EFFECT OF HUMIC ACID CONCENTRATION AND SPRAYING STAGES ON SEEDS YIELD AND ITS CONPONENTS OF ALFALFA

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ABSTRACT

A field experiment was conducted at the experimental fields in the collage of Agricultural Engineering Sciences, University of Baghdad, Iraq during the period from mid. March 2019 to to end of July 2020. The main objective was to find out the effect of humic acid (H_A) concentration (0, 1, 2 and 3 $cm^3.L^{-1}$) and three stages of foliar application (vegetative, growth, flowers buds emergence and 50% flowering) on seeds yield and its components of alfalfa local variety. Layout of the experiment was R.C.B.D arranged in split-plot, with three replications. Foliar application stages were used as main-plots, while humic acid concentrations were used as sub-plots. Results showed that spraying of H_A at conc. 3 $cm^3.L^{-1}$ resulted in a significant increment in number of florets per raceme and number of pods per raceme, in contrast, % of florets abortion in and 1000 seeds weight were not significantly influenced by H_A conc. and spraying stages. Highest number of the $stems.m^{-2}$, number of racemes per stem, seed set and seeds yield (441.0) $Kg.ha^{-1}$ were obtained from plants sprayed with H_A at conc. 3 $cm^3.L^{-1}$. Plants sprayed at vegetative growth produce highest number of $stems.m^{-2}$ number of raceme per stem, number of seeds per pod and seeds yield (423.6) $Kg.ha^{-1}$. There was high positive correlation between seeds yield and each of, number of $stems.m^{-2}$, number of pods per raceme and number of raceme per stem (+0.88, +0.70** and +0.82**) respectively. There was a significant interaction between H_A concentration and spraying stages on seeds yield. The higher seeds yield (485.) $Kg.ha^{-1}$ was obtained when alfalfa plants were sprayed with H_A at the conc. of 2 $cm^3.L^{-1}$ in vegetative growth.**

Key words: florets abortion, seed set, organic acids, pods, racemes.

مجلة العلوم الزراعية العراقية -2021 :52 (6):1541-1548 خربيط

تأثير تراكيز حامض الهيومك ومراحل الرش في حاصل البذور ومكوناته في الجت

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المستخلص

أجريت هذه الدراسة في حقول كلية علوم الهندسة الزراعية – جامعة بغداد للفترة من منتصف آذار 2019 – نهاية شهر تموز 2020 وذلك لدراسة تأثير تراكيز حامض الهيومك (0، 1، 2 و 3) سم^3.لتر$^{-1}$ ومراحل الرش (مرحلة النو الخضري، مرحلة ظهور البراعم الزهرية و 50% تزهير) في حاصل البذور ومكوناته لصنف الجت المحلي. نفذت التجربة بترتيب الالواح المتسقة على وفق تقيم (R.C.B.D) بثلاث مكررات إذ احتلت مراحل الرش الالواح الرئيسسية فيما احتلت تراكيز الحامض الالواح الثانوية. أظهرت النتائج أن رش الحامض بتركيز 3 سم^3.لتر$^{-1}$ قد تسبب في زيادة معنوية في عدد الزهيرات بالنورة وعدد القرنات بالنورة وعلى العكس من ذلك فإن كل من % لإجهاض الزهيرات وكذلك وزن الالف بذرة فإنهما لم يتأثرا معنوياً بتراكيز الحامض ومراحل الرش أعطى التركيز العالي من الحامض أعلى متوسط لكل من عدد السيقان.م$^{-2}$، عدد النورات الزهرية بالساق، عدد البذور بالقرنة وأعلى حاصل بذور بلغ (441.0) كغم.هـ$^{-1}$. كما أظهرت النتائج أن الرش في مرحلة النمو الخضري قد أعطت أعلى متوسط لعدد السيقان.م$^{-2}$ وعدد النورات الزهرية بالساق، عدد البذور بالقرنية وحاصل البذور بلغ (423.6) كغم.هـ$^{-1}$. وجد إرتباط معنوي عالي المعنوية وموجب بين حاصل البذور وكل من عدد السيقان في المتر المربع، عدد القرنات بالنورة وعدد النورات بالساق بلغ (+0.88 , +0.70** و +0.82**) بالتتابع. كما أظهرت النتائج وجود تداخل معنوي بين العاملين في تأثيرهما على حاصل البذور إذ أعطت النباتات التي رشت بتركيز 2 سم^3.لتر$^{-1}$ عند مرحلة النمو الخضري أعلى حاصل بذور بلغ (485.0) كغم.هـ$^{-1}$.**

كلمات مفتاحية: إجهاض الزهيرات، عقد البذور ، الأحماض العضوية.

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INTRODUCTION

Alfalfa (*Midicago satia*) is one of the most perennial forage legumes in Iraq. It is important in farming systems for at least two reasons, the first reason is their ability to restore fertility by fixation of atmospheric nitrogen, secondly, it has advantage over grasses and other legumes in terms of provide high quality forage for livestock (19). The increases of alfalfa pasture throughout the world has increased the need for seeds. especially in Iraq, one of the major problems facing alfalfa cultivation for seed crop is the absence of the suitable guide lines for management particularly during the critical period of seed crop. Moreover, alfalfa in Iraq normally sown for forage production, since seed production only given secondary consideration by farmer. In recent ten years ago, some Iraqi Traders started tom import alfalfa seeds from abroad without any following of regulation of introduce of foreign varieties, since these imported seeds are not adapted to local environment and probably holding dangerous at insects and diseases, despite that the price of foreign var. is low compare with the price of local var. But the Iraqi farmer still prefer to use the local seeds because they found that stands at local var. is more adapted and more persistence in the field (15). The farmers in the country, have little information about how to produce a satisfactory seeds yield per unit area. In general, seeds yield in Iraq no more tham 250 $Kg.ha^{-1}$ and its descried is very low as its compare with other countries such as U.S.A 1200 $Kg.ha^{-1}$, France 800 $Kg.ha^{-1}$, (9) and in China 100-1600 $Kg.ha^{-1}$ (25). The exhaustive removal of nutrients by frequent cutting before release the crop for seed set may lead reduction in food material reserves in roots and crown, this may be enhance new vegetative organic rather the keep those reserve towards seed set and seed formation (3). Utilization of organic fertilizer in agriculture has increased in recent years, after the efforts payed by researchers to replace the uses of organic substances instead of minerals fertilizers which became well known with their harmful effect on human health and environment pollution (Shehtm 2001). One of the used of the organic fertilizers is humic acid, this compound contains many elements which improve the soil fertility and increase the availability of nutrients and consequently increase plant growth and yield (2). Humic acid has role on the viability and increase pollen grain, it is also decrease the % of ovules abortion and contributing in transfer of carbohydrate to active regions of growth during plant reproductive stage (22). There was no study conducted in Iraq on the effect of application of humic acid on seed yield and its components of alfalfa in order to increase the availability of alfalfa seeds to meet future fodder requirements.

MATERIALS AND METHODS

A field experiment was conducted at Agricultural Experimental Station – Collage of Agricultural Engineering Sciences, University of Baghdad during the period from mid-March 2019 to end of July 2020. Soil analysis of the field shows in Table 1. The previous crop before study establishment was maize for grains. The grass stand was plowed and was fallow for three months before planting of alfalfa experiment. Layout of the experiment was Randomize Complete Block Design (R.C.B.D), arranged in split plot with three replications. Three foliar application stages [vegetative growth (two weeks after last cutting), flowers bud emergence and 50% flowering] were used as main plot, while four concentrations of humic acid (H_A) (0, 1, 2 and 3 $cm^3.L^{-1}$) were used as sub-plots. Initial fertilizers were applied for P, K and S were added before sowing as recommended (14,17,18). The purpose of adding fertilizers was to accelerate the growth of alfalfa seedling and ensures the successful establishment of the experiment field. In the mid of March 2019 seeds of local cultivar were established in 60 cm drills at seed rate 8 $Kg.ha^{-1}$ (14,16). The experimental unit was the sub-plot of 10.8 m^2 consisting of six equally spaced rows, three meters in length and 0.6 m apart. The first cutting was made on mid of June 2019 in order to remove all the annual weeds and encourage the alfalfa growth. The subsequent cutting was made when plants reached the 15-20 % flowering stage. The stands were not left for seed crop during the 1st year because in second year the stands reach the best age for seed yield (5). Last cutting was made on 13th of

May 2020 and the crop left for seed set because this time lead the plants to reach flowering on the best time for insect's pollinators Table 2. The intervals of irrigation were organized and last irrigation was done when the stands reached at beginning of pod formation stage (21). Foliar humic acid concentration (0, 1, 2 and 3) $cm^3.L^{-1}$ was carried out according to the growth stages, vegetative, flower bud emergence and 50% flowering.

Biological measurements

At completely bloom stage, twenty racemes were randomly chosen from the middle row in each sub-plot to calculate the number of florets per raceme. Quadrate (0.6 m^2) was taken from the middle rows and harvested when majority of pods had turn brown (13) to determine the number of stems per quadrate and then convert to m^{-2}. Fourty stems were chosen at random and following seed yield component were determined:

1- Number of racems.$stems^{-1}$
2- Number of pods.$raceme^{-1}$
3- Number of seeds.pod^{-1}
4- 1000 seeds weight (g) (T.S.W)=
5- Seeds yield were estimated from middle lines, drying was made at the field and threshing was made by hand.
6- Percentage of florets abortion was determined according to the following equation:

$$\textbf{Abortion}\ \% = \frac{\textbf{Number of florets} - \textbf{number of pods}}{\textbf{Number of florets}} \times \textbf{100\%}$$

Data were analyzed by analysis of variance as described by Steel and Torrie (24). Means were compared using L.S.D at the 5% level of significant. Simple correlation analysis was carried out using SPSS software version 20.

Table 1. Some chemical and physical properties of soil before planting

Property	Unit	Value
Electrical conductivity 1:1	$ds.m^{-1}$	3.8
PH	-	8.1
N	ppm	46
P	ppm	11.6
K	ppm	175
Organic matter	%	0.73
Soil texture	Silty clay loam	

RESULTS AND DISCUSSION

Number of florets per raceme, pods per raceme and % of florets abortion

Racemes in alfalfa produce many florets. In general, there are twelve to twenty-five, but only 70% of these florets or less many developed successfully into pods. Results shown in Table ?? and ?? indicated that humic and their foliar application stages had significant effect on each number of florets and pods per raceme, while % of florets abortion was not significantly influenced by H_A. Concentration and foliar application stages. Table 2 shows that plants which did not receive H_A (HA0) produce fewer florets per raceme (14.13) and fewer pods per raceme (8.56). in both parameters, increase in the concentration of H_A significantly increases of florets and pods per raceme reach the peak at concentration H_{A3} (17.95) and (11.74), respectively. Suggesting that increases in the concentration of H_A higher than used in this experiment may be useful in increase of those two parameters. Spraying of H_A at concentration 1, 2 and 3 $cm^3.L^{-1}$ compare with H_{Ao} resulted in an increase in number of florets and pods per raceme by (9.5%, 18.3% and 27%) and (16%, 28.8% and 33.9%) respectively. Such increase may be due the fact that H_A involve in maintenance of many physiological enzymes activity that leads to easy translocation of assimilate from leaf to reproductive organs, humic acid also has role in the viability and increase pollen grain this in turn may lead to increase pollination since florets which were not pollinated may be dried and then fall down (22). Similar findings were also reported by Azarpour etal. (1) in cowpea and Al-Fahdwee (7) in faba bean. While the % of florets abortion not significantly influenced by foliar application stages. Foliar application of H_A at flower buds emergence gave highest number of florets per raceme (17.4) and number of pods per raceme (11.21). while delay of spraying until 50% flowering resulted in a significant decrease in both number of florets and pods per racemes (Table 3). Such reduction probably due to that delay in spaying may reduce the benefit from the nutrient because it never gave a significant time for absorb the nutrient found in H_A.

Table 2. Effect of humic acid concentration on mean number of florets per raceme, number of pods per raceme and % of florets abortion

Humic acid conc. ($cm^3.L^{-1}$)	No. of florets per raceme	No. of pods per raceme	% of florets abortion
0 (H_0)	14.13	8.56	37.8
1 (H_1)	15.48	9.93	36.22
2 (H_2)	16.72	11.03	34.04
3 (H_3)	17.95	11.74	34.44
L.S.D 5%	0.73	0.61	NS

Table 3. Effect of foliar application stages on mean number of florets per raceme, number of pods per raceme and % of florets abortion

Foliar application stages	No. of florets per raceme	No. of pods per raceme	% of florets abortion
Vegetative Growth	16.0	10.38	34.97
Flower buds emergence	17.4	11.21	35.71
50% flowering	14.73	9.42	36.22
L.S.D 5%	1.49	0.65	NS

Number of steams m^{-2}

The number of steams is the basic component of the seed yield in alfalfa. In this field experiment results in Table 4 indicate that, there were significant effect of humic acid concentration, foliar stages and the interaction between the two factors. Plants which did not receive H_A (H_{A0}) produce significantly fewer number of stems.m^{-2} (185.6) compare to H_{A1}, H_{A2} and H_{A3}.Maximum number of stems.m^{-2} occurred at higher concentration of H_A (225.7) and it was significantly different than others except H_{A2} (218.1), indicating that folia application of H_A at level 2 or 3 $cm^3.L^{-1}$ is optimum level for promoting number of stems.m^{-2} this can be attributed to the vital role of H_A in improving cell division and cell elongation which has s direct effect on different biological operations as it affect photosynthesis and respiration, these results are in accordance with finding of El-Bassiony, etal (11), and Al-Fahdwee (7). Table 4 indicat a significant effect of spraying stages on this character as spraying stage at vegetative growth exceeded by giving the highest mean of stems.m^{-2} (224.6) and differed significantly compare with flower bud emergence (206.1) and 50% flowering (201.1) which were not significantly to each other, this may be due to spraying at vegetative growth which will provide enough time for plant to benefit from the nutrient found in the humic acid composition which will enhance the development of new stems from the crown. Table 4 shows a significant interaction between the two factors the reason behind this interaction may be due to the differences in relative response to H_A concentration in different spraying stages as it was generally noted that all concentration gave the highest mean when spraying at vegetative growth stage and started to be decreased with progress in plants growth and this decrease was more clear at high concentration compare with low concentration and control treatment.

Table 4. Effect of humic acid and spraying stages on no. of stems.m^{-2}

Humic acid conc. ($cm^3.L^{-1}$)	Vegetative growth	Flowers buds emergence	50% flowering	Mean
0 (H_0)	184.5	183.9	188.7	185.7
1 (H_1)	222.9	208.3	207.6	212.9
2 (H_2)	239.3	213.1	201.7	218.0
3 (H_3)	251.8	219.1	206.5	225.8
L.S.D 5%		15.9		10.3
Mean	224.6	206.1	201.1	
L.S.D 5%			5.6	

Number of seeds per pod and number of raceme per stem^{-1}

Number of seeds per pod (seed set) and ripe racemes per stem have been identified as the most components influencing seeds yield in alfalfa (Kharbeet, 2018). Table 5 shows that spraying of humic acid had significant effect on number of seeds per pod, plants which did not receive H_A (H_{A0}) produced lower number of seeds per pod (3.64) and it was only significantly different compare with H_{A3} (4.18) which were not significantly to each other. The reason behind the low seed set in the absence of H_A probably due to the role of H_A in the viability and increase pollen grains and increase pollen tube length, it also decrease the % of ovules abortion and contributing in transfer of carbohydrate to active regions of growth with result of Al-Fahdwee (7) in faba bean, Dawa, et al (10) in pea and Al-Dulimi (6) in green bean. Table 5 shows that there was significant effects of foliar application stages on mean number of seeds per pod. Highest seed set was obtained from plants sprayed at 50% flowering stage (4.28) and it was significantly different than vegetative growth (3.84) and flower buds emergence (3.74). This result indicates that foliar application of H_A at 50% flowering stage in alfalfa seed crop may be more relevant to increase seed set. There was no significant interaction between the two factors on this trait. Table 6 shows that number of racemes per stem were significantly influenced by H_A concentration. In the present experiment the number of racemes appear over the growing season was higher at H_{A3} (14.26) and it was significantly different than all concentration except H_{A2} (13.62). Plants which did not receive H_A (H_{A0}) produce lower number of racemes per stem (10.81) and was significantly different compare with other concentration. This result probably due to the fact that spraying of H_A to the foliage plant may increase enzymes activity that lead to easy translocation of photosynthetic product from the leaves to reproductive organs (22). Data in Table 6 shows that foliar application of H_A at 50% flowering stage produced significantly low number of racemes per stem (11.72) compare with flower buds emergence (12.87) and vegetative growth (13.34) which were not significantly different to each other. Relative increases percentage in number of racemes per stem when H_A spraying at vegetative growth and flower buds emergence as compare to 50% powering stage were 13.8% and 9.8% respectively. Such increase in number of racemes when H_A was sprayed at vegetative growth may be due to the fact that time given to the H_A is more enough to absorb and translocate within the plant tissue to take it role in bioprocesses in the plant, since enough quantity of H_A may prevent abscission of flowers and floral parts (20). Table 6 also shows a significant interaction between the two factors, the reason behind this interaction may be due to the differences in relative responses to acid conc. in different spraying stages.

Table 5. Effect of humic acid concentration, foliar application stages and their interaction on mean seed per pod

Humic acid conc. ($cm^3.L^{-1}$)	Vegetative growth	Flowers buds emergence	50% flowering	Mean
0 (H_0)	3.70	3.60	3.63	3.64
1 (H_1)	3.73	3.66	4.28	3.87
2 (H_2)	3.90	3.93	4.56	4.13
3 (H_3)	4.03	3.80	4.73	4.18
L.S.D 5%		NS		0.53
Mean	3.84	3.74	4.28	
L.S.D 5%		0.37		

Table 6. Effect of humic acid concentration, foliar application stages and their interaction on mean number of racemes per stem

Humic acid conc. ($cm^3.L^{-1}$)	Vegetative growth	Flowers buds emergence	50% flowering	Mean
0 (H_0)	11.03	10.63	10.77	10.81
1 (H_1)	12.33	12.43	10.97	11.92
2 (H_2)	14.20	13.97	12.70	13.62
3 (H_3)	15.83	14.47	12.47	14.26
L.S.D 5%		1.29		0.71
Mean	13.34	12.87	11.72	
L.S.D 5%		0.97		

Seeds yield $Kg.ha^{-1}$

Results in Table 7 reveal that foliar application H_A, foliar application stages and their interaction had a significant effect on seed yield. Plants which did not receive H_A (H_{A0}) produced lower seeds yield (283.2 $Kg.ha^{-1}$) and it was significantly different from all other concentrations of H_A. highest seed yield was produced from plants sprayed with H_A at conc. 3 $cm^3.L^{-1}$ (H_{A3}) (441.8 $Kg.ha^{-1}$) and was significantly different than other conc. except H_{A2} (419.7 $Kg.ha^{-1}$), foliar application of H_A at conc. 1, 2 and 3 $cm^3.L^{-1}$ resulted in increase in seed yield by 15.4%, 48.1% and 56.0% when compare with H_{A0}, respectively. In this field experiment it seems that most of seed yield components were influenced by H_A, number of stems per unit area, number of racemes per stem, number of florets per raceme and number of racemes pods per raceme are the most components, which show high positive correlation with seed yield (0.83, 0.82, 0.61, 0.70) respectively (Table 8). Increase seed yield after foliar application of H_A at high level 3 $cm^3.L^{-1}$ is due to increase in all components except 1000 seeds weight (Tables 2, 4, 5, 6) these results are in agreement with those reported by Al-Fahdwee (7) in faba bean Al-Dulimi (6) in green bean, Azarpour etal. (1) in cowpea, Al-Jumaily (8) on barley. Concerning the effect of foliar application stages Table 7 shows that highest seed yield were obtained when plants sprayied at vegetative growth (423.67 $Kg.ha^{-1}$) and it was significantly different compare with flower buds emergence stage (353.0 $Kg.ha^{-1}$) and 50% flowering stage (326.9 $Kg.ha^{-1}$) which were not significantly to each other. It is clear that delay in foliar application of humic acid decrease seeds yield in alfalfa plants under Iraqi condition. The reduction in this seed yield could be due to that delaying may never give a significant time for plants to absorb the H_A and transfer it to active regions from that stage to their role in metabolic process. Similar findings were also reported on Sorghum by Al-Bieruty etal (4). Table 7 shows significant inter action between the two factors, the reason behind this interaction probably due to the different responses to H_A conc. by different spraying stages as it is appear that low H_A (H_{A1}) and control treatment (H_{A0}) were not significantly influenced by different spraying stages, on the contrary of the conc. of H_{A2} and H_{A3} in which seed yield decreased significantly when spraying delay to flower buds emergence and 50% flowering stage.

1000. seeds weight (T.S.W)

This trait did not significantly influenced by H_A concentration, foliar application stages and their interaction. Despite that there was no significant effect of H_A on this trait, but it seem that low T.S.W was occurrence in plants received high concentration of H_A (H_{A3}) (2.49) g which itself produce highest number of seeds per pod (Table 5) this result may be due to high competition on assimilate during seed filling stage.

Table 7. Effect of humic acid concentration, foliar application stages and their interaction on mean seed yield ($Kg.ha^{-1}$).

Humic acid conc. ($cm^3.L^{-1}$)	Vegetative growth	Flowers buds emergence	50% flowering	Mean
0 (H_0)	306.1	270.5	273.1	283.2
1 (H_1)	358.0	314.9	308.2	327.0
2 (H_2)	485.2	399.4	374.5	419.7
3 (H_3)	454.5	427.8	352.0	441.8
L.S.D 5%		52.67		32.3
Mean	423.67	353.05	326.94	
L.S.D 5%		29.45		

Table 8. Simple correlation coefficients of seed yield component in alfalfa. SEY, seed yield, NST, number of stems per unit area, NFR, number of florets per raceme, NPR, number of pods per racemes, %FAB, % florets abortion, NSP, number of seeds per pod, NRS, number of racemes per stem, TSW, 1000 seed weight

Traits	NST	NFR	NPR	%FAB	NSP	NRS	TSW
SEY	0.83^{**}	0.61^{**}	0.70^{**}	-0.33^{*}	0.07^{NS}	0.82^{**}	-0.29^{NS}
NST	-	0.54^{*}	0.67^{**}	-0.39^{*}	-0.05^{NS}	0.78^{**}	-0.34^{*}
NFR		-	0.88^{**}	0.007^{NS}	-0.07^{NS}	0.75^{**}	-0.04^{NS}
NPR			-	-0.45^{*}	-0.04^{NS}	0.78^{**}	-0.07^{NS}
%FAB				-	-0.09^{NS}	-0.23^{NS}	0.09^{NS}
NSP					-	0.09^{NS}	0.28^{NS}
NRS						-	-0.11^{NS}
TSW							

NS= Not significant * significant at 5% level ** at 1% level

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