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Full Length Research Paper

Conjunctive use of humic acid, bio fertilizer and phosphorus augumented nutrients contents in chickpea under green house conditions.

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A pot experiment was conducted at green house (LRRI) NARC, Islamabad on loamy soil during kharif 2012 to investigate the interactive effect of humic acid (HA), Phosphorus solubilizing Bacteria (PSB) as a biofertilizer and Phosphorus (P) on P use efficiency in chickpeas. The treatments were: HA_0P_0, HA_0IP_0, $HA_0P_{75\%}$, $HA_0IP_{75\%}$, $HA_0P_{100\%}$, $HA_0IP_{100\%}$, $HA_{50mg\ kg\ -1}P_0$, $HA_{50mg\ kg\ -1}\ IP_0$,, $HA_{50mg\ kg\ -1}\ P_{75\%,}$ $HA_{50mg\ kg\ -1}\ IP_{75\%}$, $HA_{50mg\ kg\ -1}\ P_{100\%}$, $HA_{50mg\ kg\ -1}\ IP_{100\%}$ .The results of the study showed that nutrients (N, P and K) contents were significantly increased by the application of humic acid and PSB inoculation. The maximum contents of N (4.5%), P (.36%) and K (2.15%) in chickpea significantly increased with soil application of humic acid at 50 mg Kg^{-1} along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation. The nutrients availability in soil was also improved in all treatments. However, the results were statistically at par with soil application of humic acid at 50 mg Kg^{-1} along with 37.5 mg Kg^{-1} P_2O_5 (75% P) and PSB inoculation. It is concluded that combined effect of HA; P and PSB inoculation can improve nutrients use efficiency and reduce phosphorus fertilizer cost by 25% for chickpea production.

Key words: Humic acid, bio fertilizer and nutrients content

INTRODUCTION

The world population is increasing day by day (Lal, 2000), hence there is need for plenty of food crops to meet the requirement of growing population. Crops need several nutrients to reach their maximum potential yield. P is the most important nutrient required by the plants for growth and development. It is the second major essential macronutrient and plays an important role in metabolism of crop plants (Vikram and Hamzehzarghani, 2008). Most of the soils contain the substantial reserves of total P; large part of it relatively remains inert and only less than 10% of soil P enters the plant-animal cycle (Kucey et al., 1989). When P is added as fertilizer to the soil, it gets fixed. The soil microorganisms solubilise this P and make it available to the plants (Pal, 1998; Hilda and Fraga, 1999). P-solublising bacteria are relevant in this context and have the potential to be used as biofertilizer for the crops.The use of P-solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield subsequently. Strains from the genera Pseudomonas, Bacillus and Rhizobium are among the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999). Humic Acid (HA) is the active constituent of organic humus, which can play a very important role in soil conditioning and plant growth (Bendetti et al., 1996). Physically, it promotes good soil structure and increases the water holding capacity of the soil; biologically it enhances the growth of useful soil organisms, while chemically it serves as an adsorption and retention complex for inorganic plant nutrients (Brannon and Sommers, 1985). Humic acid is a naturally occurring polymeric organic compound (Schnitzer and Khan, 1972; Sposito, 1989). It is produced through decay/oxidation of organic matter through microbial action and is naturally found in soil, peat, rivers, oceans and in lignitic coals (Lawson and Stewart, 1989). It can convert elements into forms suitable for assimilation by plant due to its ability

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Table 1. Physio-chemical analysis of soil.

Soil characters	Unit	Values
pH	-	7.51
EC (1:1)	(dS m^{-1})	0.7
Organic Matter	(%)	0.78
NO3-N	(mg kg^{-1})	3.7
K	(mg kg^{-1})	88
Available P	(mg kg^{-1})	1.4
Fe	(mg kg^{-1})	4.0
Textural Class		Loam

Table 2. Effect of Humic Acid, Phosphorus and PSB application on plant P content (%).

Phosphorus	Humic Acid (0)		Humic Acid 50mg Kg^{-1}		Means
	without	Inoculation	Without	Inoculation	
0 %	0.14 h	0.16 g	0.22 e	0.25d	0.192 C
75 %	0.18 fg	0.19 ef	0.34 ab	0.36 a	0.268 B
100 %	0.3 c	0.34 ab	0.35 a	0.36 a	0.343 A
Means	0.207 C	0.23 B	0.305 A	0.325 A	

into forms suitable for assimilation by plant due to its ability to form complexes (Vaughan and Donald, 1976) and can break Fe or Al bond P in acidic soil and that with Ca in alkaline soil and release this P in soil solution and thus improves its availability for plant growth **(**Malcolm and Vaughan, 1979; Hajra and Debnath, 1987). Humic Acids have been complexed with macro and micro nutrients to overcome a particular nutrient deficiency in soil (Yingei, 1988). The physico-chemical activity, the structure and the mechanism of the stimulating effect of HA on various crops and soil conditions have been envisaged by various researchers (Malik et al., 1979). It contains 51-57% organic C, 4-6% N and 0.2 to 1% P and has potential to improve crops yield due to its capability of supplying N and P to the plants together with the improvement in the physicochemical and biological environment of the soils (Brannon and Sommers, 1985). The utilization of humic acid can be made effectively to boost up agricultural production from 25 to 40% on calcareous soil (Sharif et al., 2002).To take advantage of the facts that humic acid and PSB have substantial promise for agriculture utilization, therefore, we investigated the effect of combined usage of humic acid, P and PSB on nutrients uptake in chickpeas.

MATERIALS AND METHODS

A green house experiment was conducted utilizing loamy soil at 10 kg per pot at (LRRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The pots were assigned according to their respective treatments and arranged in completely randomized design (CRD) under three factors (HA, P levels and PSB inoculation). Humic substances have characteristics of pH 7.83, EC 0.94 and organic matter (OM) 68%; N, P and K were 3.40, 0.15 and 3.42% respectively. Basal dose of N and K was uniformly applied to all pots.

Factor 1: Humic acid levels
HA1: Control (No humic acid)
HA2: Soil application of HA 50 mg Kg^{-1}
Factor 2: PSB inoculation
Without PSB inoculation
PSB inoculation: I
Factor 3: mineral P Fertilizer
P1: Control
P2: 75% Recommended Dose of P (37.5 mg Kg^{-1} P_2O_5)
P3: 100% Recommended Dose of P (50.0 mg Kg^{-1} P_2O_5)

Treatments

HA_0P_0, HA_0IP_0, $HA_0P_{75\%}$, $HA_0IP_{75\%}$, $HA_0P_{100\%}$, $HA_0IP_{100\%}$, $HA_{50mg\ kg\ -1}P_0$, $HA_{50mg\ kg\ -1}\ IP_0$,$HA_{50mg\ kg\ -1}\ P_{75\%}$, $HA_{50mg\ kg\ -1}\ IP_{75\%}$, $HA_{50mg\ kg\ -1}\ P_{100\%}$, $HA_{50mg\ kg\ -1}\ IP_{100\%}$.

Soil and plant laboratory analysis

The composite soil samples were collected before experiment and were air dried and sieved through a 2-mm mesh screen. Standard analytical methods were follow-

Table 3. Effect of Humic Acid, Phosphorus and PSB application on Plant K (%).

Phosphorus	Humic Acid (0)		Humic Acid 50mg Kg^{-1}		Means
	without	Inoculation	Without	Inoculation	
0 %	1.38 f	1.53 ef	1.58 e	1.62 e	1.52 C
75 %	1.61 e	1.57 e	1.79 cd	1.89 bc	1.71 B
100 %	1.65 de	1.6 e	2.03 ab	2.15 a	1.86 A
Means	1.55 B	1.56 B	1.79 A	1.88 A	

Table 4. Effect of Humic Acid, Phosphorus and PSB application on plant N (%).

Phosphorus	Humic Acid (0)		Humic Acid 50mg Kg^{-1}		Means
	without	Inoculation	Without	Inoculation	
0 %	1.75 j	1.95 i	2.15 i	3.85 f	2.43 C
75 %	3.60 g	3.85 f	3.90 ef	4.25 bc	3.90 B
100 %	4.05 de	4.15 cd	4.35 ab	4.5 a	4.26 A
Means	3.13 D	3.32 C	3.46 B	4.26 A	

ed in analyzing the soil samples and chickpea plant (whole root and shoot) for the content of N, P and K contents at maturity. Determination of soil particle size distribution was carried out using hydrometer method. Soil pH was measured using digital pH meter in 1: 1 soil to water ratio. Soil ECe was measured in extract by using EC meter in1:1 soil to water ratio. Total nitrogen in chickpea was determined by the Kjeldahl procedure as described by Jackson (1958). P was determined by wet digestion as described by Benton et al., 1991.The data thus obtained were subjected for statistical analysis using MSTATC package. The basic physical and chemical analysis of soil before sowing chickpea is presented in Table 1.

RESULT AND DISCUSSION

Phosphorus content in chickpeas (%):

Phosphorus content in chickpea plant significantly increased with combined application of HA and PSB inoculation. Results from the Table 2 confirm that the P content in chickpeas increased with the application of HA and PSB inoculation. The highest concentration of P in chickpea (.36%) was observed in the treatment where HA was applied at 50 mg Kg^{-1} along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation and it was 157% higher than control. However it was similar to the treatment where P was applied 75% of recommended rate along with H.A and PSB application. The increase in P uptake might be due to the prevention of P fixation in the soil and the formation of humophospho complexes, which are easily assimilable by the plants (Raina and Goswami, 1988). Addition of humic acids and PSB to soil with P fertilizer significantly increases the amount of water-soluble phosphate and strongly retard the formation of occluded phosphate, and increased P uptake by plants Wang et al., (1995).

Potassium content in chickpeas (%):

Potassium in chick pea’s plant positively affected by HA and PSB. Data from the Table 3 reveal that the K content in chickpeas plant was increased with HA application and PSB inoculation. The highest K concentration of chickpeas plant (2.15%) was observed with the application of (HA) at 50mg kg^{-1} along with P 50mg kg^{-1} P_2O_5 (100%P) and PSB inoculation, which was 56% more than control. However statistically it is at par with treatment where H.A and P (100%) was applied without inoculation. So H.A has profound effect on K uptake, similar findings have been reported Samson and Visser (1989) that humic acid increased in permeability of biomembranes for electrolytes accounted for increased uptake of K

Nitrogen content in chickpeas (%):

Result in Table 4 reveal chickpeas N content were increased with the application of HA, P and PSB inoculation. The highest value of N in chickpea (4.5%) was observed in the

Table 5. Effect of Humic Acid, Phosphorus and PSB application on soil P (ppm).

Phosphorus	Humic Acid (0)		Humic Acid 50mg Kg^{-1}		Means
	without	**Inoculation**	**Without**	**Inoculation**	
0 %	1.28 d	1.67ab	2.78 cd	4.35 cd	2.51 B
75 %	4.27bc	4.72abc	6.23 ab	6.29 a	5.38 A
100 %	5.22ab	5.22 ab	6.35a	6.40 a	5.51 A
Means	3.588 B	3.488 B	5.115 A	5.678 A	

Table 6. Effect of Humic Acid, Phosphorus and PSB application on soil K (ppm).

Phosphorus	Humic Acid (0)		Humic Acid 50mg Kg^{-1}		Means
	without	**Inoculation**	**Without**	**Inoculation**	
0 %	81.50 f	94.00 e	83.00 f	82.50 f	85.38 C
75 %	98.00 e	103.5 d	107 cd	114 ab	105.63 B
100 %	110.5 bc	113.5 ab	116 a	116 a	113.75 A
Means	96.67 B	103.67 A	102.17 A	103.83 A	

treatment where HA was applied 50 mg Kg^{-1} along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation and it was 157% higher than control, whereas it was 5% more than the treatment where P was applied 75% of recommended rate along with H.A and PSB application (both are statistically at par). The increased N uptake was supposed to be due to the better use efficiency of applied N fertilizers in the presence of humic acid coupled with retarded nitrification process enabling the slow availability of applied N (Guminiski, 1968).

Nutrient status in the soil after harvesting

Available phosphorus (mg kg^{-1}) in soil

The residual effect of combined application of HA, PSB and phosphorus after harvesting was associated with the bioavailability of nutrients. Phosphorus content in the soil were positively affected with HA application. Results presented in the Table 5 indicate that the P content from soil were increased with HA and P application. The highest concentration of soil P (6.4 mg kg^{-1}) was recorded with the application of HA 50 mg Kg^{-1} along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation which was 22% higher than 100% P application alone. It shows that humic acid application significantly reduce the phosphorus fixation and increase its availability through chelation effect. This is in consonance with the findings of David et al., (1994), who reported slow and continuous dissolution of phosphate minerals in soil by the humic acid, which determinate the increased of P availability. The phosphatase activity from the soil is improved of the humic acids, and this fact increase the P availability, because the insoluble phosphates are converts into soluble forms (Vaughan, 1976).

Available potassium (mg kg^{-1}) in soil

Similar to N and P, Potassium content in the soil were also positively affected with HA, PSB and phosphorus treatment. Results presented in the Table 6 indicated that the K content in the soil was enhanced by increasing HA. The highest concentration of soil K (116 mg kg^{-1}) was noted in the treatment where HA 50 mg Kg^{-1}, along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation, was 42% more than control. The results are in agreement with the finding of Tan (1978) and K. Mesut (2010) who reported that humic acid helps in releasing the fixed K.

CONCLUSION

The combined effect of HA 50 mg Kg^{-1} along with 50 mg Kg^{-1} P_2O_5 (100% P) and PSB inoculation has registered the maximum P ad K availability in soil and their uptake by chickpea and it was at par with combined application of HA 50 mg Kg^{-1} along with 37.5 mg Kg^{-1} P_2O_5 (75% P) and PSB inoculation. It may be concluded that humic acid couple with PSB inoculation can improve nutrients use efficiency and therefore reduce DAP cost of phosphate fertilizer up to efficiency and therefore reduce DAP cost of phosphate fertilizer up to 25% in chickpea production.

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