INFLUENCE OF FOLIAR APPLICATION OF ZINC ON SEED YIELD AND ITS COMPONENTS IN SOME ALFALFA VARIETIES

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ABSTRACT

This study was carried out at the experimental farm College of Agriculture; Abu-Ghraib, Baghdad, Iraq during from end of march 2014 to mid of August 2015. The main objective was to find out the effect of zinc concentration (0, 30, 60 and 90) mg.L^{-1} on seed yield and its components of four alfalfa varieties (Local, Hamaddan, Vernal and Lodi). Layout of the experiment was R.C.B.D arranged within split –plots with three replications. Zinc concentration were used as main plot, while varieties were used as sub-plot. Results, showed that foliar application of zinc at concentration more than 30 mg.L^{-1} (60 and 90 mg.L^{-1}) resulted in a significant increment in the No. of ovules floret^{-1}, No. of seed pod^{-1}, No. of stems m^{-2}, No. of racemes stem^{-1}, No. of pods raceme^{-1} and seed yield. Kg.ha^{-1} in contrast, percent of ovules abortion was significantly reduced, while 1000 seed weight was not significantly influenced by Zinc concentrations. Highest seed yield (535.0 Kg.ha^{-1}) was obtained when Zinc added at level 60 mg.L^{-1}. Local var. produces higher No. of ovules floret^{-1}, No. of seed pod^{-1}, No. of stem.m^{-2}, and No. of racemes stem^{-1} and it was significantly different than other varieties except var. Hamddan. Increasing of all these traits resulted in increasing of seed yield in local var. (532.5 Kg.ha^{-1}. There was high positive correlation between seed yield and each of seed set, No. of stem. m^{-2} and No. of pods raceme^{-1} (+ 0.75, +0.66** and 0.78**) respectively. This result may be indicating that these traits could be used as good selection criteria for breeding and improving seed yield in alfalfa varieties. highest seed yield (620.2 Kg.ha^{-1}) can be obtained by seeding local variety. and spray the stands with Zinc at concentration 60 mg.L^{-1}.**

Keyword: ovules fertility, seed set, trace elements.

تأثير رش الزنك في حاصل البذور ومكوناتة في بعض اصناف الجت

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المستخلص

اجريت هذة الدراسة في حقل كلية الزراعة / جامعة بغداد – ابو غريب للفترة من نهاية شهر اذار 2014 الى منتصف شهر أب 2015 وذلك لدراسة تأثير تراكيز الزنك (0 ، 30 ، 60 و 90) ملغم. لتر$^{-1}$ في حاصل البذور ومكوناتة لأربعة اصناف من الجت هي (المحلي و همدان ,Lodi, Vernal). نفذت التجربة بترتيب الالواح المنشقة على وفق تصميم القطاعات الكاملة المعشاة بثلاثة مكررات اذا احتلت تراكيز الزنك المعاملات الرئيسية بينما احتلت الاصناف المعاملات الثانوية . اظهرت النتائج ان رش الزنك بتركيز أعلى من 30 ملغم.لتر $^{-1}$ (60،90 ملغم. لتر$^{-1}$) قد ادى الى زيادة معنوية في عدد البويضات بالزهيرة الواحدة، عدد البذور بالقرنة، عدد السيقان بالمتر المربع، عدد النووات الزهرية بالساق الواحد، عدد القرنات النوره الزهرية وحاصل البذور والى خفض معنوي في نسبة اجهاض البويضات . بينما لم تتاثر معنويا وزن 1000 بذرة. بلغ اعلى متوسط لحاصل البذور 535 كغم.هـ$^{-1}$ عند الرش بتركيز 60 ملغم. لتر$^{-1}$ من الزنك. اعطى الصنف المحلي اعلى متوسط لعدد البويضات بالزهيرة وعدد البذور بالقرنة وعدد السيقان م$^{-2}$ وعدد القرنات بالنورة وتفوق معنويا على جميع الاصناف باستثناء الصنف همدان وبلغ حاصل البذور في الصنف المحلي 535.5 كغم . هـ$^{-1}$ متفوقا على جميع الاصناف . وجد ارتباط عالي المعنوية وموجب بين حاصل البذور . وكل من عدد البذور بالقرنة وعدد السيقان م^{2} وعدد القرنات بالنورة الزهرية بلغ **+0.75 ، **+0.66،+0.78 بالتتابع لذلك ممكن استعمال هذة المكونات كمعيار لتربية اصناف من الجت عالية الحاصل من البذور . ان اعلى حاصل من البذور تم الحصول علية (620.2 كغم.هـ$^{-1}$) هو عند زراعة الصنف المحلي من الجت ورش الزنك بتركيز 60 ملغم. لتر$^{-1}$.**

كلمات مفتاحية: خصوبة البويضات، عقد البذور، العناصر الصغرى .

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INTRODUCTION

Alfalfa (*Medicago sativa* L) is one of the most perennial legume crops in Iraq and worldwide. It is well known capability of nitrogen fixation and a superior source for forage due to its high nutritional quality and herbage production (1,30). Alfalfa grown for seeds, needs different environmental conditions and specific agronomic practices (6). In Iraq, alfalfa is cultivate mainly for forage production and seed yield is considered to be of only secondary importance. The mean seeds yield under Iraqi condition is low, no more than 250 kg ha^{-1} (18). This low productivity can be attributed mainly to a lack of suitable guide lines for its management, particularly during the critical period of seed crop. Genetic diversity for seed yield and its components in alfalfa was described between and within population by Bolanos-Agilar and et al. (5), similarly genetic variation was identified for ovule fertility, ovules number and sterility (20, 31). At the experiment carried out in Agricultural Faculty in Turkey (23) to determine alfalfa seed yield and its components in sixteen variety. He found that seed yield per unit area was significantly positively correlated with the number of seeds $inflorescence^{-1}$, number of pods $inflorescence^{-1}$ and seed weight $inflorescence^{-1}$, suggested that these yield components may be good selection criteria to improve seed yield of alfalfa cultivars. All nutrients must be available in sufficient quantity in order to be able to get satisfactory seed yield (10). Zinc (Zn) is among those micronutrients which are most often deficient, availability of Zn is largely dependent on soil pH value and it is higher in acid soils, contrary to this, in alkaline soil, its availability is very low. Deficiency is more likely on soils with pH greater than 6.0 (21). Whereas most of soils in central and south of Iraq had pH more than 7.5 (4), therefore addition of Zn as foliar application to foliage rather than soil medium is great importance. Zn is involved in pollen fertility, pollen production and pollen tube elongation (19,24) so that its deficiency can cause to decrease in seed formation and subsequent yield reduction. It is also promoting nodulation in leguminous plants (26). In the present study an attempt has been made to understand the performance of reproductive growth of contrasting alfalfa cultivars under different zinc regimes.

MATERIALS AND METHODS

This experiment was performed at experimental station of the Coll. Agric., Univ. Baghdad during the period from end of March 2014 to mid of August 2015, soil analysis of the field shows in Table 1.

Table 1. Some chemical properties of the soil[*]

E.C.ds.m^{-1}	**2.8**
pH	**8.1**
Organic matter g.kg^{-1}	**12**
Nitrogen ppm	**71.0**
Phosphorus ppm	**14.0**
Potassium ppm	**190**
Zinc ppm	**1.46**
Calcium g.kg^{-1}	**210**
Soil texture	**Silty clay loam**

*** Analysis carried out in ministry of sciences and technology labs**

The previous crop before study establishment was corn (*Zea mays* L). The grass stand was plowed and fallow for four months before establishment of alfalfa study. The experiment was carried out in a (R.C. B. D) arranged in split plot with three replications. Four levels of Zinc (Zn) (0, 30, 60 and 90) mg.L^{-1} referred as Z_{n0} ,Z_{n1} ,Z_{n2} and Z_{n3} respectively as main plots (chelate EDTA 15% Zn was used as a source for Zinc) and four cultivars of alfalfa (Local, Hamaddan, Vernal and Lodi) (Table 2) were randomly assigned on each of the main treatments as a sub-plots P,K and S were added before sowing as recommended (12,14,17) in the end of march 2014 seeds of each cultivar were established in 60 cm drills at seed rate 8 kg ha^{-1} (12,13). Each sub plot consisted of five rows, each three meters along. Cutting for forage was made when plants reached the 15-20% flowering stage. The stands were not left for seed crop during the first year because in second year the stands reach the best age for seed yield (3). All plots were hand weeded during summer 2014 and subsequent time when it is necessary. Last cutting was made on 10th Mey 2015 and crop left for seed set because this time lead the plants to reach flowering on the best time for insect's pollinators (Table 3). The intervals of irrigation were organized and last irrigation was done when the stands reached at beginning of pod formation stage (22). Foliar application of nitrogen (1000 mg.$L^{-1)}$ and boron (500 mg.L^{-1}) were sprayed at beginning of flowering stage in each sub-plots (15,22). Foliar Zinc concentration (0,30,60 and 90) mg.L^{-1}) were carried out by foliar split application (the 1st application was made in the phase of vegetative growth and the 2nd application in the early flower- bud emergence (27).

Table 2. Varieties used in the experiment

Variety	Origin
Local variety	Indian or al-hijaz
Hamaddan	Iran
Vernal	Germplas resources Lab.maryland,U.S.A
Lodi	Italy .Melano,Lodi

Table 3. weather condition during April-End of July 2015 of Abu-Ghraib*

Months	Days	Temp. C		Humidity %		SLR.Avg.W/m^{-2}
		Max.	Min.	Max.	Min.	
April	1-10	30.54	14.21	62.27	17.64	298.7
	11-20	28.68	11.89	55.73	13.35	260.31
	21-30	32.43	14.77	50.70	11.90	280.30
May	1-10	36.33	19.24	44.67	10.13	268.1
	11-20	38.10	20.96	39.7	8.99	275.26
	21-30	40.0	23.29	36.52	9.61	250.60
June	1-10	43.08	25.43	29.87	8.06	269.9
	11-20	41.3	23.21	36.6	8.30	291.36
	21-30	40.5	24.80	39.79	9.37	291.31
July	1-10	44.28	26.99	32.20	6.89	290.85
	11-20	45.40	28.72	32.52	7.73	279.22
	21-30	46.75	28.80	25.72	6.32	284.75

***Meteorological data obtained from Al-Raid station, Abu-Ghraib**

Biological Measurement:

In each sub-plot when the stand reached full bloom stage, ten inflorescences were randomly chosen from the middle rows. sub sample of one floret was taken from the middle section of each inflorescence to calculate the number of ovules per floret using microscope, the ovary was removed from the calyx and corolla, the ovules dissected out and then counted. Quadrate (0.6 m^{-2}) was taken from the middle row and harvested when the majority of pods had turned brown (11) to determine the number of stems quadrate^{-1} and then convert to m^{-2}. Fifty stems were selected at random and following seed yield components were determined: -

Number of rasemes. stem^{-1}

Number of pods. rasemes $^{-1}$.

Number of seeds.pod^{-1}.

1000 seed weight (g) (T.S.W).

Seed yield was estimated from the middle lines, drying were made in the field and threshing was made by hand.

% Ovules Abortion was determined according to following equation:

Abortion % =

$$\frac{\textit{Number of ovules} - \textit{Number of seed per pod}}{\textit{Number of ovules}} \times 100$$

All data collected from this experiment were analysis according to analysis of variance method as described by Steel and Torrie (25). Means were compared using L.S.D test at 5% level of significant. Simple correlation analysis was carried out using SPSS software version 20.

RESULTS AND DISCUSSION

Number of ovules floret^{-1}, Seed set and % of ovules abortion: Seed pod^{-1} (seed set) is an important index which relate to the number of ovules ovary^{-1} and the success rates of seed set. The ovule number reflects the potential number of seeds that could be produced pod^{-1}, under Iraqi conditions, alfalfa plants produce seven to twelve ovules floret^{-1}, but only a few ovules developed successfully into seeds (16). Results shows in Table 4 indicate that foliar application of Zn at level 60 mg.L^{-1} produced highest number of ovules ovary^{-1} (8.33), seed set(5.63) and less proportion of ovules abortion (32.16) and it was significantly different from other levels of Zn except high level (90 mg.L^{-1}) .the soil analysis of this experiment (Table 1) show that the pH value was 8.1,this value of pH reduced the availability of Zn and this in turn may resulted in decrease the initiation and formation of ovules. Low seed set in plant received no Zn was probably due to inadequate supply of Zn during flowering period. Grewal and Willams (9) declare, that Zn play important role in pollen vitality and fertilization. This results in agreement with Ail (2) who found, that in field experiment foliar application of Zn at conc. more than 40 mg.L^{-1} resulted in increases in the number of ovules, seed set and reduced the % of ovules abortion .In contrast Terzic, et al (28) found, that, foliar Zn application had no influence on number of seeds pod^{-1} (seed set). There were also significant differences between varieties in number of ovules ovary^{-1} and seed set, while there were no significant differences between varieties in % of ovules abortion Table 5 shows that the Local var. produced highest No. of ovules (8.2) and highest seed set (5.29) and it was significantly differed from other varieties except var. Hamaddan and the latter was not significantly different to each other's. This is not surprising since. Local var. was more adapted to local condition. This results was in agreement with results found by Wang, et al (31) who stated, that there were significant differences in ovules number ovary^{-1} and ovules fertility among nine varieties of alfalfa. In order to breed high seed yield varieties, plants with more number of ovules ovary^{-1} may be could result in maximum seeds pod^{-1} (seed set). positive correlation between No. of ovules and seed set gave a good evidence for previous statement, (Table 9).

Table 4. Effect of zinc levels on mean number of ovules ovary^{-1}, seed set and % of ovules abortion

Zinc levels mg.L^{-1}	No. of ovules Ovary^{-1}	Seed. set	% of ovules abortion
0 (Zn_0)	7.47	4.32	42.08
30(Zn_1)	7.73	4.75	38.42
60(Zn_2)	8.33	6.63	32.16
90(Zn_3)	8.13	5.47	32.67
L.S.D	0.25	0.47	5.63

Table 5. Means of number of ovules ovary^{-1}, seed set and % of ovules abortion in four alfalfa varieties

Varieties (V)	No. of ovules ovary^{-1}	Seed set	%of ovules abortion
Local (v1)	8.20	5.29	32.92
Hamaddan (v2)	8.04	5.16	35.92
Vernal (v3)	7.72	4.83	37.58
Lodi (v4)	7.70	4.89	36.50
L.S.D	0.39	0.36	NS

Number of stems. m^{-2}: The number of stems is the basic component of seed yield.in this field experiment results in Table 6 indicate that, foliar application of Zn had a significant effect on No. of stems.m^{-2}. There were also significant differences among varieties, but there was no significant interaction. Plants which did not received Zn (Zn_0) had significantly fewer number of stems.m^{-2} (166.8) compare with Zn_1 ,Zn_2 and Zn_3 .maximum number of stems.m^{-2} occurred at highest level of Zn (Zn_3) (218.8) and it was significantly different than others except Zn_2 , indicating that, foliar application of Zn at level 60 or 90 mg.L^{-1} is optimum level for promting number of stems.m^{-2} .This results was in agreement with Grewal and Williams (8) who found , that in pot exp. Zn deficiency resulted marked reduction in shoot dry weight and chlorophyll of fresh leaf tissue .Some authors terzic et al (28) reported that application of Zn had no positive effect on the No. of stem.m^{-2} Hall et al (10) stated that none of the foliar applied micronutrients increased the number of stems in alfalfa plants. There were also significant differences between varieties in number of stems.m^{-2} local variety produced significantly highest No. of stems.m^{-2} (207.8) in comparison to other varieties except var. Hamaddan (199.0) and the latter was significantly different than vernal var. (182.1) and Lodi var. (190.7) . This variation between varieties probably due to ability of a genotype to produce more stems per unit area under different regime of Zn (8).

Table 6. Effect of zinc levels on number of stem .m^{-2} in four alfalfa varieties

Levels of zinc mg.L^{-1}	Local	Hamaddan	Vernal	Lodi	Mean
0 (Zn_0)	176.3	170.5	156.8	163.6	166.8
30(Zn_1)	192.9	183.7	167.1	172.2	179.0
60(Zn_2)	233.6	222.3	197.9	206.6	215.1
90(Zn_3)	228.5	219.5	206.5	220.5	218.8
L.S.D 5%			NS		10.07
Means	207.8	199.0	182.1	190.7	
L.S.D 5%			16.1		

No. of racemes per stem and No. of pods per raceme: It has been identified that No. of raceme stem^{-1} and No. of pods raceme^{-1} are the most two components influencing seed production in alfalfa (23). Foliar application of Zinc (Zn) up to Zn_2 (60 mg.L^{-1}) significantly increase the number of racemes stem^{-1} and number of pods raceme^{-1} (Table 7) , However, increase the level beyond that limit reduce those two parameters, but such reduction was not significant. In this study the number of raceme appeared over the growing season was higher at Zn_2 (14.49) and Zn_3 (13.28) and lowest at Zn_0 (10.33). Table 7 also shown, that Zn_2 level produced higher number of pods raceme^{-1} (11.2) but, it was not significantly different than plants received Zn_3 (10.76) but it was significantly different compare with other levels. This result might be due to role of Zinc in biosynthesis primordial for reproductive organs and partitioning of photosynthesis product toward them, which resulted in better flowering and pod formation. (26) Similar findings were also reported by Ali (2) and Verma (29). There were also significant differences among varieties in number of raceme stem^{-1}, var. Hamaddan having significantly higher racemes (13.78) than all other varieties except Local variety (13.08), while var. Lodi gave significantly lower number with other varieties (10.93) than all except var. Vernal There were also variety differences in the number of pods raceme^{-1}, Local var. produces significantly more pods (11.06) than all other vars. Except Hamaddan (10.23). var. Vernal produce significantly fewer number of pods (9.33) than all other varieties except var. Lodi (9.44). Grewal and Willams (8) stated that, alfalfa varieties had a differential response to low Zn and tolerance to Zn deficiency is considered a genetic trait, similar findings were also reported by Grewal (7).

Table 7. Effect of zinc levels on number of racemes per stem and number of pods per raceme in four alfalfa varieties

Levels of zinc mg.L^{-1}	No.of racemes per stem					No. of pods per raceme				
	Local	Hamaddan	Vernal	Lodi	Mean	Local	Hamaddan	Vernal	Lodi	Mean
0 (Zn0)	10.93	11.27	9.83	9.30	10.33	8.77	8.53	8.43	8.70	8.61
30(Zn1)	11.60	11.93	10.67	10.53	11.18	9.70	9.90	8.97	9.43	9.50
60(Zn2)	15.30	16.37	12.40	12.70	14.19	13.57	11.27	9.87	10.10	11.20
90(Zn3)	14.47	15.53	11.90	11.20	13.28	12.20	11.23	10.07	9.53	10.76
L.S.D 5%		NS			0.9		NS			0.60
Means	13.08	13.78	11.20	10.93		11.06	10.23	9.33	9.44	
L.S.D 5%		1.38					1.18			

1000.seeds weight (T.S.W) : T.S.W attribute was not significantly influenced by foliar application of Zinc. Varieties and their interaction. Despite that there was no significant effect on this trait, but it seems that high T.S.W was occurrence in plants received no Zn (2.82) which itself produce fewer number of seeds pods^{-1} (Table 4). This results may be due to the competition on assimilate during seed filling phase. Variety Hamaddan gave higher T.S.W (2.83) this is genetic trait recognize this variety, T.S.W show significant negative correlation with number of seeds per pod and number of stems per unit area (-0.31), (Table 9).

Seeds yield

Results in Table 8 reveal that foliar application of Zinc and varieties had a significant effect on seeds yield. But the interaction between these two factors do not significant, Plants which did not received Zn (Zn_0) produced lower seeds yield (412.4 kg.ha^{-1}) and it was significantly different from all other levels of Zn. Highest seed yield was produced from plant sprayed with Zinc its conc. 60 mg.L^{-1} (Zn_2) (535.0 kg.ha^{-1}) and it was significantly differed than other levels. Foliar application of Zinc at conc. 30, 60 and 90 mg.L^{-1} resulted in increases in seed yield by 12.19%, 29.72%, 26.28% when compare with Zn_0 respectively. Most component of seeds yield were affected by Zn in alfalfa seed crop. Number of stems unit $area^{-1}$, number of racemes $stem^{-1}$, number of pods $raceme^{-1}$ and seed pod^{-1} are the most components which show high positive correlation with seed yield (0.66, 0.65, 0.78, 0.75) respectively (Table 9).Increase seed yield after foliar application of Zn at level 60 mg.L^{-1} is due to increase in the number of raceme per stem, number of pods per raceme and seed set (Tables 4,5,7).increase of all these reproductive parameters might be due to role of Zinc in biosynthesis in indole acetic acid (IAA) and especially due to its role in initiation of primordial for reproductive parts and partitioning of photosynthesis towards them (26). There were also significantly differences between varieties in seed yield per unit area. Local variety was produced significantly more seed yield (532.5 kg ha^{-1}), when compare with other varieties. Vernal var. produces less seed yield (451.7 kg ha^{-1}) and it was significantly different than all varieties except var. Lodi (Table 8), which produced only (461.4 kg.ha^{-1}). Seeds yield of alfalfa is important in determining the effective distribution of new variety to farmers. Increases of seed yield in local var. probably due in increase in no. of ovules $ovary^{-1}$, seed set, no. of stem m^{-2}, no. of pods $raceme^{-1}$ and less % of ovules abortion (Tables 5,6,7). Table 9 shows positive correlation between seed yield and each of no. of ovules $ovary^{-1}$ (+ 0.57), no. of seeds pod^{-1} (0.75), no. of stems.m^{-2} (0.66), no. of pods $raceme^{-1}$ (0.78) and negative correlation with % of ovules abortion (-0.66). similar findings were also reported by Bolanso-Agnilar et al (6) and Sengul (23).

Table 8. Effect of zinc levels on 1000 seed weight and seed yield in four alfalfa varieties

Levels of zinc mg.L^{-1}	Seed yield kg.ha^{-1}				
	Local	Hamaddan	Vernal	Lodi	Mean
0 (Zn_0)	415.6	410.9	398.4	424.8	412.4
30 (Zn_1)	487.1	466.8	440.8	456.4	462.7
60 (Zn_2)	620.2	538.5	496.2	485.0	535.0
90 (Zn_3)	607.3	525.2	471.5	479.3	520.8
L.S.D 5%	NS				13.8
Mean	532.5	485.3	451.7	461.4	
L.S.D 5%	31.7				

Table 9. Simple correlation coefficients of seed yield component in alfalfa.SEY, seed yield per unit area; NOO, number of ovules per ovary; NSP,number of seeds per pod;%OAB, % of ovules abortion;NST, number of stems per area; NRS , number of racemes per stem; NPR, number of pods per raceme;NPR, number of pods per raceme ; TSW. 1000 seed weight. *$P< 0.05$; $**, P > 0.01$

Traits	NOO	NSR	%OAB	NST	NRS	NPR	TSW
SEY	0.57*	0.75**	-0.66*	0.66*	0.65**	0.78**	-0.24
NOO	-	0.81**	-0.37*	0.67**	0.71*	0.64*	-0.18
NSP		-	-0.84**	0.79**	0.79**	0.68**	-0.30*
%OAB			-	0.65*	-0.60**	0.50*	0.31*
NST				-	-0.68**	0.75**	-0.30*
NRS					-	0.60*	-0.11
NPR						-	-0.13
TSW							

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