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Response of Egyptian Clover (Variety Fahl) to Foliar Spray with Potassium Humate, Fulvate as Well as Amino Acids Mixture

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ABSTRACT

A field trial was conducted at Giza Agricultural Research Station, A.R.C., Egypt during 2013/2014 and 2014/2015 winter seasons, to study the response of Fahl Egyptian clover to foliar spray of 5 and 10 ml/L from potassium humate, fulvate as well as amino acids mixture on growth, productivity and chemical composition. Results indicated that foliar spray with 10 ml/L K-humate(10%K2o) increased significantly plant height, number of secondary branches/plant, number of heads/plant, dry leaf/stem ratio, number of seeds/head, 1000 seed weight, fresh and dry fodder yields as well as seed yield, followed by foliar spray with 10 ml/L amino acids mixture. Insignificant differences was observed between such two treatments with respect to fresh, dry and seed yields/fad. The lowest values of such traits were obtained from foliar spray with 5 ml/L K-fulvate compared to treated plants with K-humate and amino acids mixture. The maximum values of crude protein, oil (ether extract, E.E.), N, P and K% were obtained from treated plants by 10 ml/L K-humate followed by 10 ml/L amino acids mixture compared to control. The maximum value of crude fiber % was obtained from spraying plants by water (control), while the lowest value was obtained when plants sprayed with 10 ml/L K-humate.

Keywords: Egyptian clover, potassium humate, fulvate and amino acids

INTRODUCTION

In Egypt, the production of green forage is less than the demand, which affects either meat or milk production. Forage crop plays a vital role in the sustainability of agriculture production. It is an excellent plant for suppressing and controlling weeds and is environment friendly crops because minimum or no pesticides are used. Egyptian clover has been introduced with special emphasis to the agriculture sector as untraditional forage crops, the importance of such crop is the high forage productivity and quality during winter and spring season. Berseem is a major seed export crop (more than 12000 tons) and occupied about one third of the cultivated area in Egypt (between two to three millions faddans as full season and short season crops), as well as the area devoted to seed production during winter (El-Nahrawy, 2005). Egyptian clover (*Trifolium alexanrinum* L.) is the main forage crops grown in Egypt during the winter season. One of the strategies to improve forage productivity is the foliar application of humic and fulvic acids as well as amino acids mixture. Humic acid play a prominent role in various physiological and biochemical processes related to environmental stresses. Humic acid have ecological importance, as they intervene in the regulation of a large number of chemical and biological processes that occur in the natural ecosystems (Chen *et al*., 2004). Asik *et al.* (2009) reported that the lowest doses of both soil and foliar application of humic substances increased the nutrient uptake of wheat. Furthermore, El-Hefny (2010) reported significant increases in plant height, number of branches, fresh weight, leaf area/plant, total pods yield, N, P, K uptake as well as protein and carbohydrate contents in cowpea seeds with increasing the rate of humic acid application from 1, 3, 4.5 up to 6 kg/fad. Ghorbani *et al.* (2010) reported that foliar spray with humic acid has remarkable effects on vegetative growth of plant and increases photosynthetic activity and leaf area index of corn. With respect to foliar spray by fulvic acid, Xudan (1986) reported that fulvic acid sprayed on wheat plants increased grain yield by 7 to 18% over control. Khang (2011) stated that the foliar application of fulvic acid at dose of 1, 2 and 4 percent on rice and radish significantly increased plant height compared to control. Abbas *et al.* (2015) reported that soaking seeds of cowpea and pearl millet in different concentration of humic and fulvic acids significantly improved germination percentage, shoot and root length compared to control.

Amino acids are well known as biostimulants which have positive effects on plant growth, yield and significantly mitigates the injuries caused by abiotic stresses (Kowalczyk and Zielony, 2008). El-Zohiri and Asfour (2009) on potato found that spraying of amino acids at 0.25 ml/L significantly increased vegetative growth expressed as plant height and dry weight of plant.

The objective of this research was to study the effect of foliar spray by some organic acids *i.e.* K-humate, fulvate as well as amino acids mixture on growth, productivity and chemical composition of Fahl Egyptian clover.

MATERIALS AND METHODS

The present work was carried out at Giza Agricultural Research Station, ARC, Egypt during 2013/2014 and 2014/2015 winter seasons, to study the response of Egyptian clover (Fahl variety) to foliar spray with K- humate, K- fulvate and amino acids mixture.

The experiment was laid out in a randomized complete block design with four replicates. Each plot area was 12 m^2 (3x4 m). Some physical and chemical properties of the experimental site in two growing seasons are shown in the following Table.

Soil characteristics	2013/2014	2014/2015
Particle size distribution %		
Clay	39.95	40.10
Silt	36.40	36.50
Sand	23.65	23.40
Soil texture	Clay loam	Clay loam
Chemical properties:		
pH (suspension 1:2.5 soil: water)	8.11	8.07
EC(dsm^{-1})(Saturated paste extract)	1.28	1.25
$CaCO_3$%	1.14	1.11
O.M. %	1.21	1.14
Available nutrients (ppm)		
N	39.5	39.1
P	12.8	12.5
K	370	372

Fahl clover seeds were sown at the rate of 20 kg/fad on 15/10/2013 and 1/11/2014 in the first and second seasons, respectively.

The treatments are as follows:

1. Spraying with water (Control).
2. Spraying with 5 ml/L K- humate.
3. Spraying with 10 ml/L K- humate.
4. Spraying with 5 ml/L K- fulvate.
5. Spraying with 10 ml/L K- fulvate .
6. Spraying with 5 ml/L amino acids mixture.
7. Spraying with 10 ml/L amino acids mixture.

A liquid commercial product of amino acids mixture consists of (0.56% leucine, 1.91% alanine, 1.8% valine, 0.42% arginin, 8.1% glutamic, 0.62% aspartic, 2.33% lysine, 0.41% threonine, 2.36% phenylalanine, 0.40% histidin, 2.37% tyrosine, 2.55% glycine, 0.42% isoleucine, 0.29% serine, 0.46% proline), 4% N, 5% K_2O and 1% P_2O_5 were used as a source of amino acids mixture. Potassium humate and fulvate (10% K_2O) solution were used as a source of humic and fulvic acids. Elementary composition of K-humate and K- fulvate are presented in Table (1).

Table 1. Chemical analysis of potassium Humate and potassium fulvate used in this study.

Elements	Potassium humate	Potassium fulvate
pH	8.60	3.28
EC (dS/m)	55.80	57.80
O. M (%)	21.98	14.48
C%	12.75	8.40
C/N	7.33	11.50
Macro elements (%)		
N	1.68	0.86
P	0.25	0.16
K2o	10.00	10.00
Micro elements (ppm)		
Fe	101	71.60
Zn	2.45	10.40
Mn	1.29	1.65
Cu	0.39	0.25

During seed bed preparation, 150 kg P_2O_5/fad was incorporated into soil in the form of calcium superphosphate (15.5% P_2O_5). Furthermore, 50Kg ammonium nitrate 33.5% N was added before the second irrigation as a starter dose. K- humate, K-fulvate as well as amino acids mixture were sprayed two times at 20 and 35 days after sowing, the volume of water was 1 L/plot at the first time and 1.5 L/plot at the second time, 0.5% wetting agent of Tween 20 was used.

The experimental plots were divided into two equal parts ; the first was for estimating growth, fresh and dry fodder yields (ton/fad), while the second was left to the stage of flowering and seed formation to estimate yield components and seed yield (kg/fad). Other cultural practices were followed according to the methods adopted for growing Egyptian clover var. Fahl in the locality. Cutting took place at 1/1/2014 and 15/1/2015 in the first and second seasons, respectively. At cutting time, ten guarded plants were randomly taken from central area in each plot to determine some growth traits as follows:

1- Plant height (cm).
2- Number of secondary branches/plant.
3- Number of heads/plant.
4- Dry leaf/stem ratio.

An area of $2m^2$ was cutting from each plot to determine fresh and dry yields (ton/fad).

To determine number of seeds / head, 1000 seed weight (g) and

seed yield (kg/fad) plants were harvested on 21/1/2014 and 4/2/2015 in the first and second seasons, respectively.

At second season, plant samples of (100 g) were collected from each plot, weighed and oven dried at 70°C for 48h up to the constant dry weight, ground and prepared for digestion as described by Page *et al*. (1982). The digests were subjected to evaluate N, P and K% according to Cottenie *et al.* (1982), nitrogen percentage was multiplied by conversion factor of 6.25 as mentioned by Hymowitz *et al*. (1972) to calculate the protein percentage of Fahl Clover plant. Ground plant samples were subjected to estimate crude fiber and oil (ether extract, EE) percentages according to A.O.A.C. (1980).

Data were statistically analyzed according to Snedecor and Cochran (1980) and treatment means were compared by least significant difference test (LSD) at 0.05 level of significance. Bartlett test according to (Bartlett, 1937) was done to test the homogeneity of error variance. The test was not significant for all assessed traits, so, the two season's data were combined. The discussions of the results were carried out on the basis of combined analysis for the two seasons.

RESULTS AND DISCUSSION

I- Growth

Results in Table (2) indicated that all growth traits under study *i.e.* plant height, number of secondary branches/plant, number of heads/plant and dry leaf/stem ratio recorded significant affects. The maximum values of such characters were obtained from plants sprayed by 10 ml/L K- humate followed by spraying 10 ml/L amino acids mixture compared to control (spraying by water), with insignificant differences between such two treatments with respect to dry leaf/stem ratio trait.

It is worthy to mention that spraying plants by 10 ml/L K- humate increased plant height, number of secondary branches/plant, number of heads/plant and dry leaf/stem ratio compared to control by 22.22, 29.41, 27.18 and 23.61%, respectively. The lowest values of such traits were obtained when plants treated by 5 ml/L K- fulvate compared to other growth promoting substances under study *i.e.* K- humate as well as amino acids mixture.

Table 2. Plant height, number of secondary branches /plant, number of heads/plant and dry leaf/stem ratio of Fahl Egyptian clover as affected by foliar spray of K- humate, fulvate as well as amino acids mixture in two growing seasons 2013/2014 and 2014/2015.

Foliar spray treatments	Plant height (cm)			Number of secondary branches / plant			Number of heads/plant			Dry leaf / stem ratio		
	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.
Water (control)	67.9	68.8	68.4	4.19	4.31	4.25	7.42	7.51	7.47	1.41	1.46	1.44
K-humate 5 ml/L	72.9	74.1	73.5	4.67	5.00	4.84	8.33	8.67	8.50	1.62	1.67	1.65
K-humate 10 ml/L	82.6	84.6	83.6	5.33	5.67	5.50	9.33	9.67	9.50	1.76	1.80	1.78
K-fulvate 5 ml/L	70.9	71.9	71.4	4.00	4.33	4.17	7.33	7.67	7.50	1.49	1.55	1.52
K-fulvate 10 ml/L	73.7	74.6	74.2	4.33	4.67	4.50	7.67	8.00	7.84	1.65	1.70	1.68
Amino acids mixture 5 ml/L	71.7	72.8	72.3	4.33	4.67	4.50	7.67	8.00	7.84	1.55	1.61	1.58
Amino acids mixture 10 ml/L	78.0	78.9	78.5	4.67	5.00	4.84	8.33	8.67	8.50	1.70	1.73	1.72
L.S.D. at 5%	5.8	5.7	3.7	0.84	0.76	0.51	0.92	0.84	0.57	0.14	0.13	0.09
C.V.	5.0	4.3	4.7	8.50	8.10	8.30	6.20	5.80	6.00	4.50	4.30	4.40

The mechanism of humic substances action in promoting plant growth was explained by some authors, (Biondi *et al.*, 1994 and Chen *et al.*, 2004). Furthermore, Khaled and Fawy (2011), reported that, humic acid increasing cell membrane permeability, oxygen uptake, respiration and photosynthesis, enzymatic activities, phosphorus uptake, root and cell elongation, ions transport and soil microorganisms. With respect to the foliar application of amino acids mixture, Saeed *et al.* (2005) on soybean, found that, treatments of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield.

II- Yield and yield components

Foliar spray of growth promoting substances *i.e.* K- humate, K- fulvate as well as amino acids mixture had a significant effects on number of seeds/head, 1000 seed weight, fresh and dry yields/fad as well as seed yield/fad (Table 3). The highest values of such traits were scored from plants sprayed by 10 ml/L of K-humate followed by spraying 10 ml/L of amino acids mixture compared to control (spraying by water). However, insignificant differences were observed between such two treatments with respect fresh, dry and seed yields/fad. While, the lowest values of such traits were recorded from plants sprayed by 5 ml/ K- fulvate compared to foliar spray by K- humate as well as amino acids mixture. This trend could be attributed to the promoting effect of K- humate as well as amino acids mixture on growth and yield components which in tern reflected on fresh, dry and seed yields/fad. It can be noticed that spraying Fahl plants with 10 ml/L K-humate increased number of seeds/head, 1000 seed weight, fresh and dry yields as well as seed yield more than control by 23.68, 28.18, 22.50, 39.41 and 38.58% respectively. In this connection, Sadek and Sallam (2012) found that foliar spray with humic acid on barley plant resulted in substantial promotion of the studied growth parameters, kernel weight/spike and 1000 grains weight compared to control. Abbas *et al.* (2013) stated that treated mono-cut Egyptian clover (Fahl) plants with humic acid increased growth, yield and yield components traits compared with control. Moraditochaee *et al*., (2012) on cowpea plant found that foliar spray with amino acid increased number of pods/plant and seed yield. Similar results were obtained on amino acids effects for different plants by El-Shabasi *et al.*, 2005; Alaa *et al.*, 2009 and Shehata *et al.*, 2011.

Table 3. Number of seeds/head, 1000 seed weight, fresh yield, dry yield and seed yield/fad of Fahl Egyptian clover as affected by foliar spray of K- humate, K- fulvate as well as amino acids mixture in two growing seasons 2013/2014 and 2014/2015.

Foliar spray treatments	Number of seeds/head			1000 seed weight (g)			Fresh yield (ton/fad)			Dry yield (ton/fad)			Seed yield (kg/fad)		
	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.	2013/2014	2014/2015	Comb.
Water (control)	66.67	67.93	67.30	3.25	3.35	3.30	14.20	14.15	14.18	2.07	1.99	2.03	232.0	242.3	237.2
K-humate 5ml/L	75.53	76.73	76.13	3.78	3.88	3.83	15.97	16.36	16.17	2.49	2.37	2.43	297.7	320.3	309.0
K-humate 10 ml/L	82.64	83.83	83.24	4.16	4.29	4.23	17.53	17.21	17.37	2.89	2.77	2.83	325.0	332.3	328.7
K- Fulvate 5 ml/L	71.52	72.80	72.16	3.63	3.69	3.66	14.06	14.47	14.27	2.04	2.22	2.13	277.3	295.1	286.2
K- Fulvate 10 ml/L	77.53	78.53	78.03	3.82	3.87	3.85	16.59	16.74	16.67	2.49	2.77	2.63	303.7	325.0	314.4
Amino acids mixture 5 ml/L	73.93	74.83	74.38	3.71	3.77	3.74	15.22	15.75	15.49	2.33	2.77	2.55	286.7	320.3	303.5
Amino acids mixture 10 ml/L	79.83	81.03	80.43	3.91	3.97	3.94	16.94	17.47	17.21	2.83	2.70	2.77	316.3	322.7	319.5
L.S.D. at 5%	4.40	3.80	2.70	0.26	0.32	0.19	1.39	1.21	0.85	0.33	0.35	0.22	28.9	29.6	19.8
C.V.	4.30	3.40	3.90	5.40	4.70	5.10	9.40	8.70	9.10	8.70	8.00	8.40	8.4	8.3	8.4

III- Chemical composition

A- Crude protein and oil (ether extract, E.E) %

Data presented in Table (4) indicated that foliar spray with 10 ml/L K- humate increased significantly crude protein and oil contents of clover Fahl plants compared to control (spray with water), followed by spraying 10 ml/L amino acids mixture, with insignificant differences between such two treatments with respect to oil % trait. It can be noticed that spraying Fahl Clover plants with 10 ml/L K- humate increased crude protein and oil contents compared to control by 13.74 and 26.91%, respectively. In this respect Sadek and Sallam (2012) reported that foliar spray of 300 mg/L humic acid on barley plants significantly increased crude protein percentage of grains. Also, Kasraie *et al.* (2012) concluded that foliar spray of amino acids before water deficit stress increased grain protein percentage of corn.

B- Crude fiber %

Table (4) shows that crude fiber percentage of Fahl clover plants recorded a significant response due to the treatments used. The maximum value of crude fiber percentage was obtained from plants sprayed by water (control), followed by spraying 5 ml/L K –falvate. Whereas, the lowest value of crude fiber was gained when plants received 10 ml/L K- humate compared to control, foliar spray by K –falvate as well as amino acids mixture. It can be noticed that a reveres trund line was obtained between crude fiber and protein percentages with respect to foliar spray of all growth promoting substances under study. It is worthy to mention that spraying Fahl plants by 10 ml/L K- humate decreased crude fiber percent by 22.04% compared to control (spraying by water).

Table 4. Crude protein, crude fiber, oil, N, P and K (%) of Fahl Egyptian clover as affected by foliar spray of K- humate, K –falvate as well as amino acids mixture in the second season 2014/2015.

Foliar spray treatments	Crude protein (%) 2014/2015	Crude fiber (%) 2014/2015	Oil % 2014/2015	N (%) 2014/2015	P (%) 2014/2015	K (%) 2014/2015
Water (control)	14.56	23.77	4.57	2.33	0.29	1.32
K- humate 5 ml/L	15.13	22.39	5.10	2.42	0.36	1.64
K- humate 10 ml/L	16.56	18.53	5.80	2.65	0.41	1.80
K –falvate 5 ml/L	15.00	23.53	4.40	2.40	0.32	1.29
K –falvate 10 ml/L	16.13	22.25	5.33	2.58	0.35	1.36
Amino acids mixture 5 ml/L	14.88	22.49	4.67	2.38	0.36	1.55
Amino acids mixture 10 ml/L	16.25	21.62	5.43	2.60	0.38	1.68
L.S.D. at 5%	0.17	2.26	0.49	0.04	0.08	0.18

C-Nitrogen(N), phosphorus(P) and Potassium (K) %

Results in Table (4) show that N, P and K percentages of Fahl clover plants recorded significant affects as affected by foliar spray of K- humate, K –falvate as well as amino acids mixture. The maximum values of N, P and K% were obtained when plants received 10 ml/L K- humate followed by 10 ml/L amino acids mixture, with insignificant differences between such two treatments for K and P% traits. Foliar spray of 10 ml/L K- humate increased N, P and K% more than control by 13.73, 41.38 and 36.36%, respectively. In this respect, Abbas *et al.* (2013) found that application of humic acid on Fahl clover plant exhibited the highest values of N, P and K%. Such finding may be attributed to the favorite effect of humic acid in enhancing mineral nutrients uptake by plants, through its effect on the permeability of roots membranes (Mesut *et al.*, 2010).

CONCLUSION

In the light of the present results, it is clear that the maximum fresh and dry fodder yields as well as seed yield, crude protein and oil % of Fahl Egyptian clover were obtained from spraying plants with 10 ml/L K- humate followed by 10 ml/L amino acids mixture, with no significant differences between such two treatments in most traits under study, specially fresh, dry and seed yields/fad.

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استجابة البرسيم المصرى (الفحل) للرش الورقى بهيومات وفلڤات البوتاسيوم وكذلك مخلوط الاحماض الأمينية

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2- قسم بحوث فسيولوجى المحاصيل – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية – الجيزة – مصر.

3- قسم بحوث تغذية النبات – معهد بحوث الاراضى والمياه والبيئة – مركز البحوث الزراعية – الجيزة – مصر.

أجريت تجربة حقلية فى محطة البحوث الزراعية بالجيزة خلال موسمى الشتاء 2014/2013 و 2015/2014 لدراسة استجابة البرسيم المصرى (الفحل) للرش الورقى بهيومات وفلڤات البوتاسيوم وكذلك مخلوط الأحماض الأمينية بتركيز 5 ، 10 ملليلتر / لتر لكل منهم على النمو والانتاجية وكذلك التركيب الكيميائى. أوضحت النتائج الآتى:- أدى الرش الورقى بهيومات البوتاسيوم بتركيز 10 ملليلتر / لتر إلى زيادة معنوية فى كل من طول النبات، عدد الأفرع الثانوية / نبات، عدد النورات/ نبات، النسبة الجافة للأوراق / الساق، عدد البذور /نورة ، وزن الـ 1000 بذرة، محصول العلف الأخضر والجاف وكذلك محصول البذور / فدان يليها الرش الورقى بمخلوط الاحماض الامينية بدون فرق معنوى بين المعاملتين بالنسبة لمحصول العلف الاخضر والجاف وكذلك محصول البذور. بينما كانت أقل قيمة للصفات السابقة عند رش 5 ملليلتر/ لتر فلڤات بوتاسيوم مقارنة بالرش بهيومات بوتاسيوم اومخلوط الاحماض الأمينية.- وسجلت نتائج التحليل الكيميائى للبرسيم الفحل تأثيراً معنوياً. وكانت أعلى قيمة للنسبة المئوية للبروتين والزيت والنيتروجين والفوسفور والبوتاسيوم عند رش النباتات بـ 10 ملليلتر / لتر هيومات بوتاسيوم يليها معاملة رش النباتات بمخلوط الاحماض الامينية مقارنة بالكنترول (الرش بالماء). بينما كانت أعلى قيمة للنسبة المئوية للألياف عند الرش بالماء (الكنترول) وسجلت أقل قيمة للنسبة المئوية للألياف عند رش النباتات بـ 10 ملليلتر/ لتر هيومات بوتاسيوم.