Research Article

Effect of Plant Derived Humic Substances on the Yield of Chickpea Grown in Greenhouse

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Abstract | A pot experiment was conducted in experimental area of National Agricultural Research Centre, (NARC),Islamabad to see the effect of plant derived humic substances (PDHS) on chickpea crop grown in 5.50 kg soil container, consisting seven treatments, whereby HS was given either as soil applied (at the rate 0, 15,30,45,60 mg l^{-1}) and as foliar (100,150 mg l^{-1}) The experiment was laid out in CRD under greenhouse conditions. Three seeds of chickpea variety *Dhasht* was sown in each pot which was thinned to one plant. Results show that highest plant height of 45cm was obtained when HS applied at the rate of 30 mg l^{-1}as followed by 44.67cm where HS was applied @ 45mg l^{-1}. The highest number of pods/plant^{-1} (22) were obtained in HS applied at the rate of 30 mg l^{-1} followed by 22.00 at the rate of 45 mg l^{-1}. The maximum biomass wt. 14.24 g was received in 15 mg l^{-1}followed by 30 mg l^{-1} having weight of 14 g. The highest straw weight of 8.78 g was obtained in 30 mg l^{-1} treatment. The highest seed weight of 5.64g was received in 15 mg l^{-1}The highest pod length of 11.86 mm was obtained in 45 mg l^{-1}, while the highest pod diameter 17.57 mm was obtained in 45 mg l^{-1}. The highest pod weight of 8.27 g was received in 45mg l^{-1}.

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Introduction

Chick pea (*Cicer arietinum* L) crop is mostly grown on marginal lands in Pakistan characteristically of low fertility level and alkaline calcareous in reactions. The crop is grown over 936.2 thousand hectares, having total yield of 379.2 thousand tons and average yield/ hectare is 405 kg (ASP, 2014-2015). Soil organic matter contains approximately 60% humic substances (HSs), being a key components of the terrestrial ecosystem. These substances under take many chemical reactions of complex nature, in the soil (Trevisan et al., 2010). HS are naturally supramolecular structures. HS are heterogeneous molecules containing aliphatic chains, aromatic rings, polypeptides, fatty acids and sugar held together by different forces like p-p, ion-dipole, hydrogen bonds and vahn der Waals. Trevisan et al., 2010 and Piccolo, 2001. Humus is formed from fresh organic matter, agricultural by-products, animals, plants and coal through fermentation process carried out by soil micro biota under specific physical conditions like aeration, time, temperature and water (Canellas and Olivares, 2014). Humic acid and HS extracted

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from soil having different carbon sources with similar structures (Muscolo et al., 2013; García et al., 2016a). HS increase the lateral development and formation of root hair (Ramos et al., 2015).

HS play important rolein soil fertility due to their biological and physiochemical properties (Hirel et al., 2007; Brown et al., 2014). HS acts as metal complexion and ion exchange molecules (Beiraghi et al., 2014). HS enter to the plant through leaf, stem and root carrying the trace elements into the tissues being used as important ingredients of foliar fertilizers (García et al., 2016a). In the same way HS of low molecular weight readily enter to the plant and help in promoting nutrient uptake (Garcia-Mina et al., 2004). HS also act as hormone due to their properties and activities (Vaughan and Malcom, 1985; Nardi et al., 2000, 2002). HS stimulates plant growth by nutrients uptake and root and plant growth and yield are increased. (Zandonadi et al., 2016) HS form complexes with metallic ions, which facilitate in bioavailability of micronutrients like copper, zinc, iron;and macronutrients e.g. phosphorous especially when these nutrients are scarce in the soil (García et al., 2016b) Localized targeted and non-targeted effects of HS takes place at cell membranes which initiate molecular and biochemical processes at post-transcriptional levels in roots and shoot (Van Oosten et al., 2017).

Materials and Methods

A pot experiment was conducted at NARC Islamabad (33°43′17″ N; 73°02′35″E) to see the effect of PDHS on chickpea. (Khan et al., 2013). (M. Susic et al; 1991). There were seven treatments. Hs @ 0, 15,30,45,60 mg l^{-1}as soil application 100,150 mg l^{-1} as foliar application. Soil was collected from NARC, was ground and sieved through 2mm mesh, filled in plastic pots of 5.5 kg capacity. The soil was analyzed before plantation through ammonium bicarbonate-diethylene triamine penta acetic acid (AB-DTPA method developed by Soltanpour and Schuwab, 1977. Extractable P was measured colometrically through spectrophotometer while, K was determined by flame photometer. Micronutrients like (Zn), (Mn),(Cu) and (Fe) were analyzed by atomic absorption spectrophotometer (Perkin Elmer, 800; Perkin Elmer, Waltham, MA). Three seeds of chickpea were sown in each plot which was thinned later on to one plant in completely randomized design. Seeds were sown in Oct and harvested in April. Humic substances were extracted from sunflower waste materials. Data on plant height, number of pods per plant, biomass, straw weight, seeds weight, pods length, pods diameter and pods weight were collected as mean of triplicates. Statistical analysis of the data was done through statistics 8.1 Analysis of variance (ANOVA) was used to measure the variance among the treatments, while the least significant difference (LSD) was used to compare the difference among the treatments means.

Table 1.1: *Plant height, number of pods/ plant, weight of fresh biomass, weight of fresh straw.*

(HA mg/l)	Plant height (cm)	No Pods/ plant	fresh biomass weight (g/plant)	Fresh straw weight
T1	36.66^{d}	18^{b}	11.26^{b}	6.90^{b}
T2	41.33^{abc}	21^{ab}	14.24^{a}	8.60^{a}
T3	45.00^{a}	22^{a}	14.06^{a}	8.78^{a}
T4	44.66^{a}	22^{ab}	13.35^{ab}	7.910^{ab}
T5	43.33^{ab}	20^{ab}	12.54^{ab}	7.59^{ab}
T6	40.00^{bcd}	20^{ab}	12.34^{ab}	7.96^{ab}
T7	38.66^{cd}	19^{b}	12.31^{ab}	7.80^{ab}

T1: 0, T2: 15, T3: 30, T4: 45, T5: 60, T6: 100 foliar, T7: 150 mg l^{-1} foliar; Data are mean of (N: 3); Mean followed by the difference letters are significantly different from each other at p≤0.05.

Results and Discussion

Physicochemical properties of soil

The results of analysis is listed in Table 1, showing that the soil is sandy loam having low organic matter of 0.48%, slightly alkaline pH 7.98 and 0.43 EC ds m^{-1} having no problem of salinity. Phosphorus is deficient and K is marginal.

Table 1: *Physio-chemical properties of soil.*

Soil texture class	pH	EC	P	K	O.M	Zn	Cu	Fe	Mn
	(1:1)	dsm^{-1}	mg/kg		%	(AB-DTPA extract.) mg/kg			
Sandy loam	7.98	0.43	0.29	100	0.483	0.30	0.36	9.8	2.5

EC: Electrical Conductivity, O.M: Organic Matter, AB-DTPA: Ammonium Bicarbonate-Diethlyne Triamine Pentaacetic Acid.

Agronomic data

Plant height (cm): The results showed that highest plant height of 45cm obtained with HS applied @30 mg l^{-1} being 12.73 % increase over control followed by 44.67cm height which was 17.78% increase over control, where HS was applied @45.HS applied @

60 mg l^{-1} gave 43.33cm plant height and increase was 14.93%. The control treatment has value of 36.72 cm. Our results are at par with (Kahraman et al., 2017; Behnoush Rasaei et al., 2012; Ali et al., 2017) and (Bayrak, 2010)

Table 2: Plant Height, Number of pods/ plant, Weight of fresh Biomass, Weight of fresh Straw

(HA mg/l)	Plant height (cm)	NoPods/ plant	fresh biomass weight (g/plant)	Fresh straw weight
T1	36.66^{d}	18^{b}	11.26^{b}	6.90^{b}
T2	41.33^{abc}	21^{ab}	14.24^{a}	8.60^{a}
T3	45.00^{a}	22^{a}	14.06^{a}	8.78^{a}
T4	44.66^{a}	22^{ab}	13.35^{ab}	7.910^{ab}
T5	43.33^{ab}	20^{ab}	12.54^{ab}	7.59^{ab}
T6	40.00^{bcd}	20^{ab}	12.34^{ab}	7.96^{ab}
T7	38.66^{cd}	19^{b}	12.31^{ab}	7.80^{ab}

T1=0, T2=15, T3=30, T4=45, T5= 60, T6=100 foliar, T7= 150 mg l^{-1} foliar.Data are mean of (N=3). Mean followed by the difference letters are significantlydifferent from each other at p≤0.05.

Table 3: *Weight of fresh grains, pods length, pods diameter and pods weight.*

	Plant parameters			
HA rate (mg l^{-1})	Weight of fresh Grains	Pods Length (cm)	Pods Diameter (mm)	Pods Weight g
T1	4.363^{b}	10.30^{b}	10.66^{c}	5.49^{c}
T2	5.636^{a}	11.04^{ab}	12.03^{ab}	7.47^{ab}
T3	5.286^{a}	11.59^{a}	12.26^{ab}	8.06^{ab}
T4	5.440^{a}	11.78^{a}	12.53^{a}	8.27^{a}
T5	4.936^{ab}	11.60^{a}	12.21^{ab}	8.07^{ab}
T6	4.436^{b}	10.91^{ab}	11.28^{bc}	6.40^{bc}
T7	4.376^{b}	10.50^{b}	10.813^{c}	5.49^{bc}

T1: 0, T2: 15, T3: 30, T4: 45, T5: 60, T6: 100 foliar, T7: 150 mg l^{-1} foliar; Data are mean of (N: 3); Mean followed by the difference letters are significantly different from each other at p≤0.05.

Number of pods per plant: The highest number of 22.33 pods were received in treatment where 15 mg l^{-1}HS were applied and 21 % increase over control followed by 22.0 pods using HS@ 45 mg $l^{-1}$and 18 % increase over control. The HS applied @ mg l^{-1}contained 21 pods/ plant and increase was 17% over control. The control has 18 pods per plant. The experimental results of (Topalak and Ceyhan, 2015) are similar to our results.

Biomass and straw weight (g/plant): The highest biomass of 14.24 g was received by 15 mg l^{-1}which was at par with 14.04 g in the treatment where 30 mg l^{-1}HS. The control value was 11.27 g. The maximum straw weight 8.78 g was obtained in the treatment where HS was used @ 30 mg l^{-1}followed by 8.60 g weight in the15 mg l^{-1}treatment. The control treatment weight was 6.90 g.

Seed weight and Pod weight(g/plant): The highest grains weight of 5.64 was received by the HS applied @ 15 mgl^{-1} followed by 5.44 through 45 mgl^{-1}application. The control has 4.36 g value, (Bayrak, 2010), (Ceyhan et al., 2012), (Ceran and Onder, 2015), (Topalak and Ceyhan, 2015; Behnoush et al., 2012) reported that application of humic acid increased the grain yield of chickpea.

The maximum pods weight 8.27 g was obtained in the treatment 45 mgl^{-1} followed by 8.07 g by the treatment where HS were applied @60 mg l^{-1}. The control has value of 5.49 g.

The data indicated that application of HS overall increased the plant height, number of pods/ plant, pods weight and biomass due to the HS acting as metal complexion and ion exchange molecules where more nutrients would have taken up taken by plants and ultimately growth and yield could be increased as shown by (Beiraghi et al., 2014). Similarly, with the application of HS lateral root development and formation of root hair would have been increased as results are coinciding with (Ramos et al., 2015). Humic substances have biological effects on plants against the antioxidant, salt and drought stresses, being safe, plants grow well and their vigor and strength is increased (Canellas and Olivares, 2014). Moreover, HS entry to the plant through leaf, stem and root, carrying the trace elements into the tissues, so HS are considered as important ingredients of foliar fertilizers (García et al., 2016a). Humic substances affect plant metabolic processes, Nardi et al., 2002 and Nardi et al., 2000 influence respiration, Nardi et al., 2007, helps in protein synthesis Carletti P. et al., 2008. play role in the chlorophyll content and electrons transport of photosynthesis process, Thomas S.M. et al., 1978.

There are different systems in the plants working in a way that they are interlinked with each other and send messages among them for the growth and yield like the extant results indicate that HS trigger effect-oriented action via involvement of different but integrated mechanisms, which function as sequential events of complex networks at transcriptional and post-transcriptional levels (Garcia-Mina et al., 2004; Mora et al., 2014)

Pods length and diameter (mm): The highest pods length being 11.79 cm was received in the treatment 45 mg $l^{-1}$and the nearest value was 11.60 received in the treatment 60 mg l^{-1}. The control has value of 10.30 cm.. Plant with maximum diameter (12.53 mm)was noted with application of 45 mgl^{-1}HS followed by 12.26 mm with application of HS at the rate of 30 mg l^{-1}whereas in the control treatment diameter of 10.66 mm was recorded.

Author's Contribution

Ahmad Khan: Conducting experiment, write up, formatting and data analysis.
Raza Ullah Khan: Editing and fine tuning of article and suggestion for improvement.
Sadia Khan Bahawood: Data recording and laboratory analytical work.
Muhammad Zameer Khan: Editing and fine tuning of data and statistical analysis.
Fayyaz Hussain: Supervised and guided in data compilation and finalizing manuscript.

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