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EFFECT OF HUMIC ACID ON GROWTH, PROTEIN AND MINERAL COMPOSITION OF PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R.BR.] FODDER

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Abstract

Based on shortage of organic fertilizers in arid regions, the present study aimed to evaluate alternative sources of organic fertilizers that required in small quantity and that to be easy in shipment, and universally available. After an extensive literature search, we were prompted to evaluate the effect of humic acid (HA) on millet [*Pennisetum glaucum* (L.) R.Br] by using 7 different concentrations (0, 5, 10, 15, 20, 25, and 30 kg ha^{-1}) because HA, which is recognized as an exogenous growth regulator/soil activator, and its effects have been evaluated in its limited quantities only on fruits and vegetables. This study evaluated the effects of soil application of high levels of HA on the field crop. The experiment was conducted following a randomized complete block design. The results of the study indicated that the increasing level of HA to certain level (20-25 kg ha^{-1}) constantly and significantly ($p<0.05$) increased plant growth (i.e., plant height, number of leaves $plant^{-1}$, leaf area index (LAI), green and dry matter yield). The increase in growth was further confirmed by the chemical analysis of crude protein and mineral content. Based on the results, this study recommends application of 20 to 25 kg ha^{-1} of HA to soil for improving millet crop yield and quality in arid and saline conditions.

Introduction

Arid lands around the world generally have poor soil quality that is characterized by a small amount of organic matter, high salinity, and severe hot conditions, which adversely affect various molecular, biochemical, and physiological plant processes, including growth and production (Ashraf & Harris, 2013). Studies conducted by Kumar *et al*., (2009), and Neelam *et al*., (2011) have revealed that adding organic matter to soil mitigates most problems associated with arid lands, thus resulting in a good soil structure (soil aggregates) that facilitates cultivation, and enhances transport of nutrients and water to crops. In addition, organic matter supports beneficial microbes that help in increasing yield, plant nutrients uptake, and disease resistance in plants.

Various sources of organic matter have been previously identified as useful for soil amendments. For example, composted animal manure, kitchen wastes, garden wastes, and green manure can be incorporated into the soil (Borjesson *et al*., 2012, Rehim *et al*., 2012, Soomro *et al*., 2013). However, Park *et al*., (2011), recommend careful selection and sustainable organic source for soil amendment. For this reason, in this study, we utilized humic acid (HA) based on the principle that it is a major organic constituent of soil (humus). HA producers claim that 1 kg of HA is as much beneficial as 1 ton of cattle manure because manure needs a lot of time for humidification, the form that can be utilized and assimilated by plants (Anon., 2012). Moreover, farmers in remote arid areas are in need of organic sources that could be easily transported. Demir & Cimrin (2011) and Zhang *et al*., (2013) have evaluated humic acid up to a very small amount (1 to 3 kg ha^{-1}) that was found beneficial to plants and soil by decreasing the impact of drought and salinity stresses and improving seed germination, as well as improving plant growth and development, while still there is a question that what will be the effect of large quantities of HA. On the other hand, Hartz & Bottoms (2010) have reported that HA was ineffective in enhancing nutrient uptake and crop productivity.

To verify the uncertainty regarding the effect of humic acid incorporation in soil as organic amendment, the present study was conducted using various levels of HA (from low to very high levels) on millet crop.

Materials and Methods

Experimental site and materials: The field experiment on the millet crop was conducted from September 2011 to February 2012, following a randomized block design-at the Arid Land Agriculture Farm (climatic conditions are presented in Fig. 1) of King Abdulaziz University, located in Hada Al-Sham (21.79999°N, 39.72929°E), Makkah Province, Saudi Arabia. Based on the nutrient requirements of the crop (Kumar *et al*., 2009; Neelam *et al*., 2011) and the analytical results of the soil collected from the site (Table 1), it was supposed that the field could successfully support crop growth without the addition of any fertilizers. Seeds of millet (Yemeni variety) and powdered humic acid (Humintech, GmbH, Germany) were obtained from a local market in Jeddah, Saudi Arabia.

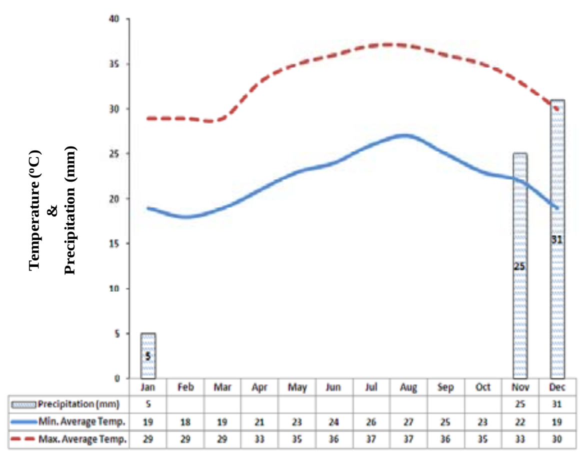

Fig. 1. Daily average minimum and maximum temp (°C), and rainfall record at the trial location.

Table 1. Soil properties (0-30 cm) initially and after the crop harvest.

Soil properties	Initial values	After the crop harvest		
		Control	H_4	H_5
Bulk density (g cm^{-3})	1.34	1.36	1.15	1.15
pH	7.81	7.60	7.40	7.42
Organic matter (%)	2.00	1.16	2.01	2.01
CEC ($cmol_c$ kg^{-1})	13.21	12.51	16.12	16.11
EC (dS/m)	1.74	1.78	1.60	1.54
Sand (g 100 g^{-1})	75.7	74.2	74.4	73.6
Silt (g 100 g^{-1})	22.0	23.5	23.0	23.6
Clay (g 100 g^{-1})	2.3	2.3	2.6	2.8
Soil texture	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand
Total N (%)	0.11	0.07	0.10	0.11
Quantity (mg kg^{-1})				
P	49.4	37.0	48.5	50.5
K	288.2	231.0	338.0	348.1
Ca	4156	4120	4142	4154
Mg	240	210	268	268
Fe	46.10	18.0	44.6	46.1
Cu	1.64	1.41	1.86	1.88
Zn	2.85	2.60	2.92	3.10
Mn	10.8	9.4	11.2	12.0

H_4 = 20 kg per ha humic acid; H_5: 25 kg per ha humic acid

Treatment application and cultural practices: Millet crop was grown for forage purposes twice (using two adjacent fields with similar soil properties in same location). Seeds were hand planted on field capacity at a depth of 1.5 to 2 inches two times subsequently on September 26, 2011 and December 2, 2012 in the growing season (2011/12). Each subplot consisted of 6 rows of 6-m length; the spacing between rows was 40 cm and between plants was 15 cm. The experimental layout was a randomized complete block design (RCBD) with triplicates. HA was applied as a side dress on both sides of the plant, along the rows after the emergence of the crop (10 d after sowing) using seven levels (0, 5, 10, 15, 20, 25, and 30 kg ha^{-1}). The experimental plots received furrow irrigation using underground saline water (TDS = 2,000 ppm) for 1 h every 3 d until the end of the experiment. Weeding was performed once after first 20 day of emergence; no pesticides were used during the course of the experiment.

Procedures for data recording

Soil physical and chemical properties: Soil bulk density (BD) was determined according to the method described by Blake & Hartge (1986). For other soil properties, at the start of the experiment, 12 soil samples were taken from each of the experimental field at a depth of 0-30 cm and were mixed as a composite sample to determine the initial soil properties. After completion of the experiment, random soil samples were collected from the control, as well as the H_4 and H_5 subplots for comparative analysis of various soil properties. The soil samples were oven-dried at 65°C for 2 d, and crushed to a size of approximately 2 mm. Soil texture was determined using the sieve method, in which 100 g of soil was separated into sand, silt, and clay using the United State Department of Agriculture (USDA) grading sieves according to the manufacturer's instructions (Humboldt Mfg. Co., USA), then each part was weighed to determine their relative proportions. Based on the observed proportions, soil texture was determined according to the USDA soil texture triangle. Soil pH and salinity were determined in a soil suspension (soil and deionized water at a ratio of 1:2) by using pH and salinity meters. The pH meter (inoLab, GmbH, Germany) was calibrated using standard solutions of pH 4.010 and 10.010, with product codes Z655112 and Z655155, respectively (Sigma-Aldrich Ltd., Gmbh, Germany), whereas the salinity meter (Model FE28, EDT Instruments Ltd., UK) was calibrated using standard saline solutions. The cation exchange capacity (CEC) was measured following the method of Page *et al.,* (1982), and organic matter using the Walkley-Black method (Nelson & Sommers, 1996). Nitrogen content was quantified using a Perkin-Elmer CHNS/O Analyzer (Model 2400) following the manufacturer's instructions (PerkinElmer, Inc., USA). All other elements (P, K, Ca, Mg, Fe, Cu, Zn, and Mn) were first extracted according to application note of Nham (2010), for each plots and were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES) ICP-OES according to the method described by Bakhashwain *et al.,* (2013) for determination of different elements.

Recording data on growth parameters: Data on plant height and the average number of leaves were collected from 10 individual plants (at heading stage, 70 d after sowing) using the procedure described by Daur *et al*., (2011). In addition, the same plants were used for calculating the leaf area index as described by Daur & Bakhashwain (2013) using the following equation:

$$LAI = [Leaf_L \times Leaf_W \times 0.68] \quad x \quad \text{Leaves number plant}^{-1}/\text{GA plant}^{-1}$$

In which 0.68 is a correction factor previously described by Payne *et al*., (1991).

Fresh forage and dry matter yields were determined according to the method described by Daur & Tatar (2013). Subsequently, the dry matter samples were analyzed for crude protein and mineral composition.

Protein and mineral analysis: The plant dry matter of each plot was analyzed for N content by using a Perkin–Elmer CHNS/O Analyzer (Model 2400), following the manufacturer's instructions (PerkinElmer, Inc., USA). Crude protein (CP) content (%) was calculated based on nitrogen content of the dry matter, using the equation as follows: CP (%) = nitrogen (%) × 6.25. P, K, Ca, Mg, Mn, Cu, and Fe were determined using a Varian ICP-OES, following the procedure described by Bakhashwain *et al.,* (2013). All chemicals used in the experiments were of analytical reagent grade (Merck, Darmstadt, Germany), and three replicates were performed for each sample.

Statistical analysis: Data collected on various parameters were statistically analyzed using the software SAS 9.2.

Results

Effect of HA on millet growth: Table 2 presents the significant differences in the crop growth parameters ($p<0.05$) using various HA levels. Taller and similar plant heights were recorded for both H_4 and H_5 levels of HA, which were statistically comparable to that observed using H_3 and H_6 levels of HA. The highest number of leaves per plant were recorded for H_4 and H_5, and a maximum LAI was recorded for H_5, although this was statistically similar to the H_4 treatments. Green fodder and dry fodder yields were significantly higher in the H_5 treatment compared to all other HA levels, and these values were statistically similar to those observed in the H_4 treatment.

Protein and mineral content of millet: The effect of various HA levels on CP and mineral content in millet forage are presented in Table 3. Significant differences in CP and mineral content were recorded among different HA levels, whereas P and Cu levels were non-significant ($p<0.05$). The highest CP values were observed in the H_4 and H_5 treatments, which were statistically similar to those observed in the H_3 and H_6 treatments. The N content was highest in the H_4 and H_5 treatments. The K content was highest in the H_5 treatment, although it was statistically similar to that observed in the H_6 treatment. The highest Ca concentration was observed in the H_5 treatment, although this was statistically similar to treatments H_3 to H_6. Mg level was highest in the H_6 treatment, which was statistically similar to treatments H_4 to H_5, whereas Mn and Fe were highest in the H_6 treatment.

Table 2. Effect of HA levels on various growth parameters of millet

HA levels	PH (cm)	LN plant^{-1}	LAI	FFY (kg ha^{-1})	DMY (kg ha^{-1})
H_0	62^{d}	6.8^{d}	1.496^{d}	30680^{e}	5890^{d}
H_1	69^{c}	8.6^{c}	2.024^{cd}	32225^{d}	6187^{cd}
H_2	75^{b}	9.1^{bc}	2.178^{c}	34068^{c}	6541^{c}
H_3	80^{ab}	10.2^{b}	2.244^{bc}	35844^{b}	6982^{b}
H_4	84^{a}	11.5^{a}	2.842^{a}	38201^{a}	7335^{a}
H_5	84^{a}	11.5^{a}	2.920^{a}	38211^{a}	7350^{a}
H_6	79^{ab}	10.1^{b}	2.120^{c}	33922^{c}	6513^{c}

HA = Humic acid; H_0, H_1, H_2, H_3, H_4, H_5 and H_6 means 0, 5, 10, 15, 20, 25 and 30 kg HA ha^{-1} respectively.
PH = Plant Height; LN = Leaves Number; LAI = Leaf Area Index; FFY = Fresh Forage Yield; DMY = Dry Matter Yield
Across each column different superscript indicate significant ($p<0.05$) variation between the mean values

Table 3. Effect of HA levels on protein and mineral profile of millet.

HA levels	CP	N	P	K	Ca	Mg	Mn	Cu	Fe
(kg ha^{-1})	(g/kg dry weight)						(mg/kg dry weight)		
H_0	64.06^{b}	10.25^{b}	2.38	15.10^{e}	2.88^{c}	2.90^{c}	52.02^{e}	50.96	262.00^{e}
H_1	64.25^{b}	10.28^{b}	2.40	18.24^{d}	3.60^{b}	3.12^{c}	60.00^{d}	52.80	262.24^{e}
H_2	64.88^{b}	10.38^{ab}	2.39	23.44^{cd}	3.72^{b}	3.34^{bc}	63.10^{cd}	53.10	267.00^{e}
H_3	66.75^{ab}	10.68^{b}	2.39	24.80^{c}	4.01^{ab}	3.48^{b}	65.31^{c}	52.40	282.11^{d}
H_4	70.00^{a}	11.20^{a}	2.43	25.11^{b}	4.44^{a}	3.71^{ab}	74.11^{b}	52.94	298.30^{c}
H_5	70.00^{a}	11.20^{a}	2.43	29.04^{a}	4.46^{a}	3.84^{a}	74.20^{b}	54.00	316.10^{b}
H_6	69.38^{a}	11.10^{a}	2.42	29.02^{a}	4.45^{a}	3.85^{a}	86.12^{a}	54.10	334.10^{a}

HA = Humic acid; H0, H1, H2, H3, H4, H5 and H6 means 0, 5, 10, 15, 20, 25 and 30 kg HA ha^{-1} respectively
Across each column different superscript indicate significant ($p<0.05$) variation between the mean values

Discussion

The results of this study showed significant effect of HA on millet growth parameters up to 20 or 25 kg ha^{-1} level of HA. However, no significant effects were observed using higher concentrations beyond the fore mentioned levels. Similarly, an increment in HA levels resulted in an increase in CP and mineral content in the plants. These results could be divided into a two-way effect of humic acid-improvement in growth and chemical composition, both of which are correlated (Daur *et al*., 2011). These effects could be attributed to the improvement in soil properties after HA application (Table 1). For example, soil compaction was reduced, as observed in the decrease in bulk density. In addition, Table 1 indicates that HA sustained soil nutrients compared to that in the control. Furthermore, the enhanced growth was linked to the increase in nutrient uptake observed in this experiment.

Although the enhanced nutrient uptake may be due to the polyelectrolyte and macro-ionic nature of HA that increases osmotic process, which in turn enhances ion exchange, improves root nutrient uptake, and increases transport through the cell membrane. However, HA is a complex source of plant nutrients and in addition, it serves as a substrate for beneficial microbes in the soil. Previous reports by Khaled *et al*., (2012), Bakhashwain *et al.,* (2013), and Du *et al.,* (2013) using small amounts of HA support our results, in which HA imparted a positive effect on water retention in soil, improved leaf N content and nutrient uptake, and higher rates of photosynthesis. Similarly, Demir & Cimrin (2011), Humintech (2012), Khaled *et al*., (2012), and Zhang *et al*., (2013) have used small quantities of HA and reported an enhancement in plant growth parameters, plant tolerance to salinity and drought stress, and conservation of soil moisture because of improved soil or plant properties. The non-significant results for P and Cu content in the millet dry matter (DM) could be attributed to the initial high content of these minerals in the soil.

Conclusions

The application of humic acid to soil resulted in an increase in N content and improved nutrient uptake in millet, which in turn enhanced crop growth and DM production. In addition, comparison of the results of the control with some HA treatments show that humic acid is a good organic source that could be used to decrease soil salinity and maintain soil fertility. The results of this study show that the optimum level of HA in soil was 20 to 25 kg ha^{-1}, applied as a side dressing to the crop rows. Further exploitation of HA is required in different climatic conditions and using different crops, with more focus on its comparison with manure and other organic fertilizers. Furthermore, a metabolomic study may be useful in investigating the specific molecular processes and pathways associated with nutrient uptake and growth enhancement using HA.

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