ISSN(Online) : 2319 - 8753
ISSN (Print) : 2347 - 6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 5, May 2015

Effect of Humic Acid Application on Growth and Chlorophyll Contents of Common Bean Plants (*Phaseolus vulgarisL.*)Under Salinity Stress Conditions

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ABSTRACT: Pot experiment was carried out at the research greenhouseof the King Abdul-Aziz University, Jeddah, Saudi Arabia to study the effect of humic acid (HA)on biological aspects (plant height, number of leaves and root length and plant growth biomass as well as chlorophyll contents) of common bean plants (*Phaseolus vulgaris L.*) under salinity stress conditions at 15, 30 and 45 days after planting (DAP).Four different salinity levels (0, 25, 50 and 100 mMNaCl) combined with and without HA supply at 3g L^{-1}) in split plot design with three replicates were assigned. Results indicated that HAsupply significantly increased plant height, number of leaves, root length, shoot and root fresh and dry weights as well as chlorophyll contents of common bean than control plants at 15, 30 and 45 DAP, respectively. While, no significant responding was found for shoot fresh weigh at 15 DAP and root fresh weight at 30 DAP.Salt stress significantly declined the plant morphological and fresh and dry biomass as well as chlorophyllcontents and leaf area in shoot leaves. Application of HA markedly mitigated the adverse effects of a biotic stress on all studied biological aspects. Lastly, humic acid supply significantly increased the relative growth rates of shoot and root under saline conditions and humic is considered as a promising soil amendment to overcome adverse effects of salinity stress.

KEY WORDS:humic acid, salinity stress, relative growth rate, common bean(*P. vulgaris L.*)

I. INTRODUCTION

All plants are subjected to multitude of stresses through-out their life cycle. Salinity stress is one of the major a biotic factors limiting legume plant growth and yield in arid and semi-arid regions [1; 2 and 3] and salinity has direct harmful effects on numerous plant species [4; 5 and 6].It could be caused by (i) salts accumulated in the top layer of the soil due to over-irrigation, (ii) poor irrigation water which contains considerable amounts of salts (iii) neighborhood to the sea, and (iiii) the capillarity rise of salts from underground water into the root zone due to excessive evaporation.Further, salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes [7]. Under saline conditions, plants are stressed in three ways; (1) water deficit due to reduced water potential in the root zone, (2) nutrient imbalance by depression in uptake and/or shoot transport (3) phytotoxicity of ions such as Na^+ and Cl^-[8;9 and10]. This is attributed to the fact that Na^+ competes with K^+ for binding sites for cellular function [11].

Humic acid (HA) are the main fraction of humic substances (HS) and the most active components of soil and compost organic matter.HA can enhance nutrient availability and improve chemical, biological, and physical soil properties [12; 13; 14and 15].The direct and indirect beneficial effects of HA on plantgrowth and development are their effect on cell membranes which lead to the enhanced transport of minerals, improved protein synthesis, plant hormone-like activity, promoted photosynthesis, modified enzyme activities, solubility of micro-elements and macro-elements, reduction of

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ISSN (Print) : 2347 - 6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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active levels of toxic minerals and increased microbial populations [16]. In addition, HA introduced as good accumulators of toxic heavy metals [17 and 18].However, there are few studies about the changes induced by the application of HAon plant structures and process in abiotic stresses, especially salinity conditions[19; 20; and 16).

The common bean (*Phaseolus vulgaris L.*) is the most important and vital leguminous crops in the semi-arid areas among the Asian countries i.e., China, Iran, Japan, and Turkey are the major producers of the common bean. Besides providing high quality protein for human consumption.The main goal of this dissertation was the assessment of humic aid supply in combination with salinity levels on morphological parameters and plant biomass of common bean plants.

II. MATERIALS & METHODS

Experimental site:

This investigation was carried out during the summer of 2013 at the research greenhouse at the horticultural unit of King Abdul-Aziz University, Jeddah,Saudi Arabia. The green house is assigned at the following conditions; photoperiod (16 h light and 8 h dark) and the average temperature was 25°C/21°C day/night.Randomly, the soil material was collected from surface layer (0–15 cm) at the private agricultural farm, North of Jeddah city from an area of 1 ha.Soil sample was air dried.

Plastic pots measuring 50 cm in height and 20 cm in diameter.Each pot was filled with 5 kg of soil and soil characterization was done according to standard procedures: particle-size distribution using the pipette method [21], soil field capacity [22], total carbonate [21], soil pH (saturated soil paste)[22], total soluble salts (electrical conductivity of saturated soil paste extract) [23]. Concentrations of available nitrogen, phosphorus, and potassium were determined as described by Hesse[24]. Available nitrogen was extracted using KCl (2.0 M), available phosphorus was extracted and determined using the Olsen method (extracted using $NaHCO_3$ [0.5 M] at pH 8.5 and determined colorimetrically after treating with ammonium molybdate and stannous chloride). Available potassium was extracted using ammonium acetate (1.0 M) at pH 7.Some physical and chemical properties of the investigated soils are shown in Table 1.

Table 1: Physical and chemical properties of the investigated soils.

pH 1:2.5	EC dSm^{-1}	Ions meq 100 g^{-1} soil							
		Cations				Anions			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO3^{=}$	HCO_3^{-}	Cl^{-}	$So_4^{=}$
7.5	0.5	1.6	0.72	2.69	0.4	0	1.15	2.32	1.94
Mechanical analysis %				Texture	Sp %	OM %	Available nutrients (mg kg^{-1} soil)		
C. sand	F. sand	Silt	Clay				N	P	K
8.00	86.60	4.37	1.03	Sandy	60.5	0.23	23.9	4.69	260

Experimental layout and salt treatment:

The experiment consisted of four salt treatments as the following;0, 25, 50 and 100mMNaCl combined with and without humic acid (HA).HA(made in Germany) in a solid form as potassium humate (85%) was applied to the soil at the rate of 3g L^{-1} and given as liquid to the soilat three equal doses. The chemical analysis of humic acid is shown in Table 2.

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Table 2: Some chemical composition of humic acid.

Humic Acid %	EC dSm^{-1}	pH	OM %	CEC [Cmol (p+) kg^{-1}]	Macronutrients (%)			Micronutrients (mg kg^{-1})		
					N	P	K	Zn	Fe	Mn
85	0.90	7.0	70	450	5.00	0.5	4.0	248	409	244

The experiment was set up in split plot design with a completely randomized block design, and each treatment was replicated three times.Prior to planting, all seeds were inoculated with rhizobium strain (*Rhizobium leguminosarumbiovarphaseoli*). Finally, NaCl treatments were initiated 10 days after sowing.

Seed germination and growth:
Nine seeds of common bean cultivarStrike (*Phaseolus vulgaris, L.*)obtained from Hollandwere sown in each pot and these were thinned to 3seedling in each pot 10 days after planting. During the growing period, the seedlings were frequently irrigated with water previously prepared with different concentration of NaCl salt. Moisture content was monitored regularly by supplying the plants with equal amounts of water to ensure that the pots were not moisture deficient.

Plant growth measurements and sampling:
After 5 days after NaCl salt treatments, measurements were taken three times (at15 days intervals), each time, plant morphological parameters such as plant height, number of leaves, root length, shoot and root fresh weight were measured.
Roots were immersed in tap water and then in de-ionized waterto clean them and thenroot and shoot were dried in an oven at 70^{o}C for 48 h. Dry root and shoot biomass were recorded. Leaf chlorophyll contents were measured using Minolta SPAD chlorophyll meter and leaf area (cm^2/plant)was calculated by the following equation:[(Fresh weight of leaves x 10 x punch area (cm^2)/fresh weight of 10 disks] x100.
Finally, the relative growth rate (RGR) was estimated during two periods at 30 and 45 days after planting (DAP) using the following equation:

$$(RGR) =(\ln W_2 – \ln W_1/t_2\text{-}t_1)$$

Where, W plant weight (mg), t the time (days), and the subscripts 1 and 2 are initial and the 2^{nd} sampling of biomass.

Statistical analysis:
Data were statistically analyzed using descriptive statistics and analysis of variance (ANOVA). Based on a two-way ANOVA, the effect of HA application and NaCl salt treatments as well as their interactions were evaluatedaccording to the procedure outlined by Duncan[25] using CoStat (Version 6.303, CoHort, USA, 1998–2004). Means of treatments were considered significantly different using the least-significant-differences test (LSD) at the confidence level of 5% according to Gomez and Gomez [26].

III. RESULTS AND DISCUSSION

Plant morphology:
A glance on Table 3, the summary of the statistical analysis for the morphological parameters clear that soil application of humic acid (HA) significantly ($P\leq 0.05$)increased plant height, number of leaves and root length at 15, 30 and 45 days after planting (DAP),respectively. While, no significant differences were observed for root length at 15 and 30 DAP, respectively.
The analysis of variance for studied parameters during the dissertation appeared that salinity stress had adversely reduced the plant height, number of leaves and root length of common bean measured at 15, 30 and 45 days after planting (DAP) as salinity increased. Differences in studied parameters were highly significant ($P\leq 0.01$) among the salinity levels with the exception of number of leaves at 15 DAP (Table 3).This reduction under high salt stress (100 mMNaCl) as observed in this dissertation explains a toxic impacts from high ion concentration in plant tissues.

Corresponding with the obtained results, there were previous studies have reported that plant height in legumes such as *P. vulgaris* was declined under salt stress [1 and27].

It was clear that plant growth markedly influenced by all applied salinity levels with a variance degree (Table 3). With the low level of salinity treatments, application of HA had pronounced effect on plant height, number of leaves and root length. On the other hand, the reduction was observed with the highest salinity treatment receiving no HA applications. Nevertheless, it was noticed that the application of HA maximized the adverse effect of salinity stress and the performance of morphological parameters of common bean plants meanwhile not to the level of plants grown under appropriate conditions (non-saline). The observed mitigating effects were true for all recorded plant growth parameters namely plant height, number of leaves and root length.

Table 3:Effect of HA application in combination with different salinity levels (mMNaCl) on plant height, number of leaves and root length of common bean plants.

	Parameters	Plant height (cm)			No of leaves			Root length (cm)		
		Days after planting (DAP)								
Treatments		15	30	45	15	30	45	15	30	45
Without	0	92.30b	157.80c	181.00b	5.33	8.50a	10.56a	6.58b	9.18b	9.75b
	25mM	83.00d	155.20c	175.56c	4.33	7.00c	9.56b	6.00c	6.57d	7.33cd
	50mM	80.12e	101.00f	152.00e	4.00	6.33d	8.56d	4.83f	6.23e	6.75d
	100mM	70.56f	88.30g	132.00f	3.67	6.00e	8.00e	5.30d	4.67g	5.83f
With HA	0	103.20a	172.00a	184.00a	5.33	8.00ab	10.60a	7.83a	9.93a	11.42a
	25mM	90.80c	166.80b	181.00b	6.33	7.67b	9.50b	5.50cd	7.50c	7.83c
	50mM	81.30d	146.20d	164.00d	4.67	8.00a	8.95c	3.83g	4.83f	6.50e
	100mM	68.50g	135.30e	132.00f	4.00	7.67b	8.56d	5.03e	3.83h	4.58g
Mean values as affected by HS application treatments										
Without		81.50b	125.58b	160.14	4.33	6.96b	9.03b	5.68	6.66	7.42b
With HA		85.95a	155.08a	165.25	5.08	7.89a	9.17a	5.55	6.52	7.58a
Mean values as affected by different salinity levels (mMNaCl).										
0		97.75a	164.90a	182.50a	5.33	8.25a	10.58a	7.21a	9.56a	10.59a
25mM		86.90ab	161.00b	178.28b	5.33	7.34ab	9.53ab	5.75ab	7.04a	7.58ab
50mM		80.71b	123.60c	158.00c	4.34	7.17b	8.76b	4.33c	5.53b	6.63b
100mM		69.53c	111.80d	132.00d	3.84	6.84c	8.28c	5.17b	4.25c	5.21c

Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test.

Plant biomass measurements:

The obtained results during the study revealed that plants received HAsignificantly mitigated shoot and root fresh weights (g plant^{-1}) at 15, 30 and 45 DAP respectively as compared to untreated plants (Table 4). Contrary, no significant responding was found with respect to shoot fresh weigh at 15 DAP and root fresh weight at 30DAP, respectively. Furthermore, dry shoot and root biomass were significantly enhanced by HAaddition as compared to the control at three plant growth intervals (Fig.1). Meanwhile, shoot/root ratio tended to enhance due to HAaddition comparing with the control but there is no significant differencesat three harvests. There was an increase in shoot/root at 30 DAP which then dropped markedly at 45 DAP (Fig.1).

The observed enhancement of plant growth using HA application had been reported earlier to be due to increase elements uptake such as N, P, K, Fe, Zn, and Mn nutrients [13; 14 and 15]. Moreover, enhancement of photosynthesis,

ISSN(Online) : 2319 - 8753
ISSN (Print) : 2347 - 6710

International Journal of Innovative Research in Science, Engineering and Technology

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chlorophyll density and plant root respiration has resulted in greater plant growth with HA application [28]. This performance in the plant growth most probably was due to enhance in moisture retention and the improvement of nutrients supply in the root zone [29 and 30].

Table 4: Effect of HA application in combination with different salinity levels (mMNaCl) on fresh biomass (g plant^{-1}) of common bean plants.

Parameters		Fresh shoots (g plant^{-1})			Fresh roots (g plant^{-1})		
		Days after planting (DAP)					
Treatments		15	30	45	15	30	45
Without	0	4.70	7.14b	10.54b	0.22ab	0.3b	0.38a
	25mM	3.67	6.14c	9.19c	0.17c	0.26c	0.29bc
	50mM	3.00	3.97e	5.93f	0.15d	0.18d	0.25d
	100mM	2.70	3.00f	4.00h	0.11f	0.13e	0.16e
With HA	0	3.44	7.66a	11.78a	0.26a	0.32a	0.4a
	25mM	4.46	6.20d	7.51e	0.21b	0.27c	0.32b
	50mM	3.70	6.82bc	8.61d	0.14de	0.17d	0.28c
	100mM	3.13	4.00e	4.98g	0.12e	0.14e	0.18de
Mean values as affected by HS application treatments							
Without		3.52	5.06b	7.42b	0.16b	0.22	0.27a
With HA		3.68	6.17a	7.97a	0.18a	0.23	0.30b
Mean values as affected by different salinity levels (mMNaCl).							
0		4.07a	7.40a	11.16a	0.24a	0.31a	0.39a
25mM		4.07a	6.17b	8.35b	0.19b	0.27b	0.31b
50mM		3.35b	5.40c	7.27c	0.15c	0.18c	0.27c
100mM		2.92c	3.50d	4.49d	0.12d	0.14d	0.17d

Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test.

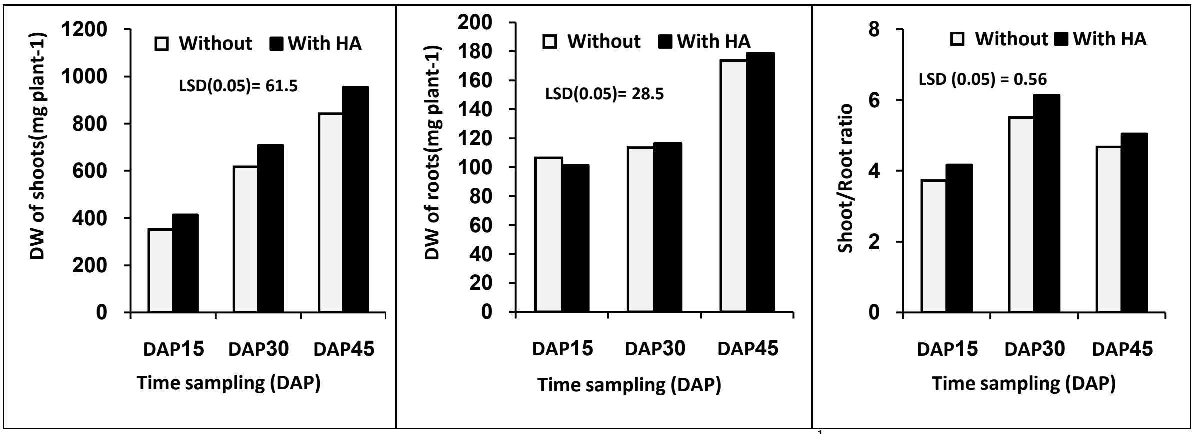

Fig 1: Effect of humic acid application on dry shoot and root biomass (mg plant^{-1}) and shoot/root ratio of common bean plants.

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ISSN (Print) : 2347 - 6710

International Journal of Innovative Research in Science, Engineering and Technology

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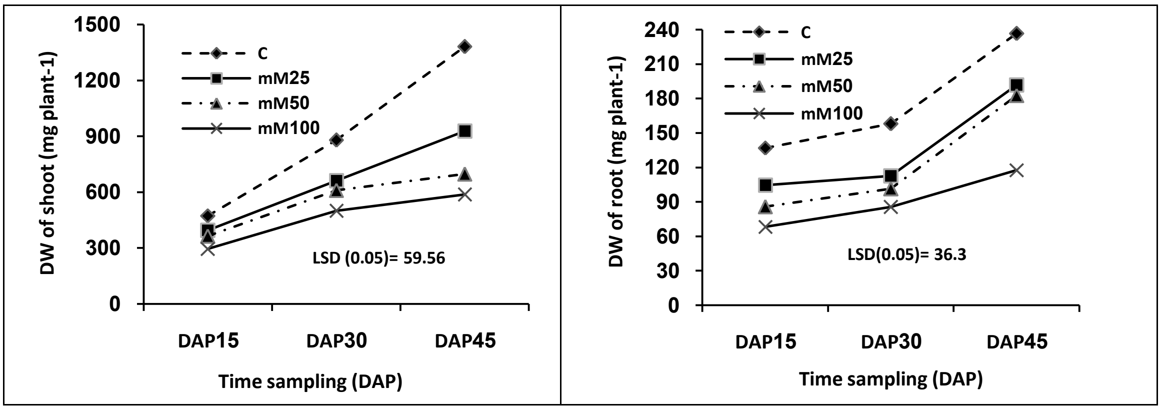
The effect of salts stress on fresh and dry biomass of common bean plants were examined at 15, 30 and 45 DAP, respectively (Table, 4 and Fig. 2). Increasing salinity level above control significantly reduced all plant growth parameters namely fresh shoot and root biomass at 15, 30 and 45 DAP, respectively (Table 4). Further, there was a negative relationship between salinity levels and dry biomass which was significantly correlated with the salinity levels (Fig. 2). The reduction in fresh weight was 25, 12 which then jump up to 32% as the salinity increased from optimal 25, 50 and 100 mMNaCl at 45 DAP, respectively. Shoot/root ratio were also significantly different as the level of salinity increased (Fig. 2). The best shoot/root ratio was 6.01 occurred in 50 mMNaCl salinity at 30 DAP. On the other hand, the lowest ratio was 3.46 found in non-saline (control). Further, it was notable that there was an increase in shoot/root ratio at 30 DAP which declined markedly at 45DAP as shown in Figure 2. It seems that the observed reduction in plant biomass may be due to a combination of slower growth and development. This has been attributed to the osmotic stress, restricted photosynthesis mainly because of salt induced changes in photosynthetic apparatus and sinks capacity [4 and 31]. In *Phaseolus vulgaris*, concentration of 50 mMNaCl caused stunted growth due to salt-induced reduction in photosynthesis [32].

Results collected in Table (5) reveal the combined effect of HA application rates and salt stress levels on dry shoot and root and shoot/root ratio at 15, 30 and 45 days after planting, respectively. The highest descending was associated with the salted plants with highest salinity stress (100 mM) in absence of HA application. On the other hand, the plants grow at HA only give the highest values of dry shoot and root biomass and shoot/root ratio during the different plant growth intervals. It was notable that the application of HA alleviated plant growth and improved biomass measurements of green bean plants. This highest mitigating effect of HA supply on fresh shoot reached about 45.19 % at 50 mMNaCl treatment. Moreover, the highest alleviation effect of HA supply on fresh root was reached about 12.5 % at 100 mMNaCl treatment. In this concern, HA might show anti-stress effects under salinity conditions and it may enhance the uptake of nutrients and reduce the uptake of some toxic elements [20 and 33]. However, there are very few researches of HA application and its effect on the condition of salinity [19].



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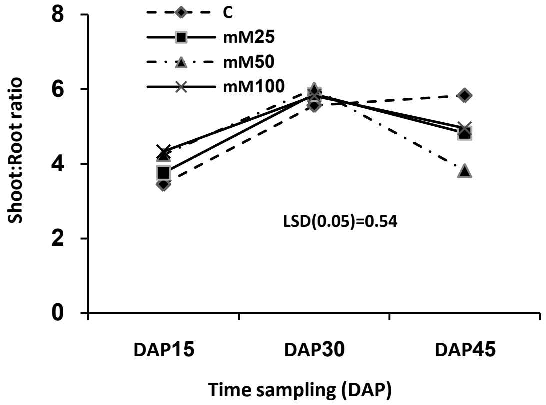

Fig 2: Effect of different salinity levels (mMNaCl) on dry biomass (mg plant^{-1}) and shoot/root ratio of common bean plants.

Table5:Effect of HA application in combination with different salinity levels (mMNaCl) on dry biomass (mg plant^{-1}) of common bean plants.

Parameters		Dry shoots (mg plant^{-1})			Dry roots (mg plant^{-1})			Shoot/Root ratio		
		Days after planting (DAP)								
Treatments		15	30	45	15	30	45	15	30	45
Without	0	460.00b	850.00b	1336.67b	137.30a	150.00b	200.00b	3.35	5.45c	5.81a
	25mM	330.00e	570.00d	835.00d	98.83d	111. 67d	185.00e	3.34	5.09d	4.39d
	50mM	343.30d	600.00e	695.23e	83.33f	108.33e	188.00d	4.12	5.98ab	3.95e
	100mM	273.30g	450.00g	500.00g	66. 67g	82.00h	121.45g	4.09	5.49c	4.55c
With HA	0	486.70a	910.00a	1425.00a	136.67b	160.00a	206.35a	3.56	5.69ab	5.85a
	25mM	460.00b	753.30c	1020.00c	110.23c	113.33c	193.56c	4.17	6.65a	5.27b
	50mM	386.60c	620.00e	700.00e	88.33e	102.50f	189.64cd	4.38	6.05ab	3.69f
	100mM	320.00f	550.00f	675.00f	70.1fg	89.25g	125.56f	4.56	6.16b	5.38ab

Mean values followed by the same letter are not significantly different at the 5% probability level according to LSD test.

Chlorophyll readings (SPAD) and Leaf area parameters:
A glance Figure 3, generalstrong increases in values of chlorophyll readings (SPAD) and leaf area (cm^2) were observed in almost plants treated with HA as compared to untreated plants under all NaClsalt treatments.Humic acid may be caused an enhancement in the synthesis of the chlorophyll [12] and/or delayed chlorophyll degradation in the two different types of leaves, primary and lateral shoot leaves.

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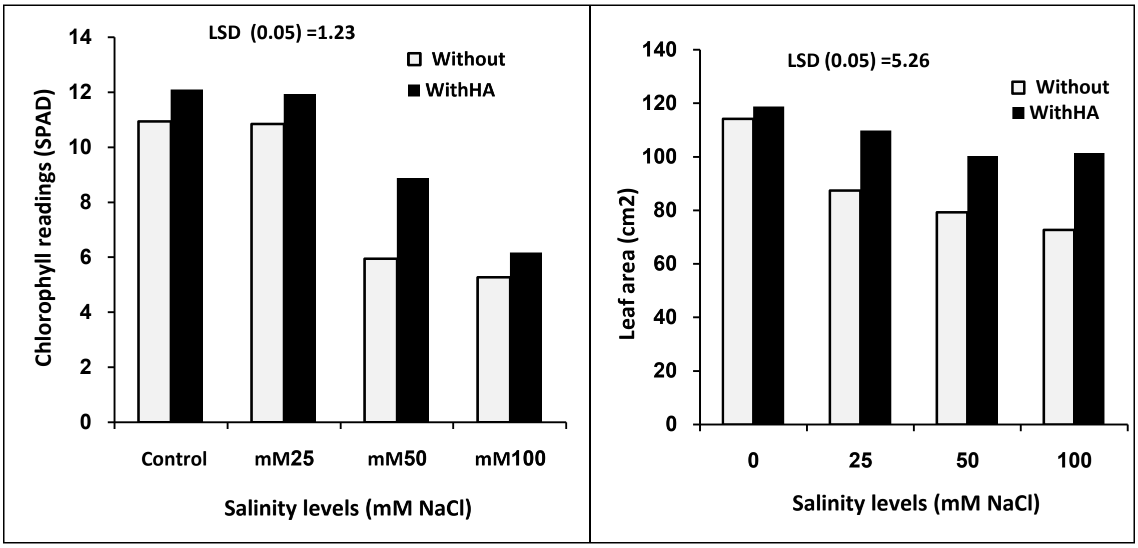

Fig 3: Effect of HA application in combination with different salinity levels on leaf area (cm^2) and chlorophyll readings of common bean plant.

In respect of use HA application in combination with the different salinity levels, results obtained in the current experiment seem to suggest that even application of the HA was able to increase SPAD values under salt stress treatment and consequently leaf area in the common bean leaves (Fig. 3). It seems that HAcan facilitate respiration and photosynthesis processes via modified functioning of mitochondria and chloroplasts [12 and 34]. So, the negative effects of a biotic stresses on plants can be alleviated by the use of HA[16; 34 and 35).

Relative growth rates (RGR):

Alternatively, relative growth rate (RGR) has been used as a relative basis on which to compare growth rates of plants. A crossthe effect of HA and NaClsalt treatments on relative growth rate(RGR) of common bean parts (shoot & root), Figure 4 illustrate that the relative growth rate of plant parts highly significant influenced by HAapplication under the different salinity levels.

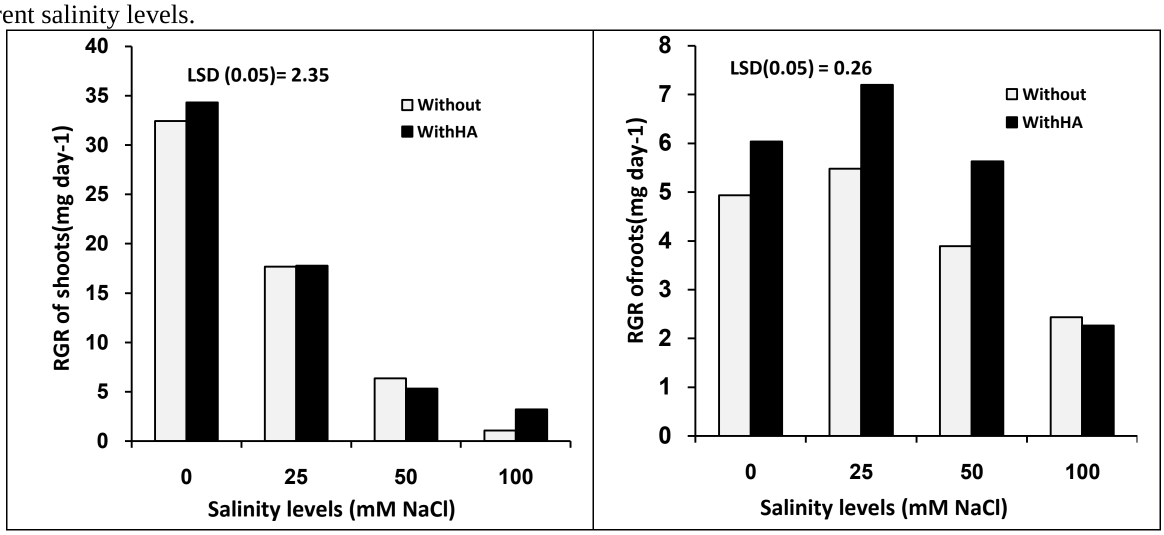

Fig 4: Effect of HA application in combination with different salinity levels on relative growth rate (RGR) for common bean plant parts (shoot & root).

ISSN(Online) : 2319 - 8753
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International Journal of Innovative Research in Science, Engineering and Technology

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The results presented show that the application of HAhad promoting effects on relative growth rate and alleviating ones on damages caused by the salt stress. The highest relative growth rate of shoot was occurred with the HA supply under non-saline conditions as compared with the others. Contrary, the highest relative growth rate of root was observed when HAsupply under the saline condition of 25mMNaCl and then declined considerably thereafter.

In addition,there are closely linear relationshipsamong the relative growth rates of plant parts (shoot & root) and whole plant (RGR) for common bean plant exposed a biotic salinity stress.A highly significant relation (r^2 = 0.97, n = 24, P> 0.05), can be plotted in relative growth rate of shoot versus relative growth plants received HA under salinity stress. Meanwhile, scatter plot indicated that there is a significant relation can be predicted in relative growth rate of root and relative growth rate of whole plant (r^2 = 0.51, n = 24, P> 0.05) supplied with HA under salinity stress conditions.

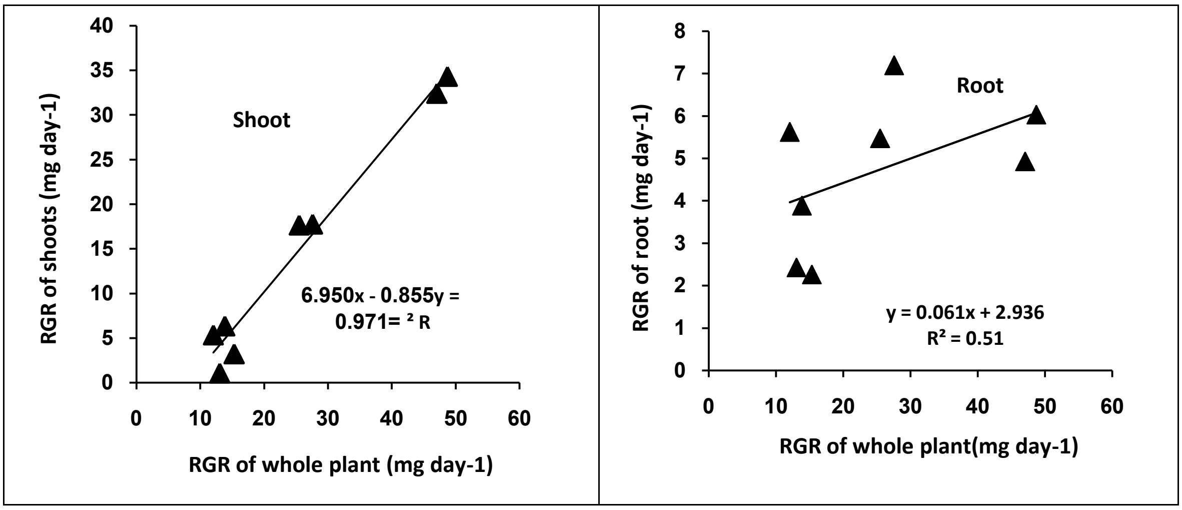

Fig 5: Relative growth rate for shootsand roots of common bean (RGR) versus relative growth rate of whole plant (RGR).

Under steady state conditions, the relative growth rate among plant parts should be equal [36].The higher relative growth rates of shoot, the faster total growth rate of the plant. Shoots growth rate for common bean was more rapid than roots growth performance as exposed by a biotic stress conditions.

VI. CONCLUSION

Results obtained in the present work confirmed that humic acid was able to produce some positive effects in common bean*(Phaseolus vulgaris, L.)*. In particular, significant improving fresh and dry biomass andchlorophyll contents as well as relative growth rate have been observed due to humic acid application under different salinity stress. Consequently, supply of humic acid markedly alleviated the negative effects of a biotic stresses on common bean plants and is considered as a promising soil amendmentto overcomeadverse effects ofsalinity stress.

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ISSN(Online) : 2319 - 8753
ISSN (Print) : 2347 - 6710

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Vol. 4, Issue 5, May 2015

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