

INTERACTIVE EFFECT OF HUMIC ACID AND NPK APPLICATION ON THE LEAF YIELD AND CONCENTRATION OF NUTRIENT ELEMENTS IN FLUE CURED VIRGINIA TOBACCO

Hamid Gul^{*}, Manthar Ali^{*}, Seema Shah^{**}, Syed Asif Shah^{*} and Hafiz Munir Ahmed^{***}

^{**} Pakistan Tobacco Board Mardan, Pakistan

^{*} Pakistan Tobacco Board Mansehra, Pakistan

^{***} Nuclear Institute for Food & Agriculture (NIFA), Peshawar, Pakistan

ABSTRACT: An experiment was conducted to investigate the effect of humic acid (HA) applied at 0, 0.5 and 1.0 kg ha⁻¹ with standard dose of NPK fertilizer (40-60-80 kg ha⁻¹) on the cured leaves yield (kg ha⁻¹) and concentrations of macro nutrients Nitrogen (N), Phosphorus (P) and Potassium (K) in Flue Cured Virginia (FCV) Tobacco variety Speight G-28 at the Tobacco Research Station Khan Garhi Mardan, Khyber Pakhtunkhwa (Pakistan), during 2013. Results revealed that humic acid (HA) at rate of 1 kg ha⁻¹ along with NPK fertilizer significantly ($P < 0.05$) affected the cured yield by producing 3115 kg ha⁻¹ compared to control treatment where no HA and NPK fertilizer were applied (1375 kg ha⁻¹) and NPK alone (2710 kg ha⁻¹). HA alone or in combination with NPK did not exhibit significant impact on concentration of N, P and K up taken by plants however, HA applied @ 1 kg ha⁻¹ with the standard dose of NPK had increased the concentration of the macro nutrients N, P and K, numerically. Therefore, humic acid application along with NPK, may be useful in enhancing the leaf yield through enhancing the availability of NPK fertilizer to plant system.

Key words: Concentration, Flue Cured Virginia Tobacco, humic acid (HA), macro-nutrients, NPK (nitrogen-phosphorus-potassium), leaf yield.

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a member of the nightshade family, *Solanaceae*. There are more than 76 species in the *Nicotiana* genus, located in America (largely in Andean South America and Mexico), Australia, South Pacific Islands, and one in Namibia [14] but only two species of *Nicotiana* (*Nicotiana tabacum* L. and *Nicotiana rustica* L.) are widely grown commercially all over the world.

Tobacco is one of the important cash crops of Pakistan contributing annually over Rs.89 billion in the national exchequer. It is grown on around 0.25% of the total irrigated land of Pakistan and 60% of the tobacco growers are located in the province of Khyber Pakhtunkhwa, producing 95% of Flue Cured Virginia over an area of 30,000 hectares in the districts of Swabi, Mardan, Charsadda, Buner and Mansehra.. The yield of FCV tobacco in Pakistan is about 3290 kg ha⁻¹ [21] and it happens to be the only crop in Pakistan whose yield per unit area can be compared to developed countries including USA [27].

Yield increase is due to adoption of a number of improved cultural practices, i.e. the use of complex fertilizer, optimum plant population, weeds management, use of standard pesticides, topping/de-suckering and use of hygrometer during curing [12].

Due to increased cost of production, the quality along with the quantity of the tobacco production is most affected. The supplementation of chemical fertilizers with cheaper lignitic coal derived humic acid could reduce cost of production without compromising on yield. Humic acid (HA) is a natural product, which is present in Pakistan's lignitic coal in reasonable concentrations and is used in agriculture and industry but on limited scale [10]. Humic substances are formed through the process of humification of organic materials as by-product of microbial metabolism and are found in soil, coal, sediments water, peat, and organic matter [32]. Pakistan is rich in coal and study have shown that Pakistan low rank weathered coal have sizeable amount of

humic acid which can be used effectively as organic fertilizer to boost up agriculture production.

Humic acid (HA) forms complexes with K, Ca, Na, Mn, Zn, Fe, Cu and various other elements to over-come their deficiency in soil [1]. Schnitzer and Rauthan [24], White and Chaney [35] reported that with the addition of HA, plant growth and uptake of N, P, K, Ca⁺², Mg⁺², Fe⁺³, Zn⁺³ and Cu were increased. Yingei [37] reported that HA forms chelates and can regulate the supply of micronutrients needed for plant growth and development. It exerts a stimulatory soil conditioning and growth promoting effect when applied in combination with chemical fertilizer due to its chelation properties that holds the nutrients by ions and releases them as and when required by plants [15]. Humic acid has a great potential to stimulate microbial activities and have significant and practical applications regarding transport and availability of micronutrients and assimilation of N by plants [13]. It is also reported that small amounts of HA increased the uptake and translocation of Ca⁺², Mg⁺² and Fe⁺³ [18]. The integrated use of organic and chemical fertilizers not only increased each other's efficiency, but also helped in the substitution of chemical fertilizers [11]. The use of lignitic coal derived HA @ 0.5 kg ha⁻¹ alone and in combination with half and full doses of NPK increased wheat grain and total dry matter over the control [26]. Humic acid addition to a soil increases the rate of absorption of mineral ions on root surfaces and their penetration into the cell of plant tissue where the plants show more active metabolism and increased respiratory activity and all of these activities are attributed to the intervention of the quinone groups of humic acid [22]. It helps to restore the soil fertility by promoting certain physico-chemical and biological activities which increases much needed soil humus and if added to acidic soil and alkaline soil, it generally reduces P fixation and solubilizes P in soil to make it available for plant growth [3]. The effects of humic acid on plant growth are long-lived compared to any other source [30]. Sarir [23] advocated that application of humic acid at

the rate of 200 g ha⁻¹ enhanced availability of plant nutrients and improved yield and yield components of maize crop. Olk and Classman [20] found that the addition of HA to the vermiculite soil reduced K fixation and resulted in greater total extractable K and highly labile K as well as greater plant uptake. Sharif [25] reported increases in concentration and accumulation of micronutrients in the wheat crop. Emonue [7] investigated that HA plays an important role in the solubility, mobility and accumulation of trace elements in marine environment. Benedetti *et al.* [2] carried out experiments to estimate the agronomic value of three plant species grown in pots on two soils of different fertility and reported that NPK with 3% of HA caused significant increase in yield compared to the control. Xue *et al.* [36] reported increased yield, uptake and translocation of nutrients. Keeping in view these factors, the said study was designed to investigate the effect of humic acid alone at two rates and in combination with standard NPK fertilizer on leaf yield and concentration of N, P and K macro nutrients in a FCV tobacco variety Speight G-28.

MATERIALS AND METHODS

The effects of humic acid and various levels with a fixed dose of NPK fertilizer on the yield, and uptake macro-nutrients N, P and K (concentration) in Flue Cured Virginia (FCV) tobacco variety Speight G-28 was studied at the Tobacco Research Station, Mardan Khyber Pakhtunkhwa (Pakistan), during 2013. The experiment was conducted in Randomized Complete Block Design (RCBD) in factorial arrangement replicated thrice. The treatments were:

T1: Control (No HA and NPK fertilizer)

T2: 0.5 kg ha⁻¹ HA alone

T3: 1.0 kg ha⁻¹ HA alone

T4: Standard dose (40-60-80 kg ha⁻¹) NPK fertilizer alone

T5: Standard dose of NPK + 0.5 kg ha⁻¹ HA

T6: Standard dose of NPK + 1.0 kg ha⁻¹ HA

Tobacco nursery for the experiment was sown on raised seed beds in the month of December 2012. Land was thoroughly prepared by deep ploughing and ridges were made at a distance of 90cm. Healthy seedlings were transplanted on the ridges with plant to plant distance of 60cm. All the recommended cultural practices and plant protection measures were adopted.

Mature leaves of 10 plants from each treatment were harvested in five subsequent pickings, stitched, labeled and cured in flue curing barns. After curing, the cured leaves were weighed and cured yield in kg ha⁻¹ was worked out. Cured leaf samples were collected from each picking and made composite sample and subjected to further chemical analysis.

Analysis of plant samples

After collection, the plant samples were washed with distilled water, air-dried and then put in oven at 70°C for 72 hours. Samples were digested with nitric acid (HNO₃) and perchloric acid (HClO₄) following procedure of Walsh and Beaton [33]. The concentration of P and K were determined. Phosphorus was analyzed in the plant digest using spectrophotometer and K with flame photometer.

Total Nitrogen

Total N in plants was determined by Kjeldhal method of Bremner [4]. Plant samples (0.2g) were digested with 3 ml of conc. H₂SO₄ in the presence of 1:1g digestion mixture containing K₂SO₄: CuSO₄ and Se in 100:10:1 ratio. After cooling, the digest was distilled with 20 ml of 40% NaOH into 5 ml boric acid mixed indicator solution. The distillate was titrated against 0.005 M HCl. Total percent Nitrogen was estimated as below;

$$\text{Total N (\%)} = \frac{(\text{Reading for sample} - \text{reading of blank}) \times \text{N of HCl} \times \text{meq. N} \times 100 \times 100}{\text{Weight of plant samples (0.2)} \times 20}$$

Where: N of HCl Normality of HCl (0.005)

Meq N: milli equivalent of N (0.014)

Statistical analysis

The data were statistically analyzed following Steel and Torrie [31] approach for Randomized Complete Block. The mean values were plotted in the bar chart to study the effect of HA in combination with standard dose of NPK on the cured leaf yield and concentration of macro nutrients (N, P and K).

RESULTS AND DISCUSSION

Significant differences were illustrated in respect of cured leaf yield, while non significant impact of HA treatments with standard dose of NPK for Nitrogen (N), Potassium (P) and Phosphorous (P) in Flue Cured Virginia (FCV) variety Speight G-28 were recorded. However, numerically increased impact of the HA and NPK was witnessed for the later parameters that would have directly improved the ultimate goals viz. high leaf yield with better quality (if rich in potash). The detail of the results is discussed as under:

The effect of HA and NPK on the cured leaf yield (kg ha⁻¹) of Speight G-28:

The effects of both humic acid (HA) and NPK fertilizer were significant (P < 0.05) Table-1. The results showed higher cured leaf yield was achieved (2244.3 kg ha⁻¹) @ 0.5 kg ha⁻¹ and it improved even further (2479 kg ha⁻¹) @ 1.0 kg ha⁻¹ HA application compared to control (1375 kg ha⁻¹). NPK application alone yielded 2710 kg ha⁻¹ while the interacted application of a standard dose of NPK with HA @ 0.5 and 1 kg ha⁻¹ produced 2972.3 and 3115 kg ha⁻¹ cured leaf yield, respectively higher than the control, HA and NPK applications alone (Table-1 & Fig-1). Therefore, it is deduced the application of HA in combination with NPK will improve the uptake of fertilizer. These results endorse the findings of [26, 23 and 37]. Humic acid is a bioactive organic biological slow-release fertilizer and together with the chemical fertilizers, forms an organic-inorganic complex fertilizer which holds the humic acid as the core. This can effectively improve the supply of nutrition [34].

The effect of HA and NPK on the Nitrogen concentration (%) of Speight G-28:

The interacted effect of the humic acid with NPK fertilizer on the accumulation of the nitrogen content in the leaves was witnessed non-significant (P > 0.05) yet the impact of the NPK efficiency showed up ward trend for nitrogen concentration from 1.36 to 1.61% with the increase of the HA application

from 0.5 to 1.0 kg ha⁻¹ (Fig-2 & Table-2). The N concentration recovered from the control was 1.30 % and it increased from 1.34 to 1.37% when HA was added @ 0.5 and 1 kg ha⁻¹, respectively, while it was estimated at 1.36% @ standard dose of NPK alone, less than HA treatment alone @ 1.0 kg ha⁻¹. These results are supported by findings of Siavash & Esimi [29]. This numerical increase in the N concentration in tobacco leaves may increase leaf yield. The concentration of N in the plant will increase the height that can be attributed to the affect of nitrogen on vegetative growth, cell division in plant organs, especially stems and provides suitable conditions for stem elongation [5].

The effect of HA and NPK on the Phosphorus concentration (%) of Speight G-28:

The results showed that the effect of HA with NPK fertilizer and interaction effects of HA x NPK were non-significant (P>0.05) Table-1. The availability of P in control treatment, at te standard dose of NPK alone and all HA application treatments, remained stagnant at 0.22 %, while the availability of P was increased in the interacted treatments of NPK and HA from 0.22% to 0.23% Table-2 & Fig-3. This also revealed that P already in the soil is rarely available to plant system. However, the concentration of phosphorous increased with the application of HA and NPK fertilizer thereby unveiling the fact that humic acid application has the role in the uptake of the P fertilizer and reported to have significantly increased the concentration of P [26]. Phosphorus is generally less available to plant system and once it is taken up by the plant, then it has enormous role like energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement/mobilization within the plant

and plant development especially root system.

The effect of HA and NPK on the Potassium concentration (mgkg⁻¹) of Speight G-28:

The statistical analysis showed that the effect of HA, NPK fertilizer and their interaction effect were non-significant (P>0.05). Potash concentration in control treatment was 24.10 mg kg⁻¹; while it had risen to 25.10 mg kg⁻¹ when HA applied @ 1.0 kg ha⁻¹. The concentration of potash was estimated 25.49 mg kg⁻¹ when NPK applied, but the concentration rose to maximum 30.10 mg kg⁻¹ when applied in combination with HA @ 1.0 kg ha⁻¹ Table-2 & Fig-4. The results clearly depicted that the addition of HA to the soil reduced K fixation and resulted in greater total extractable K as well as greater plant K uptake [20]. The quality of tobacco very much dependent on potassium nutrition, the potassium is required for carbon and nitrogen metabolism and activates enzymes involved in carbohydrate metabolism and increases resistance of tobacco. Flue-cured tobacco with high potassium content has good burning quality, good aroma and is safer to smoke [28, 9, 8, 19, 16 and 6]. Potassium is one of the most mineral elements in flue-cured tobacco. The potassium content in the good-quality tobacco is over 2% [17] and its availability can be increased through the application of HA [38].

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded from this study that application of NPK fertilizer with humic acid increased the yield of FCV tobacco significantly, and application of 1.0 kgha⁻¹ of humic acid may be useful with full dose of NPK fertilizer (40-60-80 kg ha⁻¹) for higher yield of FCV tobacco with better nutrient profile.

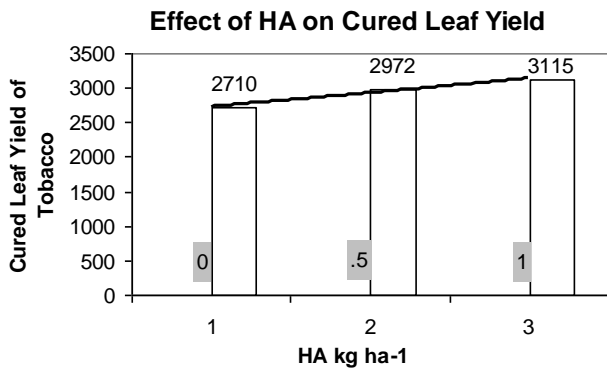


Figure-1

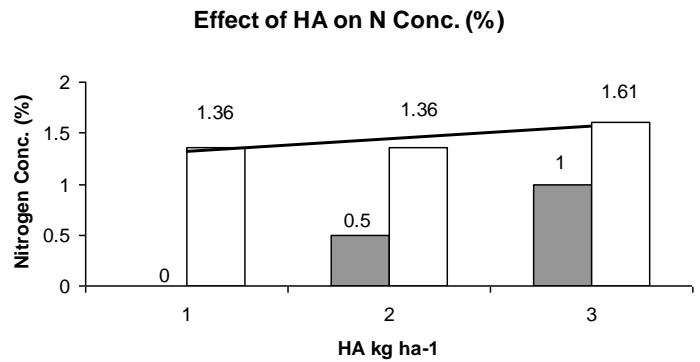


Figure-2

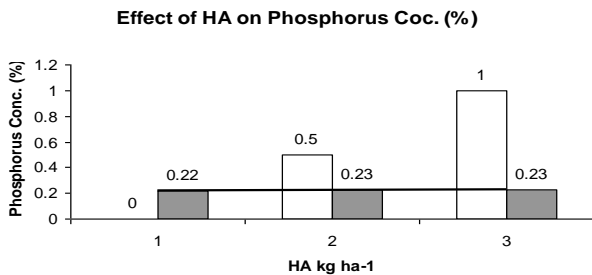


Figure-3

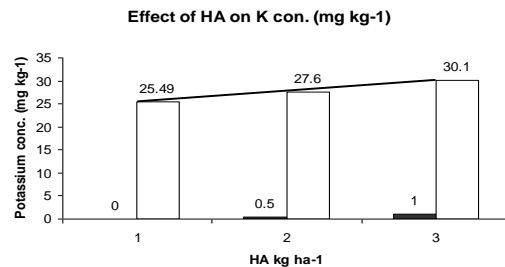


Figure-4

Table-1: Effect of HA and NPK levels on the cured yield (kg ha⁻¹) of tobacco variety Speight G-28.

Humic Acid kg ha ⁻¹	Control	Full Dose NPK Kg ha ⁻¹	Mean
0	1375.0	2710.0	1981.7 c
0.5	2244.3	2972.3	2600.7 b
1.0	2479.0	3115.0	2804.0 a
Mean	2032.8 b	2932.4 a	-----

Table-2: Effect of HA and NPK levels on concentrations of nutrient elements in leaves of tobacco variety Speight G-28

Nutrient elements	HA kg ha ⁻¹	Control	Full Dose of NPK kg ha ⁻¹	Mean
Nitrogen (N %)	0	1.30	1.36	1.34 ns
	0.5	1.34	1.36	1.35 ns
	1.0	1.37	1.61	1.45 ns
Mean		1.33 ns	1.44 ns	-
Phosphorus (P %)	0	0.22	0.22	0.22 ns
	0.5	0.22	0.23	0.23ns
	1.0	0.22	0.23	0.23 ns
Mean		0.22 ns	0.23 ns	-
Potassium (K mgkg ⁻¹)	0	24.10	25.49	25.03 ns
	0.5	23.40	27.60	25.57 ns
	1.0	25.10	30.10	28.03 ns
Mean		24.20 ns	27.73 ns	-

LITERATURE CITED

- [1] Barron P. F. and Wilson M. A., "Humic acid coal structure study with the magic angle spinning", *Nature*, **13**: 289-293, (1981).
- [2] Benedetti, A., Figliolia A., Izza C. and Indiatì R., "Fertilization with NPK and humate NPK. Plant yield and nutrient dynamics", *Suolo-Planta, Italy*, **2**: 203-214, (1992).
- [3] Brams E., "Effect of humic acid on the phosphorus fixation by soil", *Plant Sci.*, **39**:465, (1973).
- [4] Bremmer J.M., Nitrogen total. *In: D. L. Sparks. (Ed.) Methods in Soil Science*, (1996).
- [5] Castelli F., Miceli F. and Piro F., Effect of of harvesting and curing method on tobaccoburley at different nitrogen fertilizer and plant densities. *Agronomia*, **24**: 308-316, (1990).
- [6] Duan B.B., Li Y. F., Rong H., Ma D. P. & Ning, "Effects of chloride ions content on combustibility of reconstituted tobacco", *Modern Food Science and Technology*, **29**: 52-755, (2013).
- [7] Emonuele M., "Determination of trace metals complexed with HA in Antarctica marine sediments", *CSB*, **9**: 67 – 70, (1997).
- [8] Gao C. L., Zhou J. H., Qiu Y., Li Q., Zhang Z. and Huang T., "Effect of nitrogen and potassium stress on physiological properties and nicotine of flue-cured tobacco after topping", *Crop Research*, **27**: 449-452, (2013).
- [9] Guo Z. K., Yang Q., Yao Q. H., Wan X. Q., and Yan P. Q., "Study on HAL1 modified tobacco with high leaf potassium content", *Acta Tabacaria Sinica*, **12**: 46-51, (2006).
- [10] Hai S.M. and Mir S., "The lignitic coal derived HA and the prospective utilization in Pakistan's agriculture and industry", *Sci. Tech. & Dev.*, **17**: 32-40, (1998).
- [11] Hussain T., Jilani G. and Iqbal M. Z., "Integrated use of organic and inorganic fertilizer in rice-wheat cropping system", *Pak. J. Soil. Sci.*, **3**: 19-23, (1988).
- [12] Imtiaz A., Muhammad A., Raza H., Rehman A., Khaliq I., Ihtisham M., Iqbal A. and Anees M., "Performance of Flue Cured Virginia Tobacco", *International Journal of Basic & Applied Sciences*, **14**:1-3, (2014).
- [13] Jilani G., Hussain T. and Ammed R., "Substitution of organic manure with humic substances to enhance microbial activity", *Abstracts of 5th National Congress on Soil Sci., Agric. Uni. Peshawar, Pakistan*, (1994).
- [14] Knapp S., Clarkson J.J. and Chase M.W., "Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae)", *Taxon*, **53**: 73-82, (2004).
- [15] Linchen D. J., "Humic acid-Fe uptake by plants", *Joplin and Soil*, **50**:663-670, (1978).
- [16] Liu W. X., Yan H. H., Zhou Y., Lin L. T. and Lin Z. L., "Research progress of potassium nutrition of tobacco improving k content in flue-cured tobacco leaves", *Crop Research*, **21**:736-740, (2007).
- [17] Marchand M., Etourneau F., and Bourrrie B., Influence of different varieties of potash fertilizer on tobacco production and chemical composition", *Chinese Tobacco Science*, **18**: 6-11, (1997).
- [18] Mylonas V. A. and McCants C. B., "Effect of Humic acid on growth of tobacco" *Plant and Soil*, **45**: 485-490, (1980).
- [19] Niu R. B. and Nie Z., "Study on the potassium content distribution regular pattern of different position within the flue-cured tobacco leaf", *Crop Research*, **23**:194-196,(2009).
- [20] Olk, D.C and Classman K.G., "Reduction of K fixation by two HA fractions in vermiculite soils", *J .Soil.Sci.Soc.*, **59**:1250-1258, (1995).

- [21] Pakistan Tobacco Board. Statistical bulletin, Government of Pakistan, (2013 & 2016).
- [22] Petronio P., Vitorovic D. and Jablanovic M., "Investigation of the biological effect of HA." *Acte Bio., Med.Exp.*, **7**:21-25, (1982). [23] Sarir M. S., "Utilization of natural resources for increased crop production", National seminar on sustainable management of natural resources in Pakistan, Univ. of Peshawar, (1998).
- [24] Schnitzer M. and Rauthan B. S., Effect of humus on the growth and nutrient content of the cucumber. *Plant and Soil* **63**: 491-495, (1981).
- [25] Sharif M., "Effect of lignitic coal derived HA on growth and yield of wheat and maize in alkaline soil", Ph.D. Dissertation, Deptt. of Soil and Environmental Sciences, Univ. Agric. Peshawar, (2002).
- [26] Sharif M. and Sarir M. S., Utilization of humic acid as manure for increase crop production", *Scientific Khyber*, **15**:113-125, (2002).
- [27] Sheraz A., Mohammad F., Ahmed Q. and Khan M. A., "Assessing Genetic Variation for Morpho-Agronomic Traits of Some Native and Exotic Fcv Tobacco Genotypes in Pakistan", *American-Eurasian J. Agric. & Environ. Sci.*, **14**: 428-433, (2014).
- [28] Shu H. Y., Yang T. Z., Cao G. Q., Ling H. & Tian B. M., "The relationship between tobacco economic characters, nicotine content and potassium content", *China Agricultural Science Bulletin*, **23**:275-278, (2007).
- [29] Siavash M. S. & Esimi M., "Determining of the relation between nitrogen fertilizer usage amount and Tobacco leaf chlorophyll content", *Rasht Tobacco Research Center*, pp. 2-4, (2002).
- [30] Sibanda H. M. and Young S. D., "The effect of humic acid and soil heating on the availability of phosphorus in oxide-rich trop. Soils", Special publication No.9 of British Ecological Society, Oxford, UK, (1986).
- [31] Steel, R.G.D and Torrie J.H., "Principles and procedures of statistics. A biometrical approach", McGraw. Hill, New York, (1980).
- [32] Stevenson F.J., "Humus Chemistry: Genesis, Composition, Reactions", (2nd ed.) Wiley and Sons Ltd. New York, p. 496, (1994).
- [33] Walsh L. M. and Beaton J. D., "Soil testing and plant analysis", *Soil Sci. Am. Inc.*, Madison. WI, (1977).
- [34] Wang Y. X. and Qin H. J., "Function of humic acid to control the release of nitrogen and potassium and to activate phosphorus", *Humic Acid*, **4**:27-32, (2009).
- [35] White M. C. and Chaney R. L., "Zn, Cd, and Mn uptake by soya bean from coastal plain soils", *Soil Sci. Soc. Am. J.*, **44**: 308-313, (1980).
- [36] Xue S.C., Liu D.C., Tong D.Y., Han J.M. and Li Y.R., "Studies on the effects and mechanism of HA compound fertilizer", *J. of Hebei. Agri. Univ. China*, **17**: 24-27, (1994).
- [37] Yingei W., "Humic acid resin treatment of Copper and Nickel", *Hanjing Bashu*, **7**: 21-22, (1988).
- [38] Yuting He, Wang C., Li B., Wang W., Li B., Xiang J. and Lei Bo., "Effect of humic tobacco", *Journal of Agricultural Science*, **6**: 8-13, (2014).