائب و الكربوهيدرات الكلية الذائبة وسجلت أعلى القيم بواسطة معاملة الكينتين بتركيز50 جزئ فى المليون كما أدت المعاملات إلى حدوث نقص فى مستوى الأكسين الداخلى وحدوث زيادة فى السيتوكينين وأعلى مستوى من السيتوكينين نتج من معاملة إزالة الرأس يليها معاملة CPPU بتركيز 20 جزئ فى المليون.

أظهرت النتائج أن معاملات الستوكينين (الكينتين و CPPU) كانت أكثر تأثيرا على الشيخوخة من معاملة إزالة الرأس وأن الكينتين كان أكثر تفوقا من CPPU فى هذا الشأن. وقد أختلف تأثير CPPU بإختلاف التركيز. التركيز الأقل (10 جزئ فى المليون) كان أكثر تأثيرا على محصول البذرة/النبات فى حين أن التركيز الأعلى(20 جزئ فى المليون) أدى إلى كسر سكون البراعم الجانبية وحدوث نمو واضح بها.