Ass. Univ. Bull. Environ. Res. Vol. 22 No. 1 March 2019

AUCES

APLICATION OF KINETIN TO AMELIORATE CADMIUM TOXICITY ON GROWTH AND YIELD COMPONENTS OF MAIZE (*ZEA MAYS L.*) PLANTS IN SAUDI ARABIA

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ABSTRACT:

Maize is the most widely distributed crops of the world. During the past two decades in Saudi Arabia, maize becomes one of an important source of feed for human and animals. The present experiment was conducted to investigate the effect of soil drench of two concentrations (100 and 500 µmol) of cadmium chloride on growth, mineral contents, water relations and productivity of *Zea mays*. Also, the study was extended to evaluate the effect of grain presoaking with kinetin to ameliorate the toxicity effects exerted by $CdCl_2$. Generally, cadmium treatments led to significant decrease growth parameters (shoot and root dry weights, leaf area and shoot height), photosynthetic pigments (Chl. a, Chl. b and carotenoids), water relations (relative water content, transpiration rate and leaf conductance), ion contents (sodium, potassium, calcium and cadmium) and yield components (grain yield, number of grains and harvest index) of maize plants as compared to control plants. Grain presoaking in kinetin induced remarkable increases in all above studied parameters, except decrease cadmium content, of maize plants treated with different concentration of $CdCl_2$ as compared to treated plants with $CdCl_2$ alone. Total soluble sugars and protein content in yielded grains were significantly decreased in maize plants irrigated with $CdCl_2$, but application of kinetin alleviates or ameliorates the toxic effects of CdCl2. Of particular interest that application of kinetin was positively correlated with some growth, yield and water relations and negatively correlated with cadmium content of maize plants irrigated with $CdCl_2$ solution.

KEY WORDS: ***Zea mays-Kinetin-Cadmium- Growth- Water relations***

INTRODUCTION

Corn is considered one of the main grain crops in the world in general and Saudi Arabia in particular because it is important in feeding the human, animal and poultry. It enters the dry feed industry by up to 80% and the bread industry by 25% glucose, fructose and oil. The whole grain contains 72% carbohydrate, 13% water, 8.9% protein, 3.9% fat and 1.2% ash .The maize grains are rich in fat, protein and starch in particular, and they form an important part of human food in many developing countries in Africa and Asia (Dahmardeh, 2011). Saudi Arabia has been interested in growing maize plants. The cultivation of maize in the soils is successful with good drainage and ventilation, ranging from acidic to moderate. It is necessary to plant

many elements, including phosphorus, nitrogen and potassium. These elements, together with many other elements, are added to agricultural soils in the form of organic or chemical fertilizers before soil tillage (El- Naggar *et al*., 1991; Rahmat *et al*., 2017). In some cases, some farmers may be forced to use water from industrial waste in irrigation. This is the element of cadmium, which is considered one of the most important toxic elements, and its accumulation in the soil in large quantities has negative effects on the growth and productivity of some (Misra *et al*., 1994; Al-Hakmi, 2007and Singh *et al*., 2017).

Burstron *et al.* (1986) explained that ionic cadmium greatly helps to inhibit the chromosome content of leaves of lettuce plants by directly influencing the production of 5-aminolaevuli acid. The increase in cadmium concentrations in the soil leads to a decrease in the content of chlorophyll and carotenoids in addition to the water content, the transpiration rates and the ionic content of the calcium and potassium elements in the leaves of the carrot plants (Stobartet *et al*., 1985; Gadallah, 1999). Abo-Hamed *et al.* (1987) reported that diffusion of different varieties of wheat at a concentration of 50, 100, 200 ppm of kinetin significantly helps to increase the growth rates and yield of these plants under different concentrations of cadmium chloride if compared to cadmium-treated plants only. Vodnik *et al.* (1999) showed that this hormone plays a prominent role in increasing the ionic content of nitrogen, potassium and calcium in cadmium chloride-treated grains. Producing polysaccharides and protein content of bean seeds grown in contaminated soil with cadmium and lead was recorded by Robertson *et al*. (1999) and Srivastav *et al.* (2017). The influence of growth regulators has been studied to overcome the toxic and harmful effects caused by these heavy polluting elements of soil as a result of continuous irrigation in terms of their role in delayed aging and yellowing of plant leaves and increasing their tolerance to severe interstitial conditions (Aldesuquy, 2000) note that the role of cytokintin in plant resistance to the toxicity of heavy metals is unclear and further study is needed. This study aimed to study the effect of kinetin in reducing or eliminating the toxic effects of cadmium chloride in the growth and productivity of maize plants in Saudi Arabia.

MATERIAL AND METHODS

Plant and growth conditions

This study was conducted at the Research Station of the Faculty of Science, King Saud University, during the last week of April to the last week of September 2016. Healthy uniformed maizegrainswere surface sterilized in a 7% sodium hypochlorite solution for 5 minutes and washed directly with distilled water and dried on sterile filter paper which was divided into two sets. First set: the grains soaked in the distilled water for 3 hours. Second set: the grains soaked in a 50-ppm solution of kinetin (based on previous preliminary experiments) for 3 hours. Immediately after the period of soaking, the seeds were washed with sterile water and 5 grains were placed in each plastic pot (30 cm in size) containing 7 kg soil (sand:clay, 2: 1 v/v).

After two weeks of planting, the plants were thinned in each pot to one plant, leaving the homogenous plants separated. The plants in the first and second sets were divided into three groups: 1st was irrigated with normal water (control), 2nd irrigated with a 100 µM $CdCl_2$ solution, the 3rd set was irrigated with 500µM $CdCl_2$ solution. The experiment was left under normal environmental conditions in the greenhouse. After three weeks of planting, all plants were fertilized with 35 g N of ammonium nitrate (N / m2) and 35 g P of super phosphate (P / m2). Harvesting (ten plants per each treatment) was carried out 45 and 90 days after planting.

Growth measurements

At each harvest, dry weights of shoots and roots were determined. Leaf area was measured using a leaf area meter (Licor-460, NE, USA). The length of the plant was measured from the surface of the earth directly to the top of the plant.

Yield and yield components

The average grain weight of the five plants was calculated in each treatment and the harvest index was calculated according to the following equation:

Harvest index = {Economic yield (grain yield) / above ground dry matter *100

Determination of Photosynthetic Pigments

Photosynthetic pigments were extracted and determined from the leaves of wheat plants by using the method of Hare (1997) method. The extraction was measured against a blank of pure 95% ethyl alcohol at three wavelengths of 452, 644 and 663 nm. The absorbance readings were followed with a spectrophotometer (Unico UV-2100 spectrophotometer).

Relative water content (RWC)

RWC was estimated from fresh, turgid and dry weight of leaf discs, as described by the methods of Srivastava *et al.* (2010).

Measurements of transpiration rate and total leaf conductance

Transpiration rate and total leaf conductance of the *Zea mays* leaves were measured using Li-Cor, 6400XT, Lincoln, NE, USA.

Elements content analysis

A known weight of the dry grounded material of maize leaves for each treatment was digested in a digestion flask containing a triple acid mixture (HNO3:H2SO4:60% HCl4, with a ratio of 10:1:4; respectively) for analysis of sodium, calcium and potassium. Potassium (K) sodium (Na) and calcium (Ca) was assayed using a Flame photometer (Corning 400, UK). Cadmium (Cd) was estimated using an atomic absorption device.

Determination of total soluble sugars

Total soluble sugars (TSS) in dry grounded material of grains were determined after the extraction of dry seeds for each treatment in 10 ml of 80% (v/v) ethanol at room temperature with periodic shaking for 24 hours. TSS was determined using the method of Bradford (1976) with some modifications of the method of Abdel-Fattah *et al*. (2002). A calibration curve using pure glucose was made, which is linear over the tested range, with correlation coefficients higher than 0.999.

Determination of total soluble protein

Total protein in extracted grounded material of grains of each treatment was determined according to the method of Allam *et al*. (2003). A calibration curve was constructed using bovine serum albumin (BSA) and the data were expressed as µg BSA/mg dry matter.

Statistical analysis

All subjected to one-way ANOVA using the SPSS 10.0 software program. Means and standard errors were calculated for ten replicate values. Means were compared by the Duncan's multiple range test and statistical significance was determined at 5% level.

RESULTS AND DISCUSSION

Growth criteria

The results shown in Table (1) revealed that irrigation of maize plants with $CdCl_2$ had a significant decreased on growth rate parameters (shoot height, leaf area, dry weight of shoots and roots) as compared with control experiment. These effects increased with increasing cadmium chloride concentrations. On the other hand, when the maize grains were soaked in 50 ppm of kinetin, a significant increase in both the dry weight of the root and the stem as well as the length of the plant and the leaf area of the irrigated plant and non-irrigated with cadmium chloride compared with the other non-treated treatments.

These results showed that the effect of irrigation with cadmium chloride was more influential in the second harvest after 90 days of plantingin all studied treatments. Statistical analysis confirmed that these increases are significant. These findings are consistent with some previous studies by scientists (Gadalla and Ghorbal, 1999; Srivastav *et al*. 2017; Hashem, 2014 and Singh *et al.* 2017). Cadmium is one of the most toxic elements in terms of its effect on the growth and productivity of important agricultural crops (Bazzaz *et al*., 1992; Toppi and Gabrielli, 1999 and Yousefi *et al.*, 2018). The negative effect of cadmium ion on plant growth is due to its negative effect on the water content of the plant as indicated by previous studies on different types of plants (Zhang *et al*., 2013 and Benavides *et al.*, 2005). The use of kinetin to reduce the toxic effects of cadmium through the positive cycle in increasing the area of leaves and food content and increase the enzymes of oxidation and reduction in the leaves of plants. On the other hand, the treatment of the kinetin grains led to increased growth rates of maize-treated maize plants compared to plants treated with cadmium chloride only (Heikal *et al*., 1982; Robertson *et al*., 1999and Al-Hakimi, 2007).

Photosynthetic pigments

The results indicated in Table (2) showed that irrigation of maize plants with $CdCl_2$ (100 and 500µmol) had a negative effect on the content of plant pigments (chlorophyll a, chlorophyll b, carotenoids and total chlorophyll) in maize plant leaves during two stages of growth as compared to plants not treated with $CdCl_2$ (control). On the other hand, the presoaked of Maize grains inkinetin resulted in a significant increase in the content of photosynthetic pigments of maize plants when compared to plants treated only with $CdCl_2$.

This increase was markedly significant in chlorophyll a.The results of this study showed that the kinetin plays an important role in reducing the toxicity of $CdCl_2$ on the content of photosynthetic pigments of maize plant leaves during plant growth stages. These results were agreed with (Gadallah, 1999 and Singh *et al*., 2017).They found that kinetin has a direct relationship with increased rates of transpiration as well as water content and nutrient content as confirmed by the findings reached during this study.

Water relations

Kinetin has an important role in controlling the opening and closing of holes, as well as increasing the water content and increasing the degree of root connection of plants. In general, presoaking of maize grains with kinetin at 50 ppm induced a drastic increase in relative water content, total leaf conductance and transpiration rate of maize leaves treated with or without cadmium chloride when compared to control plants (Table 3). These effects were apparent with high concentrations of cadmium chloride. In this connection, these results have been interpreted by scientists Zhang and Schmidt, 1999; Yousefi *et al*., 2018)

Table 1. Effect of kinetin on the growth criteria of *Zea mays* plants growing under different levels of Cd toxicities

Days after sowing	Treatments		Growth Parameters			
	$CdCl_2$ (µmol)	Kinetin 50 ppm	Shoot dry Wt. (g/plant)	Root dry Wt. (g/plant)	Shoot height (cm/plant)	Leaf area (cm^2/Plant)
45	Control (0.0)	_	2.55	0.325	35.4	99.8
		+	2.98	0.408	50.3	155.5
	100	_	2.12	0.205	30.8	80.3
		+	2.38	0.306	41.2	142.6
	500	_	2.05	0.178	23.7	65.7
		+	2.24	0.199	30.6	120.5
	LSD (5%)		0.24	0.056	5.1	11.5
90	Control (0.0)	_	4.37	0.554	50.9	300.3
		+	5.10	0.830	66.2	390.5
	100	_	4.05	0.491	48.0	277.6
		+	4.99	0.730	55.3	335.2
	500	_	3.81	0.388	40.6	215.4
		+	4.11	0.495	51.3	278.2
	LSD (5%)		0.39	0.084	8.5	30.8

Table 2. Effect of kinetin on the photosynthetic pigments content of the of maize plants leaves grow under different levels of $CdCl_2$

Days after sowing	Treatments		Photosynthetic pigments ($mg.g^{-1}$fwt.)			
	$CdCl_2$ (µmol)	Kinetin 50 ppm	Chl.a	Chl. b	Carotenoids	Total pigments
45	Control	-	150	130	45	325
	(0.0)	+	180	144	56	380
	100	-	135	120	39	284
		+	155	135	51	341
	500	-	121	110	32	263
		+	110	105	40	255
	LSD (5%)		9.66	7.99	4.25	11.6
90	Control	_	190	140	60	390
	(0.0)	+	220	155	70	445
	100	-	150	138	55	343
		+	180	150	64	335.2
	500	-	160	130	70	400
		+	195	145	95	335
	LSD (5%)		10.44	8.11	8.90	39.8

Table 3. Effect of kinetin on water relationships of maize leaves plants growing under different $CdCl_2$ levels.

Days after sowing	Treatments		Water relations		
	$CdCl_2$ (µmol)	Kinetin 50 ppm	Total conductivity ($mmol.m^2.s^{-1}$)	Transpiration rate ($mmol.m^2.s^{-1}$)	Relative water content (%)
45	Control	-	255	80.5	35.3
	(0.0)	+	310	110.8	46.1
	100	-	145	75.2	30.2
		+	180	100.5	41.8
	500	-	99	66.2	28.3
		+	120	80.2	35.8
	LSD		20.66	15.15	3.28
90	Control	-	195	60.3	30.2
	(0.0)	+	289	66.5	40.1
	100	-	130	55.8	25.3
		+	155	50.3	32.4
	500	-	80	45.6	20.8
		+	110	40.9	28.6
	LSD (5%)		8.33	5.66	2.80

Table 4.Effect of kinetin on ionic content of the *Zea maize* leaves growing under different concentrations of $CdCl_2$

Days after sowing	Treatments		Ion contents (mM.g-1 dwt.)			
	$CdCl_2$ (µmol)	Kinetin	K^+	Na^+	Ca^{++}	Cd^{++}
45	Control	-	0.140	0.055	2.71	0.049
	(0.0)	+	0.151	0.068	2.89	0.032
	100	-	0.129	0.042	2.33	3.23
		+	0.133	0.049	2.50	2.92
	500	-	0.099	0.038	1.99	5.51
		+	0.111	0.041	2.19	3.87
	LSD (5%)		0.025	0.008	0.08	0.98
90	Control	-	0.255	0.155	3.11	1.11
	(0.0)	+	0.310	0.183	3.80	0.870
	100	-	0.210	0.133	2.98	5.88
		+	0.240	0.158	3.30	4.12
	500	-	0.151	0.098	2.77	11.32
		+	0.189	0.120	3.24	8.65
	LSD (5%)		0.059	0.038	0.177	1.88

Water relations

Table 5.Effect of kinetin on productivity and yield components of growing maize plants under different concentrations of $CdCl_2$

Days after sowing	Treatments		Yield components			
	$CdCl_2$ (µmol)	Kinetin	Number of grains / corn	Dry wt. of grains (g/corn)	Grain yield (g/plant)	Harvest index (%)
120	Control	-	105.4	3.15	5.10	33.6
	(0.0)	+	140.3	4.88	7.10	59.8
	100	-	88.4	2.88	4.99	28.5
		+	125.1	3.11	6.86	39.1
	500	-	55.8	1.98	3.10	21.8
		+	88.5	2.64	4.36	31.3
	LSD (5%)		18.5	0.99	1.25	6.44

Table 6.Effect of Kinetin on the amount of soluble total sugars and protein content in yielded grains of maize plants growing under different concentrations of $CdCl_2$

Days after sowing	Treatments		Yield components	
	$CdCl_2$ (µmol)	Kinetin 50 ppm	Total soluble sugars (mg.g^{-1} dwt.)	Total soluble protein (mg.g^{-1} dwt.)
120	Control	-	25.15	66.10
	(0.0)	+	28.10	82.55
	100	-	18.95	55.18
		+	23.66	67.30
	500	-	14.90	28.13
		+	19.88	45.16
	LSD (5%)		3.44	11.33

Ion contents

The results recorded in Table (4) showed that soil irrigation with cadmium chloride (100 and 500µmol) resulted in a decrease in the content of potassium, sodium, calcium and increase cadmium in the leaves of maize plants as compared to control plants. This increase was significantly remarked at 500 µM cadmium chloride. In this context, the kinetin had an important role in increasing the content of potassium, sodium and calcium, and a significant decrease in cadmium in the leaves of maize plants compared to their contents of non-treated plants. Many researchers have agreed with these finding(Ibrahim, 1998; El-Naggar and Allam1991, Zhang and Schmidt, 1999;Srivastavet al. 2017and Singh et al., 2017). The increase of these elements with kinetin is due to increased ATP content and activation of ATP-ase in plasma dinners of plant cells thereby increasing the potassium and calcium content in plant cells. Vodniket al., (1999) and Hashem (2014) has shown that Kinetin has a prominent role in increasing membrane membrane fluidity. Through these results, it was found that the Kinetin plays a major role in increasing the resistance of the maize plants to the cadmium toxicity by increasing the production of protein in the plant roots (Phytochelatins) and/or by accumulating in the surface of the leaves (David et al.1995). Kinetin may increases the storage gaps of cadmium increase the vacuolar storage of Cd++ in the plant (Chardonnens et al., 1998).

Yield and yield components

The results recorded in Table (5) showed that the irrigation of plants with cadmium chloride solution had a significant decreased on the plant yield of grain number, weight, yield and harvest index of maize plants compared with non-treated cadmium chloride Zea maysplants. These results have been agreed with many investigators (Hare et al. 1997;Toppi and Gabrielli 1999; Benavides et al. 2005 and Yousefi et al., 2018) concluded that cadmium chloride has a negative impact on the productivity of most agricultural crops through direct impact on transpiration, water relationships and nutrient content ,antioxidant enzymes, and also the content of plant pigments. This was confirmed by the results obtained in

this study. In contrast, David et al.(1995) and Srivastav et al.(2017) showed no significant negative effect on yield productivity with low concentrations of cadmium chloride. These differences in plant response to Cd were due to plant type and soil type as well as plant genetics.

In general, soaking of maize grain in kinetin resulted in a significant increase in the yield parameters (weight and number of grains - grain productivity - and harvest parameters) of treated plants with cadmium chlorides compared with plants treated with cadmium chloride only. These results showed that the kinetin plays an important role in increasing vegetative growth and leaf area, improving water relations an increasing the transport of nutrients such as potassium and calcium, as well as reducing cadmium transfer to the stem and leaves. This was evident from the results achieved, which was directly reflected in productivity with many researchers (Gadalla and Ghorbal, 1999; Al-Hakimi, 2007 and Singh et al. 2017).

Total protein and soluble sugars content in yielded grains

The results recorded in Table (6) showed that cadmium chloride had a negative effect on the amount of soluble sugars as well as the protein content in the grain produced by the maize-treated maize crop compared to the control plants. In the treatment of soaking grains in kinetin, the reduction of the negative effect of cadmium chloride in terms of increased soluble sugars and protein content of grains compared to plants treated with cadmium chloride only. These results have been agreed with (Gadallah and Ghorol 1999; Hashem 2014 and Singh et al. 2017). The chemistry has a significant role in reducing aging and increasing chlorophyll content, thus increasing the production of a large amount of soluble sugars and increasing the content of amino acids and nitrogen and thus increasing the protein in the grains of some plants, (Aldesuquy 2000 and Yousefi et al. 2018).

Coefficient between the effect of kinetin and some studied traits

The results shown in Table (7) show that there is a strong positive correlation coefficient between both the effect of kinetin and some vegetative traits such as dry leg weight r = 0.77 dry root weight r = 0.65 and leaf area r = 0.85, The soluble sugars r = 0.89 and soluble protein r = 0.80 and some characteristics of water relations such as water content of the paper r = o.91 and the rate of transpiration r = 0.80 and on the other hand the correlation coefficient was negative between the effect of kinetin and cadmium content r = - 0.88 in the leaves of maize growing plants under Effect of cadmium chloride toxicity.

Table 7. Correlation coefficient (π) between the effect of kinetin and some growth parameters, productivity and plant relationships of growing maize plants under different concentrations of $CdCl_2$

Parameters	R
Shoot dry weight	0.77**
Root dry weight	0.65*
Total leaf area	0.85**
Shoot height	0.80**
Harvest index	0.69*
Total soluble sugars	0.89**
Protein content in grains	0.933**
Relative water content	0.91**
Total leaf conductance	0.71*
Transpiration rate	0.80**
Leaf cadmium content	- 0.88**

Values with * and ** are significant at P≤ 0.05 and 0.01 respectively

CONCLUSIONS

This study has clearly concluded that grain presoaking with kinetin improved plant growth, productivity of maize plants grown under different concentrations of cadmium chloride by increasing photosynthetic pigments, transpiration rate, improving relative water content and ion contents like calcium, potassium and sodium (decreasing cadmium content in plant leaves), accumulation of sugars and protein contents in yielded grains of maize plants. In future, this study will be extended to include further investigations on the effect of kinetin on some metabolic pathways, different enzymes and endogenous hormonal levels.

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استخدام الكينيتن لتخفيف سمية كلوريد الكادميوم علي نمو وإنتاجية نباتات الذرة الشامية في المملكة العربية السعودية

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الملخص العربي :

ترجع أهمية البحث في ان الذرة الشامية تعد من اهم المحاصيل في المملكة العربية السعودية ومصدراً هاماً من مصادر الأعلاف الرئيسية. ويهدف هذا البحث الى تتبع الدور الذي يلعبه الكينيتين لتخفيف او تلطيف السمية الناتجة من الري بتركيز مختلفة من كلوريد الكادميوم علي نمو وإنتاجية هذا النبات .بصفة عامه أظهرت النتائج بان معامله حبوب نباتات الذرة الشامية بالكينيتين قبل زراعتها كان له اثر إيجابي في إزالة التأثير السلبي الناتج من الري بكلوريد الكادميوم علي دالات النمو (الوزن الطازج والجاف للمجموع الجذري والخضري، طول الساق، طول الجذر) والمحتوى الأيوني (البوتاسيوم والصوديوم والكادميوم) وكذلك العلاقات النباتية (الجهد التوصيلي للورقة، معدل النتح والمحتوي المائي للورقة). وأوضحت النتائج إن للكينيتين دورا هاما في زيادة المحتوى الصبغي للأوراق نباتات الذرة الشامية المعاملة بكلوريد الكادميوم مقارنة بالنباتات المعاملة بكلوريد الكادميوم فقط. وتوصلت النتائج إن نقع حبوب الذرة الشامية في الكينيتين ادي الي زيادة معنوية في الكتلة الحية للحبوب والمحتوي البروتيني والأيوني للحبوب الناتجة للمحصول. على الجانب الأخر، تبين أن معاملة الحبوب بكلوريد الكادميوم أدى إلي خلل في تغير الاتزان الهرموني للحبوب وذلك بزيادة في حمض الأبسيسيك ونقص ملموس في هرمونات النمو (أندول حمض الخليك والجبريلينات). وخلصت نتائج هذه الدراسة على أن نقع حبوب نباتات الذرة الشامية في الكينيتين أدى إلى تأثير معاكس تماما لما تسببه سمية كلوريد الكادميوم.

الكلمات المفتاحية: الذرة الشامية–سمية –كلوريد الكادميوم –الكينيتين–النمو–الإنتاجية –المحتوى المائي.