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IMPROVING FABA BEAN SEED YIELD, PROTEIN AND CHLOROPHYLL CONTENT BY FOLIAR APPLICATION OF HUMIC ACID

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ABSTRACT

Humic acid (HA) can be considered as an important alternative to chemical fertilizers in sustainable agriculture. For this purpose, an experiment was conducted for two years (2014–2015) as a factorial in the form of randomized complete block design (RCBD) with three replications in Chalous city. First factor included time of application (T1 – application of HA before planting, T2 – spraying HA at middle of vegetative growth, T3 – spraying HA at early time of reproductive growth). Second factor included the amount of HA as foliar application at four levels (0 (control), 200, 300 and 400 mg l^{-1} ha^{-1}). The results of the mean comparison show that the highest seed yield was obtained by spraying HA at 200 and 300 mg l^{-1} ha^{-1} at the beginning time of reproductive growth and recorded 2821 and 2773 kg ha^{-1}, respectively. The results also show that the highest percentage of protein were recorded by foliar application of HA at 300 mg l^{-1} ha^{-1} during T2 treatment. The use of HA at 200 and 400 mg l^{-1} ha^{-1} treatments and T2 treatment had the highest protein percentage and protein yield. The results showed that the use of HA at different concentrations had a positive effect on chlorophyll a, b and total chlorophyll, seed yield and protein yield. Also, the time of application of HA had a significant impact on the measured traits. It seems that foliar application at the early period of reproductive growth has improved growth, increased flower fertility, and seed yield by increasing the absorption of nutrients. According to the results, foliar spraying with 300 mg l^{-1} ha^{-1} HA at the T3 is the most appropriate treatment for proper faba bean.

Key words: foliar application time, humic concentrations, protein yield, photosynthetic pigments, *Vicia faba*

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the world's oldest crop plants and ranks third after soybeans among the legumes (*Glycine max* L.) and peas (*Pisum sativum* L.) species [Singh et al. 2012]. Fixation of nitrogen by beans increases soil fertility for the next crop. The cultivated area of faba bean in Iran is about 35,000 ha, and its average yield is 2–4 tons of dry seeds or 15–18 tons of green pods per hectare [Majnun Husseini 2008]. The demand for this plant is growing due to population growth and declining access to other protein sources [Turpin et al. 2002]. Therefore, one of the common goals of plant breeding specialists is to increase the yield of crops, and also that of beans due to the high consumption of this nutrient. Consequently, to increase the yield of faba bean yield, the use of various organic fertilizers, including HA, without harmful effects for the environment can be useful, especially in these conditions; hence, HA is called an eco-friendly organic fertilizer [Samavat and Malakuti 2005]. Application of HA on the plant either by foliar application and soil application caused increases in the different hormones as auxin, cytokinin and gibberellin

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in the plant [Abdel-Mawgoud et al. 2007]. HA foliar application significantly increased the concentration of antioxidants; photosynthesis, respiration, and ion absorption of nucleic acids [Schmidt and Zhang 1998]. HA also increased the photosynthetic activity of the plant by increasing the operation of the Rubisco enzyme [Chamani et al. 2012]. HA is useful in various biochemical reactions in the cell wall, membrane surface, and cytoplasm, and increased protein synthesis [Saruhan et al. 2011]. HA is one of the best organic fertilizers obtained by microbial, biological, and chemical decomposition of organic matter. HA improves soil fertility and the physical and chemical properties of the soil, such as permeability, ventilation, and granulation, water holding capacity of the soil, mobility, and availability of nutrients. Increased absorption of nitrogen, phosphorus, potassium, calcium, and magnesium has been reported through the mobility of these elements [Khan et al. 2012]. HA increases the growth and height of the plant through its hormonal effects and by affecting the cellular metabolism of plants, as well as the ability to chelate and increase the absorption of nutrients [Salimon et al. 2012]. The beneficial effects of HA depend on various factors such as the type of plant, the time, method, and amount of consumption of HA. The beneficial effects of HA consumption on germination, increasing germination rate, dry root length, and weight, and reducing the average time required for germination of crops such as wheat by 54 mg l^{-1} of HA have been reported elsewhere [Sabzevari et al. 2010]. In case of beans, it has been reported that humic acid spraying has increased plant growth, the number of pods in the plant, the weight of the pods, the amount of protein and chlorophyll in the plant through increasing absorption of nutrients [El-Bassiony et al. 2010]. While in the case of peas, soil application of HA has more beneficial effects on yield than its foliar application, so that soil consumption of 15 or 30 ppm of HA has similar effects as the application of 45 ppm of this fertilizer. The consumption of HA has resulted in maximum economic performance in the pea plant [Khan et al. 2012]. In the study of the effect of HA on yield and yield components of corn, it has been shown that consumption of 3500 and 4500 grams per hectare of HA increased seed yield due to increasing the index and durability of leaf surface, yield components. HA caused the highest economic performance in corn [Ghorbani et al. 2010]. The purpose of this experiment is to apply different levels of HA at different growth times, evaluate chlorophyll activity, and finally, the quantitative and qualitative performance of faba beans.

MATERIALS AND METHODS

Test site. This experiment was performed in two crop years of 2014 and 2015 on the research farm of Islamic Azad University, Chalous Branch, with a latitude of 40 degrees and 58 minutes north and a longitude of 53 degrees and 69 minutes east and a height of +3 meters above sea level.

Treatments studied. The experiment was performed as a factorial in the form of a randomized complete block design with three replicates. Factors include time of foliar application (T1 – application of HA before planting, T2 – spraying HA at the middle of vegetative growth stage, T3 – spraying HA at early time of reproductive growth stage) and the amount of HA-application at four levels (0 – control; 200; 300 and 400 mg l^{-1} ha^{-1}). The test used the brand name Humiferst (100% organic), which had 17% total fertilizer extract, 12% HA, 5% folic acid, and 6% potassium oxide (K_2O).

Crop management. The experimental site was prepared for cultivation in early November 2013 and 2014. At this time, tillage operations, including plowing, disking, and plotting, were performed. Before planting, a soil sample from a depth of 0 to 30 cm from 8 points of the experimental farm was taken to determine the physical and chemical properties and sent to the laboratory. The results of the soil test, where the project is implemented, are presented in Table 1. Each repetition had 12 plots. The dimensions of the experimental plots were 1.50 × 3 m. The distance between the planting rows was 25 cm, and the distance between the plants on the row was 15 cm, which was applied equally to all plots. The distance between the plots was considered to be 20 cm, and the distance between the blocks was considered to be 1 m.

Measurement. Measurement of chlorophyll content in leaf specimens was based on the spectroscopic method using US company UNICO 2800 spectrophotometer using relationships 1, 2, and 3 [Arnon 1967].

(1) Chlorophyll a (mg g^{-1} FW) = (12.25 A663.2) – (2.79 A646.8),

Table 1. Physical and chemical properties of soil before planting

Depth (cm)	EC (ds m^{-1})	pH	TNV (%)	CEC (%)	OM (%)	Phosphorus (ppm)	Total nitrogen (%)	Potassium (ppm)	OC (%)	Texture
0–30	0.41	7.2	8	12.17	0.86	16.8	0.09	88	0.5	Sandy clay loam

EC – electrical conductivity, TNV – total neutralizing value, CEC – cation-exchange capacity, OM – organic matter, OC – organic carbon

(2) Chlorophyll b (mg g^{-1} FW) = (21.50 A646.8) – (5.1 A663.2),

(3) Chl T (mg g^{-1} FW) = Chlorophyll a + Chlorophyll b.

To determine seed yield in each plot, after removing the margins, a surface equivalent to one square meter was harvested from each plot, and after drying, the weight of the seeds was determined. The percentage of seed nitrogen was measured by titration after distillation using a Tecator Kjltec auto 10 analyzer [Bremner and Mulvaney 1982, Emami 1996]. Then the percentage of seed nitrogen was multiplied by a factor of 6.25, to get seed protein. Protein yield was obtained by multiplying seed yield and protein percentage.

Data analysis. Before analyzing the variance of the data, it was checked for normality test. At first, the normality of the data was investigated using the Kolmogorov-Smirnov method [Blandino et al. 2020], and after confirming it, the errors were also checked for normality. The LSD mean comparison test was performed at the 5% level. All analyzes were performed with SAS 9.4 software. The comparison chart of the average attributes with Excel 2013 software was drawn.

RESULTS AND DISCUSSION

Chlorophyll a, b and total. The results of the variance analysis of the HA effect and its foliar application time on chlorophyll are shown in Table 2. The interaction between the application of HA and the time of HA foliar application on chlorophyll a, chlorophyll b and total chlorophyll at the level of 5% has been significant. An average comparison results showed that the use of HA increased chlorophyll a. The highest amount of chlorophyll a was observed with foliar application of 300 mg l^{-1} ha^{-1} of HA at the T3 at the rate of 1.82 mg g^{-1} FW. The amount of chlorophyll a, with the application of 200 mg l^{-1} ha^{-1} at all three times of foliar application, did not differ significantly. Also, the amount of chlorophyll a with the application of 400 mg l^{-1} ha^{-1} was the same between foliar application times in T2 and T3, but the amount was higher than the use of HA in T1.Humic spraying may have prevented the decomposition of chlorophyll at the onset of reproductive growth and increased leaf surface durability. On the other hand, the positive effect of HA on photosynthetic pigments can be attributed to the increase in CO_2 uptake and photosynthetic coefficient. The results of comparing the average interaction effect of HA and its foliar application time on chlorophyll b and total are shown in Table 3. The results showed that the highest amount of chlorophyll b was observed in the foliar application of 400 mg l^{-1} ha^{-1} in the T2 of 0.95 mg g^{-1} FW. The results of total chlorophyll also showed that foliar application of 400 mg l^{-1} ha^{-1} in T3 had the highest total chlorophyll. In general, the lowest levels of chlorophyll a, b, and total were observed under conditions of non-use of HA (control). Also, in the case of using 300 mg l^{-1} ha^{-1} of HA at T3 and the application of 400 mg l^{-1} ha^{-1} in the T2, the amount of chlorophyll has increased. These increases may be due to the role of HA in increasing the activity of Rubisco enzyme and then increasing the photosynthetic activity of plants and their yield [Delfine et al. 2005]. HA also increases the permeability of cell membranes and thus facilitates the entry of potassium, which results in increased intracellular pressure and cell division. On the other hand, an increase in energy inside the cell will lead to an increase in chlorophyll production and photosynthesis [Giasuddin et al. 2007]. The results of Dawood et al. [2019] showed that HA had a positive effect on increasing chlorophyll a and b in faba bean plant. In another study, El-Bassiony et al. [2010] showed that foliar application of HA increased the

Table 2. Results of compound variance analysis of the effect of HA and its application time on chlorophyll and seed protein indices and seed yield of faba bean

SOV	df	Chl a	Chl b	Chl total	Seed yield	Protein	Protein yield
Year (Y)	1	0.07 ^{ns}	0.02 ^{ns}	0.17 ^{ns}	225209.98 ^{ns}	39.03*	5606.50 ^{ns}
Block(Year)	4	0.17	0.05	0.39	223464.22	2.73	11703.68
HA (H)	2	0.31^{ns}	0.02*	0.44 ^{ns}	824866.88**	57.18*	47724.53*
Y × H	2	0.03 ^{ns}	0.001 ^{ns}	0.04 ^{ns}	7239.95 ^{ns}	1.14 ^{ns}	850.53 ^{ns}
Time (T)	3	0.49*	0.08 ^{ns}	0.96**	726062.84 ^{ns}	54.99**	86726.68**
Y × T	3	0.03 ^{ns}	0.01 ^{ns}	0.01 ^{ns}	108360.20 ^{ns}	1.28 ^{ns}	375.00 ^{ns}
H × T	6	0.20*	0.08*	0.40*	189932.48*	5.71*	5025.38**
Y × H × T	6	0.03 ^{ns}	0.01 ^{ns}	0.07 ^{ns}	29968.79 ^{ns}	0.99 ^{ns}	457.80 ^{ns}
Erorr	44	0.07	0.02	0.15	79163.08	0.50	2570.23
CV (%)	–	20.4	18.5	19.1	11.6	4.5	13.3

^{ns} not significant, * significant at the 0.05 level, ** significant at the 0.01 level. SOV – source of variation, df – degrees freedom, CV – coefficient of variation

Table 3. Comparison results of the mean effect of HA application and its application time on faba bean chlorophyll indicators

Humic acid (mg l^{-1} ha^{-1})	Time	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl total (mg g^{-1} FW)
0	T_1	1.02 c	0.59 d	1.61 d
	T_2	1.06 c	0.67 cd	1.72 cd
	T_3	1.04 c	0.69 cd	1.74 cd
200	T_1	1.16 bc	0.88 ab	2.03 bcd
	T_2	1.29 bc	0.67 cd	1.96 bcd
	T_3	1.24 bc	0.77 bc	2.01 bcd
300	T_1	1.26 bc	0.71 cd	1.97 bcd
	T_2	1.14 bc	0.76 bc	1.89 cd
	T_3	1.82 a	0.92 ab	2.74 a
400	T_1	1.24 bc	0.70 cd	1.94 bcd
	T_2	1.43 b	0.95 a	2.38 ab
	T_3	1.44 b	0.71 cd	2.15 bc

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using LSD test. T1 – application of HA before planting, T2 – spraying middle of vegetative growth, T3 – spraying early reproductive growth

amount of chlorophyll in the bean plant by increasing the rate and rate of food absorption.

Seed yield. The results of the variance analysis of the HA application effect and its application time on seed yield showed that the interaction effect of HA application and its application time on seed yield was significant at five percent. The results of comparing the average interaction effect of HA and its foliar application time on seed yield are shown in Figure 1. The highest seed yield was obtained by spraying 200 and 300 mg l^{-1} ha^{-1} at the T3 at 2821 and 2773 kg ha^{-1}, respectively. Treatment of non-use of HA had the lowest seed yield. In general, HA has increased seed yield, and foliar application has had a better effect on seed yield in T3.The researchers said that the use of HA increases the rate of photosynthesis, the absorption of nutrients from the soil to the leaves, and the transfer of these nutrients from the leaves to the seeds, thereby increasing seed yield [Srivastava 1995]. The results of Dawood et al. [2019] showed that the use of HA increased the yield of faba bean seeds, which is consistent with the results of this study. Also, Meganid et al. [2015] and EL-Ghozoli [2003], confirmed that HA could have positive effects on improving the fresh and dry biomass of faba bean. Many researchers have concluded that HA enhances the growth, function, and

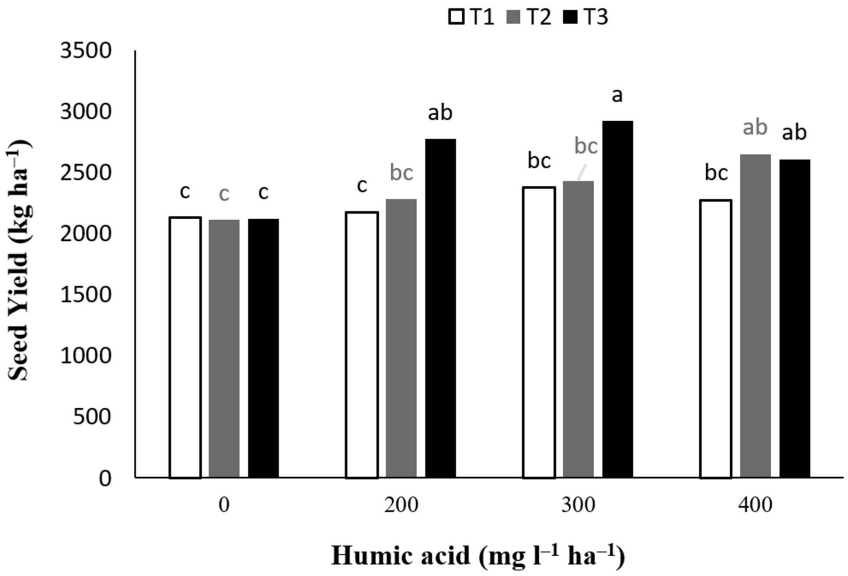

Fig. 1. The interaction between the use of HA and the time of its use on the seed yield of faba bean. T1 – application of HA before planting, T2 – spraying middle of vegetative growth, T3 – spraying early reproductive growth

Table 4. Results of comparing the average effect of HA application and its application time on the percentage and performance protein of faba bean

Humic acid (mg l^{-1} ha^{-1})	Time	Protein (%)	Protein yield (kg ha^{-1})
0	T_1	13.88 g	296.61 de
	T_2	14.94 ef	315.81 cde
	T_3	14.42 h	306.33 cde
200	T_1	12.89 h	278.96 e
	T_2	17.11 bc	390.21 b
	T_3	13.01 h	358.63 bc
300	T_1	16.29 cd	387.38 b
	T_2	20.60 a	502.60 a
	T_3	17.30 b	504.36 a
400	T_1	15.56 de	353.98 bcd
	T_2	17.20 b	455.06 ab
	T_3	15.08 ef	393.62 b

Means in each column followed by similar letter(s) are not significantly different at 5% probability level, using LSD test. T1 – application of HA before planting, T2 – spraying middle of vegetative growth, T3 – spraying early reproductive growth

absorption of nutrients by many products [Neri et al. 2002, El-Desuki. 2004, Bulent-Asik et al. 2009, Cordeiro et al. 2011, Saruhan et al. 2011]. On the other hand, Karakurt et al. [2009], El-Nemr et al. [2012], and Said-Al Ahl et al. [2016] showed that HA spraying, due to its performance in various physiological and metabolic processes, has increased plant growth, yield, and quality in many plant species. HA increased nutrient uptake, cell division, photosynthesis [Atiyeh et al. 2002] respiration, biosynthesis of nucleic acid

and enzymes, and in general, dry weight and plant yield [Ulukan 2008, Said-Al Ahl et al. 2016].

Percentage and yield of protein. The results of the variance analysis of the HA effect and its foliar application time on percentage protein and protein yield are shown in Table 2. The interaction between the application of HA and the time of foliar application of HA in percent and protein performance at the level of five percent is significant. The percentage protein and protein yield increased with HA foliar application. The highest percentage and protein yield were observed with foliar application of 300 mg l^{-1} ha^{-1} in the T2 at 20.60% (Tab. 4). The use of HA in all three treatments in T2 had the highest percentage protein and protein yield (Tab. 4). Also, the lowest protein yield was in conditions of non-use of HA. Nitrogen, phosphorus, and sulfur seem to be among the most nutritious elements in terms of the amount needed for plant growth, and one of the most critical roles of these elements is to participate in protein structure and therefore plant structure. HA and its compounds increase the ability of the plant to synthesize protein of the plant and transfer it to the seeds, both by increasing the ability of the roots to absorb these elements and intervening in enzymatic activities related to their preparation for the plant. A study by Nardi et al. [2002] showed that HA increased chelating power and the absorption of nutrients. When harvesting faba bean, the increasing yield is considered as an indicator of quantity, increasing the amount and type of protein is considered as an indicator of the quality of faba bean. In general, the tendency to increase the yield of faba bean increases the quality of the product. The composition and amount of protein affect the quality properties of faba bean, but the effect of the amount of seed protein is greater than its combination on quality properties. The results of El-Bassiony et al. [2010] showed that foliar application of HA increased the growth of the plant and the amount of protein in the plant by increasing the rate and absorption of nutrients compared to its soil consumption. Also, the results of Albayrak and Camas [2005] showed that HA increased the amount of protein in turnip forage.

CONCLUSION

The results of this study showed that the use of HA at different concentrations had a positive effect on chlorophyll content (chl a and b) and total chlorophyll, protein percentage and protein yield. Also, the time of application of HA had a significant effect on the measured traits. It seems that HA foliar application at T3 was the most optimum treatments because its improved growth, and seed yield hence we can say that foliar spraying of HA at 300 mg l^{-1} ha^{-1} during T3 is the most appropriate treatment for proper performance in the faba bean.

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