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EFFECT OF POTASSIUM HUMATE FOLIAR APPLICATION ON YIELD AND QUALITY OF SOYBEAN *(GLYCINE MAX* L.) GROWN ON CALCAREOUS SOIL UNDER IRRIGATION WATER REGIME

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Abstract

Soybean (*Glycine max* L.) is a legume native to East Asia that is widely used for its edible beans. It has numerous uses, such as fat-free soybean meal with cheap source of oils and vegetable protein, which is suitable for both humans and animals. A field study was carried out during two summer seasons 2016-2017 at El-Nubaria Agricultural Research Station, Ministry of Agriculture and Land Reclamation (MALR), Egypt, to investigate the potential of potassium humate (K-humate) to improve soybean productivity under water stress conditions. Potassium humate foliar application treatments (control, K-Humat1 = 1 gm / L, K-Humat2 = 2 gm / L and K-Humat4 = 4 gm / L) were applied twice per season with two irrigation treatments (14 and 21 days). The treatments included a 14-days irrigation treatment with 100% water demand (adequate irrigation) and a 21-days irrigation treatment (deficit or regim irrigation). Foliar application of potassium humate mitigated water stress damage in soybeans by increasing the growth and production of antioxidants such as proline and protecting both protein and chlorophyll from free radical degradation. Foliar application of potassium humate increased the growth, grain yield and oil content of soybeans under water stress conditions as a 21-days irrigation treatment. The results of the experiments showed that the foliar application of potassium humate in an amount of 4 gm / L under water stress is an effective strategy for improving soybean productivity compared to others. grain yield (1795 kg / fed.) was obtained with 2 gm / L K- humate foliar spray as a relative increase of 41% in a 14-days irrigation treatment. The value of grain yield (1344 kg / fed) was achieved with 4 gm / L K- humate in 21 days irrigation with relative increase being 41%.

Key words : Potassium humate; Foliar application; Water stress; Soybean.

Introduction

Soybean (*Glycine max* L.) is one of the most important oil seeds in the world. According to the latest USDA world statistics, soyabean acreage in 2016/2017 was nearly 120.30 million hectares, with an average yield of 2.92 tons per hectare (Foreign Agricultural Service / USDA (December 2017 Global Analysis Office). Total acreage 15.2 million hectares with an average yield of 2.84 tons of seed per hectare, in Egypt (FAO, 2010).Soya is one of the most significant oil crops that contains about 85% unsaturated fatty acids with 18-22% cholesterol free oil and 38-42 % Protein (Ali *et al.,* 2009) It has the potential to balance the gap or lack between supply and demand of protein and edible oil. On-vitro studies show that soya products can reduce the risk of breast and cancer by having bioactive substances such as lunasin peptide, Bowman Birk protease inhibitor, and isoflavones (Bouslama and Schapaughl, 1984). Soybean seeds contain on average 19% oil and 36-38% protein on dry weight, but both environmental and genetic factors can greatly affect seed production and composition (Krishnan, 2001)

Water stress states cause a variety of physiological,

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molecular, and biochemical changes that affect development and plant growth (Boutraa, 2010). A secondary water stress feature in plants is the stress-induced production of reactive oxygen species (ROS) (Azadeh *et al.,* 2014). The increased production of ROS during water stress led to progressive oxidative damage, growth suppression, and ultimately cell death (Ruiz-Lozano *et al.,* 2012). Drought is the main abiotic barrier responsible for significant production losses, as soil drying has serious effects on crop development, growth and crop yield.

Humic acids (HA) (K- Humate) are one of the core components of humic substances, HA can be used in agriculture as fertilizer, nutrient carriers, plant growth promoters and soil improvers (Bidegain *et al.,* 2000). HA have been used in the configuration of bio-stimulants to enhance the antioxidant system in plants that are exposed to environmental stress because of their phytohormonal activities (Eyheraguibel *et al.,* 2008). Under water stress, humic acid fertilization increased leaf water intake and antioxidant metabolism (Delfine *et al.,* 2005). Biostimulants have shown antioxidant activity in plants under water stress (Zhang and Schmidt, 2000), as there is evidence that the physiological fitness of plants is mainly determined by antioxidant defense systems and hormone balance (Zhang *et al.,* 2005). our goal is investigating the effect of humic acid (K- Humate) as a bio-stimulant by foliar application on yield and quality characteristics of soybeans from calcareous soils under irrigation water regime conditions.

Martials and Methods

Experimental site and conditions

The present study was carried out for two summer seasons 2016 and 2017 on the experimental farm site of the agricultural research station El-Nubaria, Ministry of Agriculture and Land Consolidation (MALR), Egypt. The geographical location of the farm is 30º 90'N, 29 ° 96'E, 25 meters above sea level. Physical and chemical properties of the test soil determined by Chapman & Pratt, 1961 and these were as follows: EC 3.9 ds/m, pH 8.5, $CaCO_3$ 27.2 %, Organic Matter 0.2%, N 700, P 23, K 280, Ca 474, Na 800, Fe 6.71, Mn 1.23 and Zn 1.00PPm. The weather data (Nubaria, El Behira, Egypt) for the two growing seasons 2016 and 2017 are shown in table 1.

The experimental plot design was placed in a randomized array of complete blocks, with three repetitions performing all experiments (two treatments of irrigation, four treatments, and three repetitions). The total number of tests was carried out on 24 plots with the same plot area of 10.5 m². Treatments included a 14-days irrigation period with 100% water demand (adequate irrigation) and a 21-days irrigation period. The 14-days treatment consisted of eight irrigation frequencies (eight irrigations per season) and the amount of water used for irrigation was equivalent to 100% of ETpan (pan evapotranspiration). The mean value of ETpan was calculated according to the data obtained daily from pan evapotranspiration (Doorenbos and Kassam, 1986). The amount of water applied for each irrigation event was measured using a flow meter (m^3 / fed. / month). The 21-days treatment consisted of four flushing frequencies (four irrigations / season). The main treatments included four different treatments with K-humate, WESKOPLUS-K consisted of humate-potassium 60% K_2O, 7% EDTA-Fe, 2% EDTA-Mn in amounts of 1 gm / L, 2 gm / L, 4 gm / L and control, these treatments were applied to the leaves twice a season. Soybean variety was purchased from Food Legumes Research Section, Agricultural Research Center, Giza, Egypt. The experiment began at the same time in two seasons (3 May) with the sowing of soybean seeds. Recommended doses obtained by the Ministry of Agriculture from nitrogen (ammonium sulphate 20.5% N, 100 kg / fed.), phosphorus (superphosphate 15.5% P_2O_5, 300 kg / fed.) and potassium (potassium sulphate 48% K_2O, 50 kg / fed.) were used during the growth phase and used as a control. The plots were watered using the surface irrigation method and then irrigated Application every 14 days or 21 days during plant development, up to 4 weeks before the final harvest. All agronomic practices remained normal and consistent in all treatments. At harvest time (3 September), a sample of 1 meter / plot plants was taken which were designed to determine characteristics such as plant length (cm), number of branches per plant, fresh weight per plant and dry weight per plant. The chlorophyll content was measured

Table 1: The monthly average (2016 and 2017 seasons) meteorological data of experiment site (Nubaria Research Station).

Month	Solar radiation [mJ/m²]	Wind speed [m/sec]	TMAX [°C]	TMIN [°C]	Relative humidity [%]	Dew Point [°C]	ETo mm/day
May	27.66	4.62	31.5	16.59	42.56	9.44	4.75
June	29.74	4.69	36.56	20.43	41.67	13.16	5.43
July	29.55	4.35	34.62	21.45	51.84	16.75	6.04
August	27.42	4.19	34.68	21.28	53.96	17.33	5.20
September	23.64	4.30	32.96	20.66	52.94	15.69	4.18
Average	27.602	4.43	34.064	20.082	48.594	14.474	5.12

in fresh leaves with the chlorophyll meter Spad 502 at 09:00 after Wood *et al.,* 1992. The content of leaf-free proline was determined according to Bates *et al.,* 1973. Yield and its ingredients were estimated as follows: number of pods per plant, seed weight per plant (g), 100 seed weight (g) and yield (kg / fed.). The percentage of carbohydrates was determined in grains according to the DuBois principle (DuBois *et al.,* 1956). The percentage of seed oil was estimated according to the Association of Official Agriculture Chemists (A.O.A.C., 1990).

Oil content was calculated as follow:

Oil content (%) =

$$\frac{\text{weight of the flask} + \text{oil} - \text{empty the flask weight}}{\text{weight of sample}} \times 100$$

The harvest samples from leaves were also taken for determination of nutrients by Cottenee's method Cottenee, *et al.,* 1982. Statistically analysis was performed to compare the means of two seasons data by using the least differences (L.S.D) (Snedecor and Cochran, 1990 and Steel and Torrie, 1980.

Results

The vegetative growth characteristics data of soybean cultivar grown under the effects of two irrigation treatments (14 and 21 days) and applied potassium humate foliar spray are shown in table 2. The results obtained showed that there were significant differences between two irrigation treatments for plant height, shoot fresh and dry weight per plant and plant height; It decreased with increasing periods of irrigation, while the number of pods per plant and the number of branches per plant did not differ significantly. It is worth noting that this is due to the effect of water stress when the time between irrigations is extended.

It is also shown that foliar application of potassium humate caused a significant increase in all growth characteristics compared to control treatment. The highest values of growth properties were obtained from the spray plants with K-Humat2 compared to those obtained from the other treatments. The lowest values of all growth characteristics were recorded by control treatment.

The interaction effect between two watering treatments and applied potassium humate foliar spray on growth characteristics was significant except for plant height and number of pods per plant. The highest levels of growth characteristics were observed by spraying K-humate 2 with a 14-days treatment. It is enhanced by spraying K-humate 4 with a 21-days treatment.

Data of biochemical characteristics showed that the differences between the two water treatments (14 and

Table 2: Effect of potassium humate foliar spray on growth characteristics of soyabean cultivar grown on calcareous soil under irrigation water regime.

Irrigation Period (Irr) (Days)	K-humate (gm/l)	Fresh weight/plant (gm)	Dry weight/plant (gm)	Pant height (cm)	Number of pods/plants	Number of branches/ plants
14 Day	Cont.	281	176	87	25	2.8
	1	307	202	90	31	3.9
	2	345	235	94	33	4.3
	4	308	204	93	30	3.3
21 Day	Cont.	271	147	80	25	2.8
	1	292	159	85	27	3.7
	2	300	166	89	29	3.8
	4	303	199	90	29	3.9
Mean values of (Irr)	14 Day	310	204	91	30	3.6
	21 Day	292	168	86	28	3.6
Mean values of (K-humate)	Cont.	276	162	84	25	2.8
	1	300	180	88	29	3.8
	2	323	201	92	31	4.0
	4	306	201	92	30	3.6
LSD at 5 %	Irr	0.542	1.079	1.075	ns	ns
	K-humate	0.250	0.514	3.260	1.988	0.1216
	Irr x K-humate	0.353	0.726	ns	ns	0.171

Combined analysis of two successive seasons.

Table 3: Effect of potassium humate foliar spray on Biochemical characteristics of soybean cultivar grown on calcareous soil under irrigation water regime.

Irrigation Period (Irr) (Days)	K-humate (gm/l)	proline (µg/g)	Protein in grain (%)	Carbohydrates in grain (%)	Chlorophyll Index
14 Day	Cont.	51.9	38.3	47.0	40.8
	1	53.6	38.9	47.9	41.5
	2	59.4	39.1	48.0	44.1
	4	57.7	39.1	47.9	42.3
21 Day	Cont.	87.0	37.5	46.6	39.3
	1	106.0	38.5	46.9	39.8
	2	108.0	38.6	47.1	40.0
	4	114.4	38.8	47.5	40.1
Mean values of (Irr)	14 Day	55.7	42.2	47.7	38.9
	21 Day	103.9	38.3	47.0	39.8
Mean values of (K-humate)	Cont.	69.5	37.9	46.8	40.1
	1	79.8	38.7	47.4	40.1
	2	83.7	38.9	47.5	42.1
	4	86.1	38.9	47.7	41.2
LSD at 5 %	Irr	0.28	0.19	0.17	0.11
	K-humate	0.22	0.12	0.22	0.14
	Irr x K-humate	0.31	0.17	0.31	0.19

Combined analysis of two successive seasons.

21 days) reached the level of significance for proline, protein, carbohydrates and chlorophyll shown in Table 3. The proline is increased in 21 days of treatment (water stress) compared to other parameters and it dropped in 14 days treatment.

On the other hand, the differences in the potassium humate leaf application levels reached the significance level and the highest levels of proline, protein and carbohydrates were obtained using K-Humat4, however, the highest level of chlorophyll produced using K-humate2

The interaction effects of potassium humate foliar application and both water treatments on biochemical properties were significant. The results also showed that the highest mean of proline was obtained by 21-days treatment with K-humate4. While protein, carbohydrates and chlorophyll appeared the highest averages by treating with K-Humat 2 for 14 days as shown in Table 3.

The effect of foliar spray with different potassium humate doses by using two irrigation treatments and their interaction on soybean yield and its components is shown in Table 4. The results describe the significant differences between the two irrigation treatments in terms of yield and its components. Grain yield and oil yield decreased with increasing irrigation time.

Potassium humate foliar spray, however, significantly affected all characteristics of the soya yield and its component. From the results obtained, it could thus be concluded that K-humate2 foliar spray is the most effective dose for obtaining the maximum grain yield (1509 kg / fed with a relative increase of 36%) and the oil percentage.

Concerning the interaction between potassium humate foliar spray and two water treatments, it should be noted in Table 4 that the highest mean weight of 100 grains, grain yield (1795 kg / fed with relative increase 41%) and oil content in 14-days treatment with additional K-humate2 leaf spray. While the irrigation treatment achieved the highest values in 21 days, mainly the grain yield (1344 kg / fed with a relative increase of 41%) was increased by adding K-humate 4-foliar spray. Increasing the yield might reflect the promoting effect of potassium humate on growth and biochemical properties as shown in Table 2 and Table 3, which could lead to an increase in yield as shown in table 4.

There are no significant differences between two water treatments on K content in leaves and Mg and Ca content in grains, but content of Ca, Mg, Na and K in grains are significantly reduced with 21-day irrigation treatment, as in table 5.

The effect of applied potassium humate foliar spray had no significant effect on the K content of leaves and grains, but there was a significant effect on the Ca, Mg and Na content of the leaves and grains and the highest levels of values were by K-. humate 4.

Table 4: Effect of potassium humate foliar spray on Yield and yield component of soybean cultivar grown on calcareous soil under irrigation water regime.

Irrigation Period (Irr)	K-humate (gm/l)	weight of 100 grain (gm)	yield of grains (kg /fed)	Oil(%)
14 Day	Cont.	26.5	1270	18.61
	1	27.8	1546	18.75
	2	30.8	1795	19.15
	4	28.1	1428	18.85
21 Day	Cont.	22.8	950	18.52
	1	25.6	1076	18.70
	2	29.0	1223	18.75
	4	31.5	1344	18.80
Mean values of(Irr)	14 Day	28.3	1510	18.84
	21 Day	27.2	1148	18.69
Mean values of (K-humate)	Cont.	24.7	1110	18.56
	1	26.7	1311	18.72
	2	29.9	1509	18.95
	4	29.8	1386	18.83
LSD at 5%	Irr	0.318	36.4	0.014
	K-humate	0.22	8.09	0.04
	Irr x K-humate	0.32	39.72	0.05

Combined analysis of two successive seasons.

The interaction effects of the potassium humate foliar application and both water treatments on the elemental content were significant for the Ca and Mg content of the leaves and the highest values in 14 days were generated by K Humat 2 foliar application, but in 21 days the highest values were generated by K-humate4 leaves application.

Discussion

Potassium humate stimulates the plant's progress through the assimilation of major and minor elements. Hussein *et al.*, (2006) and El-nasharty *et al.*, (2017) found that potassium humate-treated plants had a significant increase in the growth characteristics of soybeans compared to control plants. This observation was also recorded by Abd El-Aal and Eid (2017). The results of Mahmoud *et al.*, (2011) showed that the treatments that received HA in both soil and foliar applications caused a significant increase in plant height compared to the untreated ones. Amina and Zen El-Dein. (2016) showed that the dry weight of the shoots was increased by the application of humic acid. Was also in agreements with those of Abd El-Aal and Eid (2017). Macak and Candrakova. (2013) reported that the use of Humic significantly affects the number of pods per plant.

In general, the results obtained agree with Shaaban *et al.*, (2009), Kirnak *et al.*, (2013) and Muhammad *et al.* (2014), which shows that stress significantly reduces the chlorophyll content of the leaves during the vegetative growth stage. In addition, Meganid *et al.*, (2015) and Amina and Zen El-Dein (2016) clearly showed that chlorophyll was significantly influenced by the application of humic acid. In addition, Abd El-Aal and Eid (2017) reported that the content of photosynthetic pigments in soybean leaves increased with humic acid treatment compared to the control.

Aayudh Das *et al.*, (2017) indicated that proline is an important organic osmolyte and accumulates strongly in response to drought stress. Therefore, proline accumulation under dry stress can serve as a sink for excess products needed to maintain photosynthesis and respiration processes. In response to drought, soybean growth is limited and more carbohydrates are stored (Myers and Kitajima, 2007, Amina and Zen El-Dein, 2016 and Aayudh Das *et al.,* 2017). The data showed that in response to drought, key metabolites (carbohydrates) in soybean leaves were accumulated and decreased differently than under drought stress. Protein yields were reduced linearly with decreasing water balance.

Kirnak *et al.*, (2013), Comlekcioglu and Simsek (2011) found that potassium humate treated plants had a significantly higher yield of soybeans than control plants. In addition, Macak and Candrakova (2013) observed that applied humic acid significantly increased the yield of soybean seed. In addition, soybean grain yield was severely affected by the water stress condition and grain yield was estimated by Prado *et al.*, (2016) reduced by more than 50%. Behtari *et al.*, (2009), Kirnak *et al.*, (2013) and Behtari *et al.*, (2011) found that the oil contents at the beginning of the tube filling are significant and that they remained constant during further seed development, especially after the ripe one, such as 62 days after flowering. The high oil content in the first harvest (38 days after flowering) shows that the oil accumulation occurred before this stage. A relationship between seed weight, oil content, and protein content indicates that oil and protein levels change in response to each other's stress and seed weight change. In addition, Abd El-Mohsen *et al.*, (2013) found that among the irrigation skip agents, the maximum oil content under control

Table 5: Effect of potassium humate foliar spray on Potassium (K), Calcium (Ca), Magnesium (Mg), and Sodium (Na) concentration (%) in leaves and grains of Soyabean cultivar grown on calcareous soil under irrigation water regime.

Irrigation Period (Irr)(Days)	K-humate (gm/l)	Leaves				Grains			
		K	Ca	Mg	Na	K	Ca	Mg	Na
14 Day	Cont.	0.70	2.75	0.29	1.80	2.05	0.37	0.05	2.20
	1	0.74	3.05	0.39	1.90	2.10	0.39	0.06	2.40
	2	0.77	3.30	0.46	2.10	2.20	0.42	0.07	2.50
	4	0.75	3.10	0.40	2.00	2.15	0.41	0.07	2.45
21 Day	Cont.	0.67	2.50	0.27	1.60	1.85	0.35	0.05	2.10
	1	0.69	2.70	0.30	1.70	1.90	0.37	0.05	2.20
	2	0.70	2.80	0.36	1.75	1.95	0.39	0.07	2.25
	4	0.72	2.95	0.38	1.90	2.00	0.40	0.06	2.30
Mean values of (Irr)	14 Day	0.74	3.05	0.39	1.95	2.13	0.40	0.06	2.39
	21 Day	0.70	2.74	0.33	1.74	1.93	0.38	0.06	2.21
Mean values of (K-humate)	Cont.	0.69	2.63	0.28	1.70	1.95	0.36	0.05	2.15
	1	0.72	2.88	0.35	1.80	2.00	0.38	0.06	2.30
	2	0.74	3.05	0.41	1.93	2.10	0.41	0.07	2.38
	4	0.74	3.10	0.40	2.00	2.10	0.41	0.07	2.45
LSD at 5 %	Irr	ns	0.15	0.01	0.19	0.21	ns	ns	0.11
	K-humate	ns	0.11	0.02	0.17	ns	0.03	0.01	0.13
	Irr x K-humate	ns	0.15	0.03	ns	ns	ns	ns	ns

Combined analysis of two successive seasons.

conditions (k-humate zero) was 21.50% for standard irrigation and the oil yield decreased linear with decreasing water regimes.

Mahmoud *et al.*, (2011) found that the weight of 100 grains was increased with the use of humic acid. Gawlik *et al.*, 2016 noted that soybean seeds swelled on a base layer saturated with the solutions containing humic acid and stress factors. The results showed the significant exposure of those soybean seeds to both factors under stress condition.

Humus favors the increase in potassium content in the leaves to a sufficient extent, probably due to the potassium contained in the mineral fertilizer. Plants exposed to water stress had lower levels of macronutrients in the diagnostic sheet (Prado *et al.,* 2016). These findings have similar results as Mahmoud *et al.*, (2011) demonstrated that the K content in seeds as well as the seed yield and yield components of soybeans increased significantly due to the soil or foliar application of HA, and the increase progressed with increasing rate of HA. Humic acid had a significant positive effect on K concentrations in soybean seeds in both the soil and foliar applications.

From all the above results and discussions Humic acid (potassium humate) have been used in the configuration of bio-stimulants to enhance the antioxidant system in plants that are exposed to environmental stress because of their phytohormonal activities (Eyheraguibel *et al.,* 2008). Under water stress, humic acid fertilization increased leaf water intake and antioxidant metabolism (Delfine *et al.,* 2005). Biostimulants have shown antioxidant activity in plants under water stress (Zhang and Schmidt, 2000), as there is evidence that the physiological fitness of plants is mainly determined by antioxidant defense systems and hormone balance (Zhang *et al.,* 2005).

Conclusion

Potassium humate foliar application is useful to improve the performance of crops under water stress conditions. Foliar applications of potassium humate alleviated the stress-related damage in soyabean, particularly in the 21-days irrigation treatment, by increasing the growth and production of antioxidants such as proline and protecting protein and chlorophyll from free radical degradation. The foliar application of potassium humate increased the yield and the oil content of soybean under water stress. These results indicate that foliar application of potassium humate is an effective strategy for improving soybean productivity under water stress.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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Author contributions

All authors significantly contributed in all parts and aspects of paper.

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