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Effect of humic acid application rates on physicochemical and fertility properties of sandy loam soil grown with mung bean under different irrigation water regimes

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A field experiment was carried out to evaluate the changes in some physicochemical and fertility properties of sandy loam soil treated with three different rates of humic acid (HA) under three different irrigation water regimes. Soil bulk density and saturated hydraulic conductivity were decreased while organic matter was increased by increasing HA rates. Decreasing water regime and increasing HA rate increased soil salinity. Decreasing irrigation water regime increased nitrogen and reduced available phosphorus and potassium. Increasing HA rate increased nitrogen and available phosphorus and potassium.

Keywords: Drip irrigation, macro-nutrients, soil properties, water stress.

CULTIVATING sandy and sandy loam soils to mitigate the global food problems using least amount of irrigation water and mineral fertilizers is greatly required and recommended[1]. Also, water stress is considered to be one of the major problems in global agricultural production which leads to a huge decrease in crop yield especially in arid and semiarid regions, where there is not enough rain[2].

Based on previous studies, humic acid (HA) which is considered a vital constituent and a friendly part of soil organic structure was used to conserve water in root-zone area[3,4]. Therefore, water availability increases due to reduced run-off and deep percolation that ultimately increases crop yield. Moreover, application of HA helps to improve soil physical and chemical properties, i.e. water retention, permeability, water infiltration, drainage, aeration, structure and nutrient availability. As a result, water usage was reduced[3,4] in sandy loam soil using 10 kg ha^{-1} of granular HA which decreased soil bulk density (BD) and saturated hydraulic conductivity (SHC) while increasing water holding capacity, soil organic matter and soil nutrients under fully and water stress conditions[5]. Beside containing nutrients, humic substances can chelate soil nutrients and improve nutrient uptake, especially phosphorus, sulphur, nitrogen and zinc because they act as a sink for such nutrients[6–9].

In arid regions, water resources are limited and the majority of cultivated land contains light textured soils (sandy and sandy loam). Using HA as a soil amendment could be a practical option to increase water and fertilizer efficiencies. Therefore, the present study was aimed at evaluating changes in soil organic matter, soil bulk density, saturated hydraulic conductivity, soil pH, soil salinity, total nitrogen and available phosphorus and potassium of sandy loam soil treated with different rates of HA under different irrigation water regimes.

A field experiment was carried out for two consecutive seasons of 2015–2017 at the Agriculture Research Station of King Abdulaziz University located at Hada Al-Sham, 110 km northeast of Jeddah, KSA. The soil texture of the experimental site was classified as sandy loam. The design of the experiment was a split plot with four replications. The plot size was 6 m^2 (2 m × 3 m). The main plots comprised three irrigation water regimes. The first represented full irrigation (100%) water requirement (WR). The second and third were 80% and 60% of the first regime, and represented stress treatments. The full irrigation water requirement was calculated based on the

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cultivated crop which was mung bean [(*Vigna radiata* (L.) Wilczek]. Mung been is a nutritional field crop rich in protein; it is a drought-tolerant and short period growth crop.

The evapotranspiration was calculated from reference evapotranspiration and crop coefficient as

$$ET_c = K_c \times ET_o,$$

where ET_c is the crop evapotranspiration (mm day^{-1}), ET_o the reference evapotranspiration (mm day^{-1}) and K_c is the crop coefficient. ET_o was calculated using Penman-Monteith equation[10].

The sub-plots were treated with a commercial product of HA, with a granular presentation and a purity of 90%, produced by Pioneers Chemicals (Saudi Arabia) in three proportions (15, 30 and 45 kg ha^{-1}). These proportions were selected based on previous studies carried out in the area. Each proportion of HA was added and mixed manually in the topsoil (15 cm deep) of the corresponding block and respective repetitions. Once the soil treatments were completed, drip irrigation system was installed. On 18 December 2015 and 31 October 2016 mungbean seeds (*Vigna radiata* L.) were sown for the first and the second crop cycle respectively. After crop emergence, the water regimes were applied; moderately saline irrigation water (1800–2000 ppm) was used for irrigation until the end of each crop cycle.

Before starting the experiment, four compound soil samples covering the whole experimental area were collected from the surface layer (30 cm) using soil auger. These samples were prepared for initial soil analysis including soil texture, soil pH, organic matter (OM), soil salinity (EC), total nitrogen (N), available phosphorus (P) and potassium (K) contents. Detailed soil analyses carried out prior to the experiment are presented in Table 1. At the end of each growing season, 144 core samples were sampled from the upper 30 cm of soil surface. The samples were used to measure BD and SHC. Another 144 disturbed soil samples were sampled from the same layer and transferred to the laboratory to prepare for chemical analyses including soil pH, soil EC, OM and N, P and K contents. BD was determined by the core (5 cm height × 5 cm diameter) method[11]. Soil texture and SHC were determined as described by Klute[12]. OM was determined by titration method[13]. The soil EC was measured in 1 : 1 water extraction using the electric conductivity meter, while soil pH was measured in 1 : 1 soil suspension using Beckman pH meter[14]. Total N was determined according to the Kjeldahl method[14]. Available P was determined as described in Olsen and Sommers[15] while available K was determined as described in Carson[16] using flame emission spectrophotometry.

The data obtained for each season was statistically analysed through analysis of variance procedures to determine the significance of the treatments and the interactions. Revised least significant difference (RLSD) test was used to compare between the means after applying the statistical analysis assumptions[17].

The results revealed that decreasing water regimes increased OM in both growing seasons (Figure 1). The highest soil organic matter (1.07%) was measured under the least water regime (60% WR) in the second season. Increasing HA rates also increased soil OM and the highest value was recorded in the treatment of 45 kg ha^{-1} HA rate followed by 30 and 15 kg ha^{-1} HA rates in both growing seasons respectively. On an average, over the three irrigation water regimes and both growing cycles, application of 45 and 30 kg ha^{-1} increased OM by 16% and 6% respectively compared to 15 kg ha^{-1}. Moreover, soil OM measured in the second growing season was higher than that of the first growing season by 40%, 33.8% and 30.7% for 15, 30 and 45 kg ha^{-1} respectively.

Results of BD presented in Figure 2 revealed that 100% WR reduced BD by about 9% compared to 80% WR treatment on an average over the humic rates and growing seasons. The highest BD was recorded in 60% WR treatment (1.78 g cm^{-3} on an average over the humic rates and growing seasons). Results also revealed that increasing HA application rates reduced soil BD. BD was the highest in the proportion 15 kg ha^{-1} HA (1.7 g cm^{-3}) and gradually reduced to the minimum value in 45 kg ha^{-1} HA treatment (1.64 g cm^{-3} on an average) over the three irrigation water regimes and growing seasons.

Table 1. Initial soil analysis before starting the experiment

Parameter	Values			
Particle size analysis	Clay (%)	Silt (%)	Sand (%)	Texture grade
	11.2	19.1	69.7	Sandy loam
Organic matter (%)	0.46			
ECe (dS m^{-1})	2.3			
pH (peast)	7.50			
Nitrogen (%)	0.04			
Phosphorus (mg kg^{-1})	33			
Potassium (mg kg^{-1})	283			

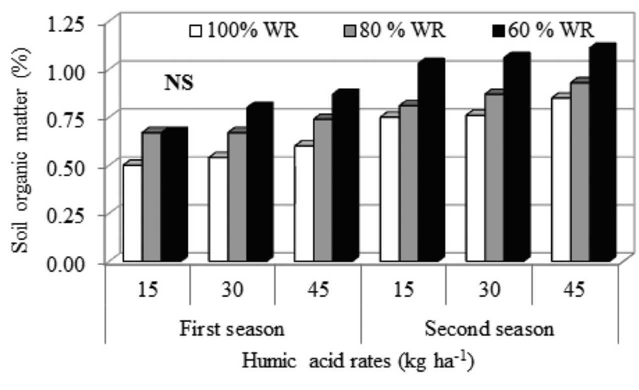

Figure 1. Soil organic matter per cent as affected by irrigation water regimes and humic acid rates after both growing seasons of 2015–16 and 2016–17.

Table 2. Means of some soil chemical and fertility properties of mung bean soil under the effects of irrigation water regimes and humic acid rates during the two growing seasons of 2015–16 and 2016–17

Treatment	pH (Susp.1:1)		EC (Ext. 1 : 1)		Total nitrogen (%)		Available phosphorus (mg kg^{-1})		Available potassium (mg kg^{-1})	
	2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017
Irrigation water regimes (WR)										
100% WR	7.49	7.11	2.56	2.46bc	0.055a	0.058	165.2a	149.1a	638.3a	641.0
80% WR	7.53	7.15	2.67	2.72b	0.070b	0.067	124.5b	110.6b	614.3a	621.6
60% AR	7.54	7.18	3.07	4.12a	0.075a	0.079	82.6c	94.2c	571.8b	586.2
RLSD (0.05)	NS	NS	NS	0.27	NS	NS	28.5	14.5	29.6	NS
Humic acid rates (HA kg ha^{-1})										
15	7.59	7.19	2.63	2.87cb	0.064	0.065	108.9b	106.5b	592.4bc	588.8
30	7.50	7.15	2.68	3.02ab	0.066	0.067	126.6a	119.0a	607.4ab	624.6
45	7.47	7.10	2.98	3.41a	0.070	0.072	136.8a	128.4a	624.4a	635.4
RLSD (0.05)	NS	NS	NS	0.35	NS	NS	17.47	11.65	25.24	NS
Interaction										
WR × HA	NS	NS	*	*	*	NS	**	*	**	NS

Means followed by the same letter(s) are not significantly different according to RLSD at $P \leq 0.05$. NS, Not significant.

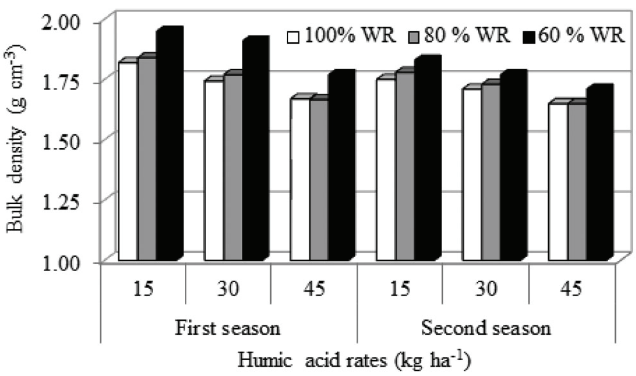

Figure 2. Soil bulk density as affected by irrigation water regimes and humic acid rates after both growing seasons of 2015–16 and 2016–17.

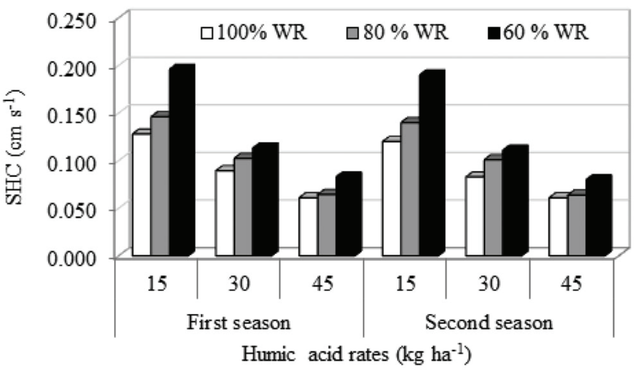

Figure 3. Saturated hydraulic conductivity (SHC) as affected by irrigation water regimes and humic acid rates after both growing seasons of 2015–16 and 2016–17.

Results of SHC presented in Figure 3 revealed that decreasing irrigation water regimes increased SHC. The highest SHC was measured in 60% WR (0.08 $cm^3 s^{-1}$) followed by 80% WR (0.075 $cm^3 s^{-1}$) and 100% WR (0.065 $cm^3 s^{-1}$) treatments on an average, over the HA proportions and growing seasons. Increasing HA rate decreased SHC. The highest SHC (0.089 $cm^3 s^{-1}$) was measured in the treatment of 15 kg ha^{-1} HA rate and gradually decreased to reach the minimum value (0.051 $cm^3 s^{-1}$) in 45 kg ha^{-1} HA rate on an average, over the three irrigation water regimes and both growing seasons.

Results of soil pH presented in Table 2 revealed insignificant differences among soil pH means in both growing seasons under all investigated water regimes and HA rates. HA rate slightly decreased soil pH. The soil pH measured in the second growing season was lower than that in the first growing season. Results of soil salinity presented in Table 2 indicate that decreasing irrigation water regime increased soil salinity especially in the second growing season where the differences in soil EC means were significant. The highest soil salinity was measured in 60% WR treatment followed by 80% WR and 100% WR treatments. Soil EC means were similar under the three investigated HA rates in the first growing season. However, in the second growing season, the least soil EC (2.87 dS m^{-1}) was measured in 15 kg ha^{-1} HA rate followed by 30 kg ha^{-1} HA rate (3.02 dS m^{-1}). The highest soil EC (3.41 dS m^{-1}) was measured in 45 kg ha^{-1} HA rate and was significantly similar to 30 kg ha^{-1} HA rate.

Results presented in Table 2 show no significant difference in total nitrogen among the three investigated water regimes; however decreasing irrigation water regime gradually increased total nitrogen content in both seasons. Increasing HA application rate resulted in a gradual increase in soil nitrogen content in both seasons, however the increase was not significant in all HA rates.

Table 3. Means of some soil chemical and fertility properties of mung bean soil under the effects of the interaction between irrigation water regime and humic acid rates during the two growing seasons of 2015–16 and 2016–17

Irrigation water regime	Humic acid rate ($kg ha^{-1}$)	EC (Ext. 1 : 1)		Total nitrogen (%)		Available phosphorus ($mg kg^{-1}$)		Available potassium ($mg kg^{-1}$)	
		2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017	2015–2016	2016–2017
100% WR	15	2.43	2.41	0.051	0.054	133.75	127.41	622.50	616.9
	30	2.92	2.14	0.054	0.059	174.50	159.53	634.25	648.48
	45	2.34	2.83	0.059	0.061	187.26	160.27	658.02	657.46
80% WR	15	2.74	2.73	0.069	0.065	114.00	105.54	602.03	591.02
	30	2.72	2.46	0.070	0.067	124.24	107.46	611.26	630.58
	45	2.54	2.96	0.071	0.070	135.26	118.67	629.52	643.17
60% WR	15	2.72	3.47	0.072	0.076	79.01	86.39	552.75	558.37
	30	2.41	4.45	0.074	0.076	81.00	89.12	576.77	594.63
	45	4.07	4.45	0.080	0.084	87.77	107.09	585.76	605.58
RLSD (0.05)		0.13	0.60	NS	NS	45.91	20.18	43.73	NS

NS, not significant.

Results of available phosphorus showed a significant reduction due to decreasing irrigation water regime in both growing seasons. The highest significant available phosphorus values were measured in 100% WR followed by 80% WR and 60% WR in both growing seasons. Available phosphorus means were significantly increased by increasing HA rate in both seasons. The highest available phosphorus values (136.76 and 128.43 $mg kg^{-1}$) were measured in 45 $kg ha^{-1}$ HA rate followed by 126.58 and 118.95 $mg kg^{-1}$ in 30 $kg ha^{-1}$ HA rate in the first and second growing seasons respectively. The least significant available phosphorus means were measured under 15 $kg ha^{-1}$ HA rate and were 108.92 and 106.45 $kg ha^{-1}$ in the first and second seasons respectively.

The highest available potassium in the first season was recorded in 100% WR and 80% WR treatments and was significantly higher than that of 60% WR treatment. Available potassium measured in the second season showed similar trend as in the first growing season, however the differences among them were not significant (Table 2). Increasing HA application rate gradually increased available potassium. The highest significant available potassium (624.43 $mg kg^{-1}$) was found in 45 $kg ha^{-1}$ HA, followed by 607.43 and 592.43 $mg kg^{-1}$ in 30 and 15 $kg ha^{-1}$ HA rates in the first growing season respectively. The highest available potassium mean value in the second growing season was 635.4 $mg kg^{-1}$ followed by 624.56 $mg kg^{-1}$ and 588.76 $mg kg^{-1}$ in 45, 30 and 15 $kg ha^{-1}$ HA rates but the differences among them were not significant.

The interaction between irrigation water regimes and HA rates significantly affected on soil EC, OM, P and K contents. Decreasing irrigation water regime resulted in a significant increase in soil EC (Table 3). The highest significant EC (4.45 $dS m^{-1}$) was measured under 60% WR with 45 $kg ha^{-1}$ HA rates in the second growing season. However, the least soil EC (2.14 $dS m^{-1}$) was recorded under 100% WR with 30 $kg ha^{-1}$ HA rate in the second growing season. Results of total nitrogen (N) showed a gradual increase due to decreasing irrigation water regime and increasing HA rate in both seasons, but the differences were not significant among all interaction treatments.

Decreasing water regimes decreased available P, and within each water regime, available P increased by increasing HA rate. The highest available P value was 187.26 $mg kg^{-1}$ measured under 100% WR with 45 $kg ha^{-1}$ HA rate, while the least value was 79.01 measured under 60% WR with 15 $kg ha^{-1}$ HA rate. Available potassium (K) behaved as described in available phosphorus. The highest available K (658.02 $mg kg^{-1}$) was measured in the treatment of 100% WR with 45 $kg ha^{-1}$ HA and significantly different from the other interaction treatments. The least available K was 552.75 $mg kg^{-1}$ measured in 60% WR with 15 $kg ha^{-1}$ HA rate (Table 3).

The increase in soil OM due to decreasing water regime could be attributed to the reduction in retained water in soil. Under low water supply, as in 60% WR, the microorganism's activity for decomposing soil organic matter decreased and accumulation of OM in soil increased. Increasing HA application rate also increased soil organic matter. Humic acid is an organic substance, so adding it to soil might increase soil organic matter[5]. The reduction in soil BD under full water requirement could be attributed to the enhancement in soil structure especially due to the presence of organic material like HA. Such conditions encourage granulation and resulted in an increase in soil volume and consequently, reduced BD[5]. The reduction of BD as affected by HA application rate could also be attributed to the improvement of soil physical properties especially soil structure and aggregation[18]. Humic compounds can help improve soil structure by increasing the amount of pore space and enhancing the air exchange, water movement, water holding capacity and root growth[4]. The reduction in soil BD was greater in the second growing season than in the first. This could be attributed to the continual improvement in soil physical properties, especially granulation due to the time and presence of soil organic matter which increases the stability of formed aggregation.

SHC of soil was reduced by increasing HA application rate. Presence of HA helps to improve soil structure and aggregation which resulted in reduction in soil pore space especially in its volume. Any decrease in volume pore space is met by reduction in SHC. Due to reduction in BD and decrease in water movement, SHC decreased by increasing HA application rate. The addition of clay or organic matter to sandy soil as a conditioner improved its hydraulic properties by limiting percolation losses, while maintaining adequate infiltration rate and water retention[1]. The reduction in soil BD directly reduced SHC as clearly indicated from the obtained results. The reduction could also be attributed to the decrease in effective mean pore radius as a result of soil expansion and/or due to improved soil structure and stability[19].

Results of soil chemical and fertility properties showed that soil salinity was increased by decreasing irrigation water regime. The increase in soil salinity could be attributed to two reasons. The first is due to the salinity of irrigation water used in the current experiment, which ranged from 1800 to 2000 ppm. Using such moderate salinity irrigation water for two consecutive seasons might accumulate salts in the soil. The second reason is the reduction in water supply according to each investigated water regime. Reducing water supply, as in 80% WR and 60% WR might decrease solubility of salts and consequently increase its accumulation in soil. Soil salinity was also increased by increasing HA rates. Humic acid may chelate soil nutrients and form complexes with various metal ions, thereby increasing the cation exchangeable capacity of the soil. Increasing cation exchangeable capacity in soils may increase soil salinity[20].

The increase in total N due to decreasing irrigation water regime is logical and expected because N is soluble in water. Therefore, increasing irrigation water, as in 100% WR, might increase solubility of N and increase its loss from soil either by leaching or by uptake via plants. On the other hand, in lower water supply (60% WR), solubility of N decreased and large amount of it remained in soil. Also, in lower available soil moisture, plants have difficulty in absorbing N from soil. As a result, N content in the soil was higher in 60% WR compared to 100% WR treatment. More than 90% of N movement in soils is caused by mass flow and hence water acts as a driving force. However, under limited water conditions, movement of N is drastically reduced and plants are unable to absorb the same as well[21].

Available P and K decreased by decreasing irrigation water regime. The reduction could be attributed to the decrease in amount of irrigation water and consequently the solubility of both nutrients as in 60% WR. However, under adequate water supply, as in 100% WR, solubility of P and K increased and resulted in an increase in their availabilities in soil[21]. Increasing HA application rate increased available P and K. Humic acid contains some nutrients in its structure and is characterized by chelating soil nutrients. So, HA improved nutrient uptake, especially phosphorus, sulphur and nitrogen, and acted as a storehouse of N, P, S and Zn[18]. Organic fertilizers like HA may be used to improve soil fertility by increasing soil organic carbon, nitrogen, sulphur and phosphorus nutrients[22].

The results of this study indicated that increasing irrigation water regime decreased soil organic matter, bulk density and saturated hydraulic conductivity. Increasing HA application rates decreased soil bulk density and saturated hydraulic conductivity but increased soil organic matter. Soil salinity was increased by decreasing water regime and by increasing HA rate. However, the increase was mainly due to decrease in irrigation water regime which increased N, while it decreased available P and K. Increasing HA rate increased NPK in soil. Using 80% WR with 45 kg ha^{-1} HA rate optimized water and nutrients in sandy soils through improvement in physicochemical and fertility properties.

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Morphological, cytological, palynological and molecular characterization of certain *Mangifera* species

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The *Mangifera* genus has more than 60 species, mostly distributed in tropical Asia. The wild relatives of *Mangifera* are considered reservoirs of potential genes that can confer tolerance/resistance to biotic and abiotic stresses. The morphological, cytological and molecular characterization of eight species was done to study the diversity and phylogenetic relationship among different *Mangifera* species. In order to study the evolutionary relationship and polymorphism among the mango species, the ITS1/ITS4 gene and partial chloroplast *psbH-trnH* genes were sequenced. Phylogenetic analysis of the nuclear and chloroplast marker revealed that the *M. indica* L. is closely related to *M. griffithii* and *M. camptosperma*, which belong to subgenus *Mangifera*. Results indicate that the taxonomic position of *M. andamanica* should be reconsidered as this species is very close to *Bouea oppositifolia* which is evident from both ITS and *psbA-trnH* rDNA analysis. The morphological traits such as tree, leaf, flowers and fruits and palynological and cytology of the genus mango were used to distinguish the species and its phylogentic status. The morphological traits among various species indicate the high level of variability which were further confirmed with ITS sequences and cpDNA. Phylogenetic analysis illustrates that partial chloroplast *psbH-trnH* gene gave better polymorphism in mango species than nuclear ITS. The pollen morphology and chromosomal counts were also done in certain *Mangifera* species to study the phylogenetic relationship.

Keywords: Chromosome, ITS, mango, pollen grains, psbA-trnH and phylogenetic analysis.

MANGO (*Mangifera indica* L.), considered as ‘King of fruits’, belongs to the family Anacardiaceae. It is an important tropical fruit believed to have originated in the Himalayan hills of Indo-Myanmar region. There are 58 listed species of the genus *Mangifera* which are further classified into several sections, based on their flower morphologies[1]. Mango (*M. indica* L.) and some other species of this genus are diploid ($2x$) with somatic number ($2n$) of chromosomes 40 (refs 2, 3). The high chromosome number, secondary association of bivalents,

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