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Response of soil applied lignite coal derived humic acid on yield and quality of spinach (*Spinacia oleracea* L.)

Akhila Nand Dubey*, Priyankar Raha and Arnab Kundu

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Abstract

A pot experiment was carried out in the net house of the Department of Soil Science & Agricultural Chemistry, Banaras Hindu University, Varanasi, Uttar Pradesh during *rabi* season of 2017-2018, to study the effect of lignite coal derived humic acid (HA) on growth and development of spinach (*Spinacia oleracea* L.) in an Inceptisol of Varanasi, Uttar Pradesh with six doses of lignite coal originated humic acid [i.e. 0.0 (H_0), 0.5 (H_1), 0.75 (H_2), 1.5 (H_3), 2.5 (H_4) and 5.0 (H_5) mg HA kg^{-1} of soil] and four doses of N (urea) fertilizer [0.0 (N_0), 18.50 (N_1), 27.80 (N_2) and 37.0 (N_3) mg kg^{-1} soil] in different combination. The plant growth parameters (*viz.* no. of leaves, fresh weight & dry weight) and biochemical parameters (*viz.* brix value, chlorophyll, nitrogen and protein content) were taken at 30, 45 and 60 days after sowing. The highest values of yield and quality parameters was recorded in the treatment, where higher level (5 mg kg^{-1} soil) of humic acid was applied with full dose of nitrogen (37.0 mg kg^{-1} soil) through urea fertilizer. The application of humic acid with nitrogen fertilizers in soil was noticed to be promising technique in increasing yield and quality of spinach.

Keywords: Lignite coal, humic acid, nitrogen fertilizer, spinach, protein

Introduction

Lignite popularly known as brown coal is a lower rank premature stage coal and an important precursor of humic acid, which would have converted to black coal under favorable geological conditions in due course of time. It is brown in colour, soft, friable, contain high *insitu* – moisture and volatile matter. India is rich in lignite which has sizeable amounts of humic acid that can be extracted and utilized effectively as organic fertilizers to boost up agricultural production. Humic acid may be utilized as nutrients carrier, fertilizer and soil conditioner (Robertson and Morgan 1995). Humic acids are polyelectrolyte macromolecular compounds originating from chemical and biological degradation of plant and animal resides and microbial cells. Humic acids play an important role in the globally nitrogen cycle through their influence on the distribution, bioavailability and ultimate fate of sedimentary organic nitrogen. Lignite coal originated humic acid can increases crop yields effective with urea due to inhibit the urease activity resulted to low rate of urea hydrolysis (Dong et al 2008). Shafeek et al (2013) reported that foliar spraying of high rates of humic acid (4g/L) recorded the high values of growth characters, i.e. number of leaves and branches, fresh weight of whole plant and its different parts as well as total yield and its components in spinach crop.

Nitrogen is commonly considered as one of the most important and limiting primary essential nutrients for the plant growth and development. The chemical fertilizers are the dominant and main source of nitrogen in crop production systems worldwide. However, N- fertilizer use efficiency is very poor and recovery of nitrogen in the soil – plant system seldom exceeds 50% of applied nitrogen (Raun et al 2002). The very low use efficiency of nitrogen is associated with its losses by leaching, denitrification and volatilization loss. The foremost challenges in front of farmers in India and abroad including improving nitrogen use efficiency, and reversing the widespread loss of soil organic matter (Fageria and Baligar 2005). Adequate supply of nitrogen can promote plant growth and increase crop production, but under excessive application of nitrogen fertilizer, especially, vegetables can accumulate high levels of nitrate and, upon being consumed by living beings, pose serious health hazards (Hord et al 2009). With the increasing amount of nitrogen in the nutritional environment of plants, nitrate content in their tissues increases (Ceylan et al 2002, Wang and Li 2004). Moreover, Demir et al (1996) reported that by increasing the nitrogen fertilizer

Department of Soil Science & Agricultural Chemistry,
Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, UP
*Corresponding author, Email: akhila.dubey1@bhu.ac.in

rate the leaf area, stem length and yield of spinach increased. Zarehie (1995) observed that by increasing the nitrogen fertilizer rate to 200 kg ha^{-1} increased the yield of spinach but that by increasing the nitrogen level up to 200 kg ha^{-1} the corresponding increase in yield was not economical. Spinach (*Spinacia oleracea* L.) is one of the most important leafy vegetable crop due to its dark green color quality and consumer acceptance and highly rich in iron and vitamin A contents. Among the methods that have been followed for improving the growth, yield, seed production and quality of spinach, the application of nitrogenous fertilizers is very common. The application of humic substances had an overall positive effect on dry matter yield of the crops and this effect was statistically significant for incorporated. In the case of permanent grassland, humic substances promoted mainly the production of the first grass cut, which has the highest grass quality among all cuts during the growing season. Humic acid may be applied as a fertilizer, plant growth promoter, nutrient carrier and soil conditioner. Therefore, a study was undertaken to assess the Response of soil applied lignite coal derived humic acid on yield and quality of spinach (Spinacia oleracea L.)

Materials and Methods

Lignite coal derived humic acid: The lignite coal was collected from Matasukh Coal Mines, Nagaur, Rajasthan. The lignite coal was air dried, crushed and sieved in 2 mm sieve. The humic acid was extracted by 0.5 N KOH (Stevenson 1994, Yuan et al 2015) from lignite coal. The extracted humic acid potassium salt was precipitated by 1 N HCl. The precipitated humic acid was dialysed and purified. The material was dried through lypholizer.

Experimental techniques: A pot experiment was conducted in net house of the Department of Soil Science & Agricultural Chemistry, Banaras Hindu University, Varanasi, Uttar Pradesh, India(25°16′10′′N and 82°59′9′′E), to evaluate the effect of lignite coal derived humic acid on growth and development of spinach (*Spinacia oleracea* L.) in an Inceptisol of Varanasi, Uttar Pradesh. The surface soil layer from 0-15 cm depth was collected from Gangetic plain of alluvial soil (Inceptisol) in research farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi Uttar Pradesh during pre-monsoon season period. The initial properties of soil is given in Table 1. Each pot was filled with 10 kg soil, irrigated with tap water and left for 3-4 days to attain field capacity. The recommended doses of P_2O_5: K_2O (50: 50 kg ha^{-1}) for spinach was applied in the each pot and with six doses of lignite coal originated humic acid [i.e. 0.0 (H_0), 0.5 (H_1) 0.75 (H_2) 1.5 (H_3) 2.5 (H_4) and 5.0 (H_5) mg HA kg^{-1} soil] and four doses of N (urea) fertilizer [0.0 (N_0), 18.50 (N_1), 27.80 (N_2) and 37.0 (N_3) mg kg^{-1} soil] according to treatments at sowing time considering the recommended dose of N fertilizer (80 kg ha^{-1}). Then spinach seed variety "All Green" (developed from ICAR- IIVR, Varanasi) was sown in month of December 2017. The experiment was conducted in factorial complete randomized design (FCRD) with three replications. Standard cultural operation and plant protection practices were followed in experiment.

Table 1: Physical and chemical properties of Initial Soil under study

Parameters	Value
Bulk density (Mg m^{-3})	1.32
Particle density (Mg m^{-3})	2.51
Porosity (%)	48.21
Sand (%)	61.53
Silt (%)	14.05
Clay (%)	24.42
Textural Class	Clay loam
Water Holding Capacity (%)	45.57
pH (1:2.5)	7.91
EC (1:2.5) (dSm^{-1})	0.301
Organic carbon (g kg^{-1})	4.97
Available N (Kg ha-1)	220.68
Available P (Kg ha-1)	25.47
Available K (Kg ha-1)	171.45

Observation and analytical techniques: Photosynthetic pigments chlorophyll was measured using chlorophyll meter (SPAD-502 plus, Minolta Co. Japan), which was presented by SPAD value. Total soluble solid (TSS) was determined using refractometer meter (M A871 Refractometer, Milwaukee Co. Europe), which was presented by °Brix value. Fresh and dry weight of leaves was measured after drying in a thermo-ventilated oven at 70°C. Nitrogen content in leaves was determined according to methods of Chapman and Pratt (1978). The protein content in leaves was measured by Lowery method (Lowery et al 1951). Data were analyzed by statistically for their test of significance as per completely randomized design, at 5% level of significance.

Results and Discussion

Crop growth and yield: The application of nitrogenous fertilizer (urea) with humic acid in different levels showed significantly enhancement in the number of leaves, fresh weight and dry weight of spinach. The effects of different levels of N fertilizer on number of leaves and fresh weight as well as dry weight of spinach were highest in 60 days after sowing (DAS) followed by 45 & 30 DAS presented in Table 2&3. At 60 DAS, the number of leaves per plant and fresh weight as well dry weight in spinach was recorded highest when 100% of recommended dose of N (37.0 mg N kg^{-1}soil) was applied followed by 75% of recommended dose of N (27.80 mg N kg^{-1}soil) and the lowest value was recorded in control. At 30, 45, and 60 DAS, application of full dose of nitrogen (37.0 mg kg^{-1} soil) increased the yield of spinach 39.25, 49.96 and 64.87%, respectively over the control. It could be concluded that, increased the nitrogen level has important role on crop growth as it is a basic element of protein, nucleic acids, chlorophyll and growth hormones (Barker et al 1974). Adequate supply of nitrogen (N) can promote plant growth and increase crop production. These results agreed with those obtained by several workers (El-Fadaly and Mishriky 1990, Zarehie 1995, Demir et al 1996, Salman et al 2000, Maryam and Naser 2007 and Nevruz et al 2014). They reported that higher N levels enhanced vegetative growth and total yield of leafy vegetables.

The treatments which received 2.5 and 5 mg humic acid kg^{-1}soil humic acid significantly enhanced plant growth and yield of spinach (Table 2 &3). At different day interval 30, 45, and 60 DAS, application of higher level of humic acid (5.0 mg kg^{-1} soil) increased the yield of spinach 17.34, 28.09 and 34.32%, respectively over the control. Moreover, humic substances are mostly used to remove or decreased the negative effects of chemical fertilizers from the soil and have a major effect on plant growth as shown by many scientists (Ghabbour and Davies 2001). Humic acid also stimulate plant growth by the assimilation of major and minor elements, enzyme activation and /or inhabitation, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Ulukan 2008). The chelating of NH_4^+-N and NO_3^--N in soil through –COOH group and $–NH_2$ group of humic acid predominant action in this case. The perusal of the data indicated that fresh and dry weight of spinach leaves significantly increased at 30, 45 & 60 DAS, respectively. The highest vegetative growth expressed as number of leaves, fresh and dry weight which were obtained by adding full recommended dose of nitrogen fertilizer (37.0 mg kg^{-1} soil) with high level of humic acid application (5.0 mg kg^{-1} soil).

Table 2: Impact of lignite derived humic acids with nitrogenous fertilizer on number of leaves per plant of spinach (*Spinacia oleracea* L.)

Tretments	Number of leaves per plant		
	30 DAS	45 DAS	60 DAS
H_0: Control	4.00	4.83	5.33
H_1: 0.50 mg HA kg^{-1}soil	4.58	5.50	5.52
H_2: 0.75 mg HA kg^{-1}soil	5.17	5.92	6.33
H_3: 1.50 mg HA kg^{-1}soil	5.75	6.25	7.42
H_4: 2.50 mg HA kg^{-1}soil	6.42	6.75	7.58
H_5: 5.00 mg HA kg^{-1}soil	6.83	7.50	8.83
SEm±	0.22	0.20	0.23
CD (P = 0.005)	0.63	0.57	0.66
N_0: Control	3.67	4.19	4.67
N_1 :18.50 mg N kg^{-1}soil (50 % of recommended dose of N)	4.38	4.90	5.43
N_2: 27.80 mg N kg^{-1}soil (75 % of recommended dose of N)	5.19	5.71	6.33
N_3: 37.0 mg N kg^{-1}soil (100 % of recommended dose of N)	5.48	6.19	6.95
SEm±	0.18	0.16	0.19
CD (P = 0.005)	0.51	0.47	0.54

HA = Humic Acid, DAS = Days after sowing

Table 3: Impact of lignite derived humic acids with nitrogenous fertilizer on fresh weight and dry weight of spinach (*Spinacia oleracea* L.)

Tretments	Fresh weight (g / pot)			Dry weight (g / pot)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
H_0: Control	17.82	29.08	32.20	1.98	3.48	3.91
H_1: 0.50 mg HA kg^{-1}soil	18.73	31.42	34.50	2.08	3.51	3.96
H_2: 0.75 mg HA kg^{-1}soil	19.62	32.82	37.31	2.18	3.59	4.27
H_3: 1.50 mg HA kg^{-1}soil	20.02	33.74	38.77	2.22	3.66	4.43
H_4: 2.50 mg HA kg^{-1}soil	20.43	35.12	40.28	2.27	3.81	4.59
H_5: 5.00 mg HA kg^{-1}soil	20.91	37.25	43.25	2.32	4.07	4.90
SEm±	0.27	0.16	0.23	0.03	0.02	0.03
CD (P = 0.005)	0.76	0.46	0.66	0.08	0.06	0.08
N_0: Control	13.33	21.67	23.34	1.48	2.37	2.99
N_1 :18.50 mg N kg^{-1}soil (50 % of recommended dose of N)	15.76	28.34	32.50	1.75	3.27	3.65
N_2: 27.80 mg N kg^{-1}soil (75 % of recommended dose of N)	18.27	30.93	35.00	2.03	3.40	3.97
N_3: 37.0 mg N kg^{-1}soil (100 % of recommended dose of N)	19.80	33.02	38.48	2.20	3.60	4.27
SEm±	0.22	0.13	0.19	0.02	0.02	0.02
CD (P = 0.005)	0.62	0.38	0.54	0.07	0.05	0.06
Interaction (H*N)						
SE m±	0.534	0.32	0.47	0.06	0.04	0.05
CD (P = 0.005)	1.518	0.92	1.32	0.17	0.12	0.15

Pot = containing 10 kg soil, HA= Humia Acid

Chlorophyll content: The chlorophyll content in leaves was significantly increased (Table 4) due to application with both the nitrogenous fertilizer and humic acid in different levels. Chlorophyll content was maximum at 45 days after sowing (DAS) followed by 60 DAS than 30 DAS (Table 4). The increase in chlorophyll content was paralleled with increasing nitrogen level. These results agreed with that the results obtained by Gao et al (1989). However, increasing N levels up to its highest level (100 % of recommended dose of N) increased fresh weight of spinach plants (Table 4), it increases chlorophyll content, accelerates plant respiration and hormonal growth responses, increases penetration of nutrients in plant membranes, etc. The humic acid operate singly or in integration. The above discussion clearly validates the suitability of humic acid as a

Table 4: Impact of lignite derived humic acids with nitrogenous fertilizer on total soluble solid and chlorophyll content of spinach (*Spinacia oleracea* L.)

Tretments	Chlorophyll content (SPAD value)			TSS (Brix Value)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
H_0: Control	23.33	26.48	26.10	1.53	2.11	2.34
H_1: 0.50 mg HA kg^{-1}soil	23.98	28.25	27.16	1.66	2.61	2.89
H_2: 0.75 mg HA kg^{-1}soil	23.29	29.20	27.56	2.13	2.83	3.15
H_3: 1.50 mg HA kg^{-1}soil	23.38	30.11	27.79	2.26	3.00	3.25
H_4: 2.50 mg HA kg^{-1}soil	23.89	30.75	28.05	2.42	3.01	3.50
H_5: 5.00 mg HA kg^{-1}soil	24.48	31.52	28.93	2.54	3.21	3.88
SE m±	0.21	0.31	0.31	0.04	0.05	0.06
CD (P = 0.005)	0.61	0.88	0.87	0.13	0.15	0.16
N_0: Control	19.85	23.52	21.52	1.54	2.06	2.4
N_1 :18.50 mg N kg^{-1}soil (50 % of recommended dose of N)	20.20	24.77	23.05	1.74	2.37	2.61
N_2: 27.80 mg N kg^{-1}soil (75 % of recommended dose of N)	20.47	25.59	24.37	1.86	2.52	2.84
N_3: 37.0 mg N kg^{-1}soil (100 % of recommended dose of N)	20.83	26.87	25.68	2.01	2.63	3.01
SE m±	0.18	0.25	0.25	0.04	0.04	0.05
CD (P = 0.005)	0.50	0.71	0.71	0.1	0.12	0.13
Interaction (H*N)						
SE m±	0.430	0.616	0.614	0.09	0.1	0.12
CD (P = 0.005)	NS	NS	NS	NS	NS	0.33

TSS = Total soluble solid

Table 5: Impact of lignite derived humic acids with nitrogenous fertilizer on nitrogen and protein content of spinach (*Spinacia oleracea* L.)

Tretments	Nitrogen content (%)			Protein content (%)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
H_0: Control	2.51	2.59	2.50	11.81	12.15	11.76
H_1: 0.50 mg HA kg^{-1}soil	2.54	2.71	2.73	11.95	12.74	12.84
H_2: 0.75 mg HA kg^{-1}soil	2.57	2.74	2.80	12.09	12.87	13.16
H_3: 1.50 mg HA kg^{-1}soil	2.60	2.82	2.89	12.21	13.27	13.59
H_4: 2.50 mg HA kg^{-1}soil	2.63	2.90	2.97	12.35	13.63	13.96
H_5: 5.00 mg HA kg^{-1}soil	2.73	3.02	3.10	12.81	14.18	14.55
SEm±	0.01	0.01	0.01	0.05	0.06	0.06
CD (P = 0.005)	0.03	0.04	0.04	0.15	0.17	0.18
N_0: Control	1.97	1.99	1.98	9.26	9.33	9.32
N_1 :18.50 mg N kg^{-1}soil (50 % of recommended dose of N)	2.12	2.35	2.40	9.95	11.04	11.27
N_2: 27.80 mg N kg^{-1}soil (75 % of recommended dose of N)	2.26	2.53	2.56	10.63	11.91	12.03
N_3: 37.0 mg N kg^{-1}soil (100 % of recommended dose of N)	2.55	2.72	2.77	12.01	12.77	13.01
SEm±	0.01	0.01	0.01	0.04	0.05	0.05
CD (P = 0.005)	0.03	0.03	0.03	0.12	0.14	0.15
Interaction (H*N)						
SEm±	0.02	0.03	0.03	0.11	0.12	0.13
CD (P = 0.005)	NS	0.07	0.08	NS	0.34	0.36

NS = Non-significant

beneficial fertilizer product. These results are in well agreement with obtained with Yildirim (2007), Unlu et al (2011), Shafeek et al (2013) and Aisha et al (2014). The highest value of biochemical quality expressed as a chlorophyll content (Table 3) was obtained by adding full recommended dose of humic acid (37.0 mg kg^{-1} soil) with high level of humic acid application (5.0 mg kg^{-1} soil).

Total soluble solid: The brix value or total soluble solid (TSS) in spinach leaves was greatly increased with improving the level of humic acid with nitrogen fertilizer (Table 4). The highest brix value was recorded at 60 DAS in H5 treatment (i.e. 5.0 mg kg^{-1}). The application of 100 % of recommended dose of N was also resulted to highest value followed by 75 % of recommended dose of N fertilizer (Table 4) and the lowest value was recorded in control (without humic acid & nitrogen). The improvement of quality parameter like °Brix value, especially by humic acid, agreed with Vercesi (2000) and in table grape (Colapietra 2000) with different humic substances.

Nitrogen content: Nitrogen plays a crucial role in the synthesis of amino acids and proteins, plant growth chlorophyll formation, leaf photosynthesis and yield of crop. As shown in Table 5, total nitrogen content in spinach was significantly increased with increasing the levels of humic acid and different doses of nitrogen fertilizer. Among all treatments, the highest value of total nitrogen and in leaves of spinach was obtained when the plant was received the higher dose of humic acid (5.00 mg HA kg^{-1} soil) in spinach crop (Table 5) at 30DAS, 45 DAS and 60 DAS, respectively. The effect of different levels of N fertilizer on nitrogen content of spinach leaves was highest in 60 days interval followed by 45 & 30 DAS presented in Table 5.

Protein Content: At different days interval *i.e.* 30, 45, and 60 DAS, application of higher level of humic acid (5.0 mg kg^{-1} soil) increased 8.47, 16.71 and 23.72 % of protein content in spinach over the control and application of full dose of nitrogen (37.0 mg kg^{-1} soil) increased 12.01, 12.77 and 13.01 %, respectively of protein content in spinach over the control. Fagbenro and Agboola (1993) reported that with addition humic acid to soil, nutrient uptake including N, P and K as well as protein content increased which is in agreement with results of this experiment. Ayas and Gulser (2005) indicated that increasing doses of humic acid increased N contents in spinach leaves. However, MacCarthy et al (2001) concluded that humates enhance nitrogen uptake, and also increase the yield and quality of various crops.

The application of humic acid in soil improved the yield contributing parameters in spinach, such as, number of leaves (66.60 %), fresh weight (34.32 %) and dry weight (25.32 %), respectively over control at 60 DAS. The application of humic acid also enhanced the quality parameters viz. chlorophyll content (10.84 %), total soluble solid (65.81 %), nitrogen content (24.0 %) and protein content (23.72%), respectively. The highest improvement of yield and quality was recorded in the application of 5 mg kg-1 humic acid (H5) in soil with the split application of recommended dose of nitrogen fertilizer (N3) through urea (50% as basal and rest 50% in split doses). In addition to these recommended dose of phosphatic (50 kg ha^{-1}) and potassic (50 kg ha^{-1}) fertilizers should be added. The application of humic acid with nitrogen fertilizers was noticed to be promising technique in increasing yield and quality of spinach.

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Ikjk'k

लिग्नाइट कोल व्युत्पन्न ह्यूमिक एसीड का पालक के वृद्धि एवं विकास पर प्रभाव को ज्ञात करने के लिए गमले में एक प्रयोग मृदा विज्ञान एवं कृषि रसायन विभाग, कृषि विज्ञान संस्थान, काशी हिन्दू विश्वविद्यालय, वाराणसी (उत्तर प्रदेश) में वर्ष 2017–18 के रबी मौसम में किया गया जिनमें लिग्नाइट कोल आधारित ह्यूमिक एसीड के 6 दरों [0.0 (एच$_0$), 0.5 (एच$_1$), 0.75 (एच$_2$), 1.5 (एच$_3$), 2.5 (एच$_4$), एवं 5.0 (एच$_5$) मिग्रा. ह्यूमिक एसीड किग्रा.$^{-1}$ मृदा, एवं नत्रजन के लिये यूरिया के चार दरों [0.0 (एन$_0$), 18.50 (एन$_1$), 27.80 (एन$_2$) एवं 37.0 (एन$_3$) मिग्रा. किग्रा$^{-1}$ मृदा, के विविध के संयोजन सम्मिलित है। पौध विकास घटकों (पत्तियों की संख्या, ताजा भार एवं शुष्क भार) एवं जैव रसायनिक गुणों (ब्रिक्स मूल्य, क्लोरोफिल, नाइट्रोजन एवं प्रोटीन की मात्रा) का मापन बीज बुवाई के 30, 45 एवं 60 दिनों उपरान्त किया गया। अधिकतम उपज एवं गुणवत्ता घटक ऐसे उपचारों में पाया गया जिनमें अधिक स्तर (5 मिग्रा. किग्रा$^{-1}$ मृदा) में ह्यूमिक एसीड की मात्रा के साथ पूर्ण नत्रजन की दर (37 मिग्रा. किग्रा$^{-1}$ मृदा) यूरिया उर्वरक के माध्यम से दी गई थी और पालक की पैदावार गुणवत्ता बढ़ाने में नाइट्रोजन उर्वरक के साथ ह्यूमिक एसीड के अल्प प्रयोग को उत्तम पाया गया।

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