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EFFECT OF PLANT REGULATORS, ZINC NANOPARTICLES AND IRRIGATION INTERVALS ON LEAF CONTENT OF ENDOGENOUS HORMONES AND NUTRIENTS IN SUNFLOWER *(HELIANTHUS ANNUUS* L.)

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Abstract

The experiment was conducted in a local nursery belongs to Najaf municipality in the province of Najaf during the spring growing season of 2018 in order to investigate the effect of plant growth regulators (IAA and GA_3) and zinc nanoparticles on sunflower leaf content of exogenous hormones and nutrient at different levels of water stress. The experiment was RCBD with three factors including irrigation intervals (3, 6 or 9 days), foliar application with 100ppm of plant growth regulators IAA and GA_3 at rates of 0:0, 1:1, 0:1 or 1:0 respectively, and foliar spray with 100ppm of zinc (control, $ZnSO_4$ or Nano-zinc ZnO). Results showed water stress (long irrigation interval) significantly decreased leaf content of IAA, GA_3 and zinc and at the mean time significantly increased leaf content of ABA, calcium and potassium. The growth regulators IAA and GA_3 individually or combined positively affected all the studied traits. Foliar application with growth regulators and their combinations reduced symptoms of water stress severity and showed an improvement in all growth traits (internal hormonal content (IAA, GA_3, ABA), mineral elements (Zn, Ca, K). findings showed that Nano-zinc application was also effective and caused a significant increase in all the above measured plant traits.

Keywords: water stress, IAA, GA_3, Nano-particles, ABA, calcium, potassium

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops worldwide. The seeds contain approximately 40-50% healthy low cholesterol edible oil and 17- 20% protein (Hussain *et al.,* 2018). Like most crops, sunflower cultivation in Iraq is limited due to water scarcity in general and plants usually suffer from water stress.

Definition of water stress may differ depend on geographical area, type of plant and environmental conditions, etc. (Gaspar *et al.,* 2002). However, one of the major reasons of water stress is the inefficient management of water resources and agricultural regulations which consequently resulting in limitation in crop cultivations and productivity (Wariach *et al.,* 2011). Generally, water stress causes changes in plant's metabolic activities and nutritional contents, especially carbohydrates. Leaf content of starch was reduced sunflower plants due to water stress, as well as plant tissues contents of natural plant hormones such as IAA, ABA, GA_3, ethylene, and cytokines were also affected (Rahman and Hasegawa, 2012). However, water stress can be successfully managed and overcome using exogenous application of plant growth regulators to increase plant tolerance to various environmental stresses (Hussain *et al.,* 2018). Plant hormones are playing major roles in plant adaptation ability to environmental fluctuations by modifying plant growth and development processes, distribution and transportation of nutrients within plant's body (REF). Among all the plant hormones, ABA is the most tested and studied in relation to environmental stresses (REF). However, Auxins (IAA), gibberellins (GA_3) and some other plant hormones were also demonstrated to have synergistic effects on response of different plants to different stress types (Peleg and Bluwald, 2011). IAA activity and behavior within stressful plants is still under discussion until now. Some researchers believe that water stress decreases plant content of this auxin, while others believe that adaptation to water stress is accompanied by increased plant content levels of IAA (Lecube *et al.*, 2014). It was also noted that the foliar application of gibberellic acid increased the efficacy of photosynthesis, modified the transpiration rate and caused an increase in pollen production in plants growing under dehydration stress (Philipson, 2003). In addition to the plant growth regulator, studies indicated that the use of nanofertilizers causes at least three-fold increase in the efficiency of nutrient use and reduces the potential negative effects associated with use of traditional fertilizers (Raliya *et al.,* 2013; Sekhon, 2014). In the comparison context, Nano-fertilizers not only provide additional stress tolerance but also are cost effective and economically reasonable as can be used in small quantities compared to excessive consumption of regular chemical fertilizers (Panwar *et al.*, 2012; Manjunatha *et al.*, 2016). This aim of this study, therefor, was to investigate the effects of foliar application with plant growth regulators (IAA and GA_3) and zinc nanoparticles in sunflower tolerance to different water stress conditions.

Materials and Methods

The experiment was conducted in a local nursery belongs the town municipality in the province of Najaf in the 2018 spring growing season. The study was to investigate the effect of plant growth regulators IAA and GA_3, and zinc nanoparticles on sunflower leaf content of exogenous hormones and nutrient at three irrigation intervals (3, 6 or 9 days). After preparation of the nursery soil and soil samples were taken for analysis (Table 1), sunflower seeds were planted in lines 50 cm between each two lines in holes 30 cm apart at rate of 3 seed per hole. Each planting line contained 3 holes (9 holes per experimental unit). A drip line irrigation system was used to irrigate the plants at rate of 9 holes per experimental unit (1.82 m^2). The experiment was RCBD with three replications and factors including irrigation intervals, foliar application with 100ppm of plant growth regulators IAA and GA_3 at rates of 0:0, 1:1, 0:1 or 1:0 respectively, and foliar spray with 100ppm of zinc (control, $ZnSO_4$ or Nano-zinc ZnO). The growth regulators and the Nano-zinc were applied as foliar spray three times during the experiment period (30 days post planting (DPP) at flower buds starting, 40 DPP when the flower buds are completed and 50 DPP on open flowers). At the end of the experiment in 21/6/2018, data were collected including leaf contents of IAA, GA_3, ABA, zinc, calcium and potassium.

Preparation of treatments (growth regulators and zinc) solutions

The GA_3 solution (100ppm) was prepared by dissolving 100 mg of gibberellin in small amount ethylene and completing the volume to one liter with distilled water. On the same way, IAA was prepared by dissolving 100mg of the powder in a small amount of ethanol and then the volume was completed with distilled water to 1 liter. The 100ppm of Nano-zinc solution was prepared in the same way using 100mg of ZnO Nano-particles in small amount of ethanol and then the distilled water, while normal zinc solution was prepared using same amount of $ZnSO_4.7H_2O$ to be solved directly in 1 liter of distilled water.

Measurement and data analysis

The IAA was extracted according to the method by Yang *et al.* (2001), GA_3 extracted according to Bhalla *et al.* (2010) while ABA extraction was according Cobat *et al.* (1968). As for the quantitative estimation of hormones, High Performance Liquid Chromatography (HPLC) device was used. The mobile phase was composed of Acetonitrile with a solution of buffer containing Phosphate (3%) at a rate of (40:60), respectively, with a flow rate of 1.2 mL/min. The separation column was of type C18-ODS (25cm* 4.6mm), and the reagent was used to detect ultraviolet radiation at the wavelength of 280 nanometers. The zinc content was estimated according to the method approved by Haswell (1991) while potassium and calcium were estimated according to Berry and Jonson (1966).

Data were analyzed by the aid of Genstat 2012, computing program and analysis of variance ANOVA was performed. Means were compared according to Duncan's multiple rang test at 5% probability level.

Results

In case of leaf content of IAA, results (Table1) showed that IAA was significantly decreased due to water stress (9 days irrigation interval) recording 8.19 ppm while plants irrigated at 3 days interval recorded the highest value of leaf IAA (10.52 ppm). Foliar application with growth regulators resulted in different leaf content of IAA as treatments differed (Table1). Plants treated with GA_3: IAA (0: 1) gave the highest IAA value (10.82 ppm) compared to the lowest (7.37 ppm) from the control. Similarly, IAA value was increased up to 10.37 ppm in the Nano-zinc treatment. Generally, the highest IAA content (12.20 ppm) in leaves was in the interaction treatment of 3 days interval irrigation and GA_3: IAA (0:1) compared to 6.56 ppm resulted from untreated plants with 9 days interval. Similar results were observed in case of Nano-zinc interacted with 3 days irrigation interval.

Table 1 : Effect of growth regulators and zinc nanoparticles on sunflower leaf contents of IAA, GA_3 and ABA under different irrigation intervals

	Treatments		Irrigation intervals (II) 3 Days	6 Days	9 Days	Means of GR
Leaf content of IAA (ppm)	Growth regulators IAA:GA3	(0:0)	7.92 de	7.63 ef	6.56 f	7.37 b
		(1:1)	11.09 ab	10.50 bc	8.37 de	10.02 a
		(1:0)	12.20 a	10.96 ab	9.28 cd	10.82 a
		(0:1)	10.88 abc	10.79 ab	8.53 de	10.07 a
	Means		10.52 a	9.97 a	8.19 b	
	Zinc	Control	9.45 bcd	9.13 bcde	7.57 e	8.72 c
		Nor. Zinc	10.26 abc	10.16 bc	8.28 de	9.57 b
		Nano-zinc	11.77 a	10.63 ab	8.72 cde	10.37 a
	Means		10.52 a	9.97 a	8.19 b	
Leaf content of GA3 (ppm)	Growth regulators IAA:GA3	(0:0)	15.87 e	14.92 ef	10.76 f	13.85 d
		(1:1)	35.12 b	35.12 b	28.80 c	33.17 b
		(1:0)	26.26 cd	23.20 d	18.03 e	22.50 c
		(0:1)	42.30 a	41.08 a	33.87 b	39.08 a
	Means		29.99 a	28.58 b	22.86 c	
		Control	24.39 abc	22.29 bc	18.52 c	21.34 c
		Nor. Zinc	31.87 a	30.65 ab	24.21 abc	28.91 b
		Nano-zinc	33.71 a	32.80 a	25.86 abc	30.79 a
	Means		29.99 a	28.58 b	22.86 c	
Leaf content of ABA (ppm)	Growth regulators IAA:GA3	(0:0)	26.67 e	32.39 cde	42.36 c	33.81 d
		(1:1)	43.42 c	51.20 b	59.83 a	51.48 a
		(1:0)	42.41 c	49.61 b	58.42 a	50.15 b
		(0:1)	38.08 cd	42.66 c	52.19 b	44.31 c
	Means		37.64 c	43.97 b	53.20 a	
		Control	31.21 f	37.38 ef	45.99 c	38.19 c
		Nor. Zinc	38.27 de	44.57 cd	55.48 ab	46.10 b
		Nano-zinc	43.45 cde	49.95 bc	58.13 a	50.41 a
	Means		37.64 c	43.97 b	53.20 a	

* Means followed by the same letter(s) within a treatment are not significantly different according to the Duncan's multiple range test ($P\leq0.05$)

As for leaf content of GA_3, the highest values were combined with 3 days irrigation interval, GA_3: IAA (1:0) and Nano-zinc treatments compared to all the other treatment types (Table1). Interaction of IAA: GA3(0:1) and 3 days irrigation interval resulted in the highest value of GA3 concentration (42.30ppm) with significant difference from that resulted in untreated plants irrigated every 9 days (10.76 ppm). Interaction of 3 days interval and Nano-zinc resulted in higher GA3 value than normal zinc treatment with the same irrigation interval.

Regarding the ABA in sunflower leaf, results (Table 1) showed a significant increase in leaves content of ABA by increasing irrigation interval. The ABA was more increased by IAA:GA3 (1:1) than other combinations as well as by Nano-zinc than normal zinc treatment. Plants irrigated every 9 days and treated with IAA:GA3(1:1) had the highest ABA value of 59.83 ppm while 3 days interval of untreated plants recorded the lowest value (26.67ppm). Similar results were recorded in the 9 days interval interacted with Nano-zinc compared to other treatment combinations.

Relative to leaf content of nutrients (zinc, calcium and potassium), values differed as treatments and irrigation intervals differed (Table 2). Generally, the shortest irrigation interval (every 3 days) recorded the highest leaf content of zinc followed by the 6 and 9 days intervals. In case of growth regulators, the IAA:GA3 (1:1) treatment resulted significantly in the highest value of leaf zinc compared to all the other combinations. In similar manner, Nano-zinc application increased leaf content of zinc more than application of normal zinc. The highest values for leaf content of zinc (15.64 and 13.32 $mg.g^{-1}$) were recorded in the 3 days intervals interacted with IAA: GA3 (1:1) and with Nano-zinc, respectively.

Calcium concentration in leaves was more affected and increased by the 9 days interval, GA3: IAA (0:1) and Nano-zinc treatments compared to their other corresponding treatments and controls. Leaf content of calcium was at highest value in the interaction treatment of 9 days irrigation interval and GA3: IAA (0: 1) resulting in 20.083 mg/g^{-1} followed by 19.673 mg/g^{-1} resulted from interaction of the same irrigation interval with zinc nanoparticles.

Table 2 : Effect of growth regulators and zinc nanoparticles on sunflower leaf content of zinc, calcium and potassium under different irrigation intervals

	Treatments		Irrigation intervals 3 Days	6 Days	9 Days	Means of GR
Leaf content of zinc ($mg.g^{-1}$)	Growth regulators IAA:GA3	(0:0)	7.88 ef	8.29 ef	6.92 f	7.73 d
		(1:1)	15.64 a	14.34 a	13.73 ab	14.57 a
		(1:0)	12.39 bc	11.17 cd	11.25 cd	11.60 b
		(0:1)	10.55 cd	9.77 de	9.40 de	9.91 c
	Means		11.62 a	10.92 ab	10.32 b	
	Zinc	Control	10.23 bc	9.55 c	9.51 c	9.76 b
		Nor. Zinc	11.31 ab	10.57 bc	9.86 c	10.58 b
		Nano-zinc	13.32 a	12.64 ab	11.60 abc	12.52 a
	Means		11.62 a	10.92 ab	10.32 b	
Leaf content of calcium ($mg.g^{-1}$)	Growth regulators IAA:GA3	(0:0)	13.05 g	12.94 g	14.80 efg	13.594 c
		(1:1)	15.42 def	14.65 fg	18.97 ab	16.347 b
		(1:0)	17.07 bcde	18.47 abc	20.08 a	18.539 a
		(0:1)	16.31 cdef	17.17 bcd	19.58 a	17.687 a
	Means		15.462 b	15.806 b	18.358 a	
		Control	13.89 d	14.10 d	16.71 bc	14.899 b
		Nor. Zinc	15.77 cd	15.47 cd	18.69 ab	16.643 b
		Nano-zinc	16.73 bc	17.85 abc	19.67 a	18.083 a
	Means		15.462 b	15.806 b	18.358 a	
Leaf content of potassium ($mg.g^{-1}$)	Growth regulators IAA:GA3	(0:0)	14.530 e	18.437 d	20.643 c	17.870
		(1:1)	17.873 d	22.310 abc	23.658 a	21.280
		(1:0)	17.373 d	21.283 bc	23.320 ab	20.659
		(0:1)	18.823 d	20.453 c	22.497 abc	20.426
	Means		17.026 b	20.6210 a	22.530 a	
		Control	15.978 c	19.050 b	21.305 a	17.026 c
		Nor. Zinc	17.133 bc	21.150 a	23.140 a	19.629 b
		Nano-zinc	18.028 b	21.665 a	23.143 a	22.529 a
	Means		17.026 b	20.6210 a	22.530 a	

* Means followed by the same letter(s) within a treatment are not significantly different according to the Duncan's multiple range test ($P \leq 0.05$)

Results (Table 6) also showed leaf content of potassium was affected more in the 9 days interval than other irrigation intervals. However, in case of growth regulator treatment combinations, The IAA:GA3(1:1) was the most effective among other combinations, as well as the Nano-zinc treatment resulted ih higher leaf potassium level than normal zinc and the control treatments. Interaction treatment of 9 days intervals interacted with treatment of IAA:GA3 (1:1) or Nano-zinc application resulted significantly in the highest values of leaf potassium level that of 23.658 mg/g^{-1} and 23.143mg/g^{-1}, respectively.

Discussion

Findings of this study showed that long interval of irrigation resulted in decreases in leaves content of IAA and GA3 with an increase in leaves content of ABA. It is possible that because water stress affects the endogenous hormonal balance, which in turn controls the forms of plant's growth and development as all plant hormones are affected by water limitation. This effect is a type of adaptation in which the plant reduces levels of IAA and GA3 with an increase in ABA to work on closing stomata during the periods of various stresses (Bano and Yasmeen, 2010). Also the decrease in IAA level under drought may be because the oxidative breakdown of IAA due to high oxidative potential caused by reactive oxygen species (ROS), stimulating of peroxide and inhibiting IAA biosynthesis (Xie *et al*., 2004). Moreover, these results confirm the inverse relationship between ABA and GA3 internal levels where reduced leaf content of GA3 was accompanied by an increase in the ABA content. This is mostly due to the effect of water stress on the genes responsible for regulating the GA3/ABA ratio (Baydar, 2002). On the other hand, ABA is considered a natural stress hormone due to its rapid accumulation in response to various stresses. It is involved in vital role of mediating many physiological processes especially plant tolerance to stress conditions (Zhang *et al*., 2006). These results were consistent with Bano and Yasmeen (2010) on wheat and Pirasteh-Anosheh *et al.* (2013) on corn plants where water stress increases the leaf content of ABA. Regarding the effect mineral elements, we find that water stress caused a significant decrease in the leaf content of zinc, but at the same time caused a significant increase in the leaf content of calcium and potassium. Water deficiency negatively affects the transmission of some nutrients from the soil solution to the plant, as mineral nutrition problems occur as a secondary effect of drought. One of these elements is zinc, which will decrease in the soil solution with a lack of moisture in it, and then the plant will face a shortage of this element and the accompanying negative effects on plant growth and development (Kafi and Rostami, 2007). Water stress also disrupts the absorption of essential nutrients due to the effect of drought (Hussain *et al.*, 2018). It is possible that the increase in calcium concentration in the leaves under stress conditions is stimulated by signal molecules such as Inositol triphosphate, Inositol hexaphosphate, diacylglycerol, and reactive oxygen species ROS (Hirayama and Shinozaki, 2010). The cellular level of calcium sensors in stress conditions has an important physiological role in regulating the genetic expression of tolerance genes in the plant (Reddy *et al.*, 2011). The increase in calcium level leads to binding to a special protein called Calmodulin forming a complex that able to activate various physiological activities and act as a signal transduction mediating the plant responses to abiotic stress (Cacho *et al*., 2013). The reason for the high leaves content of potassium under stress conditions may be due to the accumulation of potassium in the leaves of stressful plants, so that potassium participates in most physiological activities involved in the plant's response to water stress and plays a central role in the mechanism of regulating the closure of stomata, photosynthesis, osmotic adjustment of cells, maintenance of osmotic pressure, and increasing The permeability of the aquaporins proteins and in scavenge the reactive oxygen species (Wang *et al*., 2013).

The reason for the increase in the endogenous IAA content in the leaves due to the foliar application of growth regulators is that auxin stimulates the genetic cloning of a large number of genes called primary auxin genes that stimulate the plant to respond to exogenous auxin (Hagen and Guilfoyle, 2002). The mutual action between the different plant growth regulators leads to a variety of synergistic reactions that are resistant to stresses, has an important and decisive influence on the plant's response to these stresses. This action is caused by specialized mechanisms at the molecular level that regulate hormone synthesis, distribution and activities (Peleg and Bluwald, 2011). The positive effect of foliar application of growth regulators in increasing zinc concentration in leaves is mostly due to the fact that plant growth regulators make plants more efficient in absorbing mineral nutrients and using them at a higher level in physiological processes and improving shoot/root ratio (Agegnehu and Taye, 2004; Baranyiovà *et al*., 2014). In the same context, it was found that auxins increase the level of calcium in plant cells by lowering the pH and releasing calcium from the complex compounds and molecules (Taiz and Zeiger, 2002). It is possible that the role of plant growth regulators in increasing the leaf content of potassium is that IAA increases the area exposed to absorption in the roots with an increase in the length and diameter of the roots, as it is an important catalyst for the growth of roots in stress conditions (Muhammed *et al.,* 2016) which increases the absorption of nutrients including K^+.

Zinc nanoparticles have also a positive impact on the studied traits in the leaves, as it is possible that the reason for the increase in the leaf content of the IAA due to the vital role that zinc plays in the manufacture of the amino acid tryptophan, which is the initiator of the manufacture of the natural hormone IAA and increase its level within the plant, as well as controlling the biosynthesis of the auxins themselves and in particular the IAA (Garcia-Lopez *et al*. 2019; Laware and Raskar, 2014). Small size with large area may lead to increased absorption of zinc (Raddy, 2014). These results are consistent with Tadayyan *et al.* (2018), Raddy (2014) and Tiwari (2017) on the influence of nanozinc in increase the root absorption of zinc and in turn its increase of its concentration in the leaves. The increase in leaf content of calcium due to the zinc nanoparticles might be due to the adding of necessary elements which increased the production of antioxidants and so enhances the plants' tolerance to drought by increasing root growth and their ability to absorb nutrients even in abnormal conditions (Waraich *et al.*, 2011). Adequate mineral nutrition has a prominent and decisive influence in increasing the plant's tolerance to water stress (Mraschner, 2012), as nanozinc is important and effective in regulating opening and closing stomata, integrity of biological membranes, and maintaining the potassium level in the guarding cells of stressed plants (Khan *et al.*, 2004)

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