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ROLE OF COAL-DERIVED HUMIC ACID IN THE AVAILABILITY OF NUTREINTS AND GROWTH OF SUNFLOWER UNDER CALCAREOUS SOIL

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ABSTRACT

The soils of Pakistan are calcareous alkaline and poor in organic matter where the availability of nutrients is limited resulting in low crop yield per hectare. However, there are enriched deposits of low rank coal containing humic acid (HA) in large quantities which can be used as a source of organic matter. Taking advantage of this natural resource, the present study was designed to evaluate the different concentration of coal-derived HA (0, 0.5, 1, 1.5, 5 and 25 g l^{-1}) on growth of sunflower in pot experiments arranged in a completely randomized design with a basal dose of NPK and replicated four times. The results indicated that shoot (58.84 g) and root dry weight (18.24 g), soil organic matter and leaf nutrient concentration except Cu and Mn of sunflower improved significantly when humic acid was applied up to 1g l^{-1} while further increase in HA resulted suppressive effects on all studied parameters. However, soil pH decreased significantly with increasing HA that enhanced nutrient availability. It was concluded that the application of coal-derived HA with low concentration under calcareous soil can help in improving soil quality and increasing yield on sustainable basis.

Key words: Coal-derived humic acid; nutrients; growth; sunflower; calcareous soil.

INTRODUCTION

One of the important components of high quality soil is organic matter which directly affects soil physical, chemical and biological properties and its presence in sufficient quantity is indispensible for sustainable crop production and friendly environment (Bloem *et al.*, 2005). In tropical and sub-tropical areas of the world high temperature results in high decomposition rate of soil organic matter affecting soil fertility and productivity with low crop growth and yield (Giardina *et al.*, 2000; Steiner *et al.,* 2007). In developing countries like Pakistan where inputs are in short supply and removal of crop residues for fuel and feed purposes further aggravate the loss of organic matter in soil resulting in low yield. However, the enriched deposits of low rank coal containing humic acid are found in large quantities can be used as a source of organic matter by extracting its humic acid contents (Hai and Mir, 1998). It contains phenolic, acidic, amino and quinione groups those help in the availability of nutrients in calcareous alkaline soil poor in organic matter. This has aromatic structure and is soluble in alkali because it is surrogated by carboxyl, phenolic, hydroxyl, and alkyl groups which are connected together through either linkage (Mackowiak *et al.,* 2001). The application of coal-derived sodium humate under calcareous soil increased growth, yield and nutrient uptake of various crops (Sarir and Durrani, 2006; El-Nemr, 2012; Daur and Bakhashwain, 2013).

The stimulatory effect of humic substances on higher plants produce different physiological, morphological and biochemical changes (Nardi *et al*., 2002) resulting in promotion of plant growth and development (Morard *et al.*, 2011). Such stimulation helps in nutrient uptake (Dell'Agnola and Nardi, 1987) and regulating growth mechanisms in the form of high germination and improved root and shoot growth for sustainability (Dobbss *et al*., 2007).

One of the indispensable ingredients of human diet is oil and fat which is enriched with calories, vitamin A and D. Some non-conventional oil seed crop like sunflower is now being introduced for meeting the edible oil needs of the country (Ali *et al*., 2006), because Pakistan is not self-reliant in edible oil and more than 70% of its requirements are met from import. During the year 2011-12, Pakistan imported 2.148 million tons of edible oils on which Rs. 216.40 billion was spent in the form of foreign exchange, while, its local production was recorded 0.636 million tons which is less than 30%. In the domestic production of edible oils, the contribution of oil seed crops including cottonseed, canola, rapeseed and sunflower was 60.53, 1.73, 9.59 and 28.15 % (GOP, 2012). Due to high yielding capacity, sunflower is returning more income to farmers as compared to other oil seed crops because it is grown in two seasons i.e. one in spring and second in summer (Alias *et al*., 2009). Now, the high potential of sunflower as oil crop has been identified for meeting the future needs of edible oil in Pakistan because this crop can be grown successfully in a variety of climatic zones which are conducive for its production. The major climatic requirement for sunflower is the 120 days periods that must be free of frost. However, the optimum temperature for sunflower is from

20 to 25ºC but the temperature of 8 to 34ºC is also tolerable for its production (Shah *et al*., 2005). There are four agro-ecological zones of Balochistan such as Coastal, Desert, Plains and uplands where sunflower is grown in irrigated and rainfed areas except Desert. It is also grown on marginal lands in Balochistan fruitfully. According to Ahmed *et al*., (2009) who reported that sunflower is quite appropriate in the present cropping system without replacing any other major crop. However, its yield is low due to poor soil organic matter and unavailability of nutrients in calcareous alkaline soil.

The high deposit of low grade coal in Pakistan has sufficient quantity of extractable humic acid which can be used for enhancing soil fertility and crop growth. The Fuel Research Centre (FRC) Karachi, Pakistan Council of Scientific and Industrial Research (PCSIR), have devised the economical process of humic acid extraction from low grade lignite coal under the project entitled "Development of Humic Acids from Lignitic Coal and its use in Agriculture and Industry". FRC further revealed that numerous field experiments confirmed the effectiveness of HA from coal with satisfactory findings for enhancing agriculture production.

Keeping in view the importance of coal-derived humic acid under calcareous soil, the present study was designed to evaluate different levels of coal-derived humic acid on plant growth and soil properties using sunflower (*Helianthus annuus* L.) as a test crop.

MATERIALS AND METHODS

A pot experiment was conducted at Agricultural Research Institute (ARI) Sariab, Quetta, Pakistan during spring season 2011 to investigate the effect of different levels of humic acid (extracted from low grade coal) on sunflower. The experiment was designed in a completely randomized design with five levels of humic acid (0, 0.5, 1, 5 and 25 g l^{-1}) and replicated four times. Coal derived HA was kindly obtained from the Department of Soil Science, University of Agriculture Peshawar, Pakistan. Soil was collected from the research field of ARI, air dried and sieved (<2mm), then weighted @ 8 kg pot^{-1}. The soil used in the pot study was sandy loam in texture with calcareous (18.12% $CaCO_3$) alkaline (pH 8.03) nature having EC_e 1.34 dSm^{-1}, organic matter 2.11%, and CEC 7.48 me 100 g $soil^{-1}$. The pre soil analysis for macro (NPK) and micronutrients (Cu, Fe, Mn and Zn) revealed that the soil total nitrogen content was 0.150% and AB-DTPA extractable P,K, Cu, Fe, Mn and Zn concentration was from 7.31 to 157.6, 0.37, 4.22, 1.31 and 1.25 ppm respectively. Before filling the pots, the humic acid was dissolved in distill water and sprayed as per treatment following the method described by Sharif *et al*. (2002a), then the polyethylene pots were filled along with application of NPK @ 90-60-60 kg ha^{-1} in the form of urea, nitrrophose (NP) and sulphate of potash (SOP). The pots were placed under natural (open) environment. Six seeds of sunflower hybrid Hysun-33 were equally sown in each pot. The germinated seeds were thinned to two plants pot^{-1}. Tube well water was used for irrigating the pots when required. Before flowering, the plants were harvested after 68 days from the date of sowing and root and shoot dry weight was determined by drying them in oven at 70ºC for 48 hours.

Soil analysis: Pre and post soil analysis was carried out for its physicochemical properties. Soil texture was determined by hydrometer method (Bouyoucos, 1962). Electrical conductivity, soil pH and cation exchange capacity were determined by the methods described by Richards, (1954). Soil organic matter was determined by method of Walkley, (1947), nitrogen by Kjeldahl method (Bremner and Mulvaney, 1982). Phosphorus, potassium, copper, iron, manganese and zinc were extracted by AB-DTPA method (Soltanpour and Schwab, 1977). Sulphate-S was extracted from alkaline soil with 0.15% $CaCl_2.2H_2O$ (Williams and Steinbergs, 1959) using turbidimetric procedure with barium choloride (Verma, 1977).

Plants analysis: For measuring nutrients accumulation in sunflower plants, leaf samples were collected from each pot before flowering, washed and processed for wet digestion using the mixture of H_2SO_4 and H_2O_2 (Van Schouwenberg and Walinge, 1973). In the wet digest, nitrogen was determined by Kjeldahl method, potassium by flame photometer, phosphorus by spectrophotometer and micro nutrients (Fe, Zn, Cu and Mn) by atomic absorption spectrophotometer. Wet ashing with an $HClO_4$-H_2O_2 mixture was performed for total sulphur contents in leaves of sunflower using turbidimetric procedure with barium choloride (Verma, 1977).

RESULTS AND DISCUSSION

Shoot and root dry weight: The effect of different levels of coal derived humic acid on shoot and root dry weight of sunflower is indicated in Table 2. The shoot dry weight across all humic acid treatments ranged from 39.43 to 59.76 g with mean value of 50.84 g and root dry weight ranged from 14.10 to 18.88 g with mean value of 16.36 g (Table 3). Whereas, both shoot and root dry weight $plant^{-1}$ showed statistically significant response for all coal derived humic acid application. The highest shoot and root dry weight (58.84 and 18.24 g) was produced in the treatments when humic acid was applied @ 1 g l^{-1}, while the minimum was noted in the treatment supplied with 25 g l^{-1} HA. However, 0.5 and 5 g l^{-1} HA produced statistically same shoot and root dry weight. Similar results were also reported by Sharif *et al*. (2002b) who recorded 20 -23 % highest maize shoot dry weight over control with application of humic acid in combination with recommended dose of NPK. Malik and

Azam, (1985) reported an increase of 22% of wheat shoot dry matter yield with application of humic acid. It was observed that the high concentration of HA suppressed shoot and root dry weight even lower than control. It meant that HA works well under calcareous soil only when applied with lower concentration. The enhancement in sunflower root dry weight may probably be due to the positive effects of humic acid which constitute a major fraction of carbon, regulate carbon cycle, release nutrients and help in better uptake and utilization of nutrients especially nitrogen (El-Ghamry *et al*., 2009; Ulukan, 2008). Similarly, Eyheraguibel *et al.* (2008) found positive effects of humic like substances (HLS) on roots, shoot and leaf biomass of maize plants. Ortega and Fernandez (2007) reported that application of humic substances increased root dry matter, plant greenness and nutrient use efficiency in ryegrass. Our results are also supported by Katkat *et al*. (2009) who revealed that soil application of 1g kg^{-1} humus to wheat plants produced highest dry weight and uptake of nutrients as compared to higher level of humus (2g kg^{-1}).

Soil organic matter and pH: The range of soil organic matter contents was quite narrow i.e. from 2.10 to 2.15 % with mean contents of 2.13%, because HA did not affect soil organic matter and it was found statistically same over all HA levels but was higher as compared to control (Table 2 and 3). However, Soil pH decreased gradually in a descending order with increasing concentration of HA which ranged from 7.77 to 8.03 with mean pH of 7.88 (Table 2 and 3) and the lowest soil pH (7.78) was recorded in the pots supplied with 25 g l^{-1} HA. The highest soil pH (8.02) was attained in control. Reduction in soil pH may probably be due to addition of humic acid in combination with NPK fertilizers to soil because HA releases H^+ in soil solution on dissociation of its different functional groups and nitrogenous fertilizer also release H^+ when its nitrification occurs. Juan *et al*. (2008) revealed that application of both organic and mineral fertilizers significantly decreased soil pH. On the contrary, Ortega & Fernandez (2007) could not observed significant effect of C rates on soil pH and electrical conductivity.

Leaf Nutrient concentration: Leaf nutrient concentration of sunflower were affected by the different levels of coal derived humic acid (0.0, 0.5, 1.0, 1.5, 5.0 and 25.0 g l^{-1}) when applied in pots using calcareous soil (Table 2 and 3). Among the leaf macronutrients, nitrogen ranged from 2.02 to 2.28 % with mean value of 2.18%, phosphorus from 0.16 to 0.27 % with mean value of 0.22%, and potassium from 3.13 to 3.58% with mean value of 3.34%. Similarly, leaf micronutrients indicated that copper ranged from 9.74 to 10.30 ppm with mean value of 10.04ppm, iron from 55.20 to 79.49 ppm with mean value of 70.09ppm, manganese from 25.49 to 28.28 ppm with mean value of 26.78ppm and zinc from 28.64 to 35.12 with mean value of 32.94ppm. Maximum leaf nutrients were noted at two HA levels (0.5 and 1.0 g l^{-1}) in which nitrogen, sulfur, iron, manganese and zinc were statistically at par while phosphorus and potassium were significant (p=0.05) from each other but maximum copper was noted in control and minimum leaf nutrients were noted in treatment receiving HA @ 25 g l^{-1}. Statistically manganese concentration in leaf was similar at all HA levels and copper was non-significant at three HA concentration (0.5, 1.0 and 5.0 g l^{-1}) only. The increase in leaf nutrient concentration by HA might be due to improvement in cell permeability of sunflower roots as other scientists like Dell'Agnola and Nardi, (1987); Fagbenro and Agboole, (1993) indicated that HA results in the availability of mineral nutrients by enhancing interaction between root cells and active compounds in HA and thus increase cation & anion exchange capacity of soil and ultimately enhance nutrients uptake. Quagiotti *et al*. (2004) and Pinton *et al*. (1999) also reported that humic substances increased the uptake of nitrate through the interaction with plasma membrane H+-ATPase. Our results are in line with Sharif *et al*. (2002 a,b) who revealed that application of HA in combination with NPK significantly improved nutrients accumulations in wheat and maize plants. Similar results were also reported by Nikbakht *et al.* (2008) who indicated that humic acid significantly improved macro (N, P, K, Ca and Mg) and micro (Mg, Fe and Zn) nutrients contents of leaves and scapes in gerbera (*Gerbera jamesonii* L.) plants.

Correlation: Pearson correlation of shoot & root dry weight, soil organic matter and soil pH with leaf macro and micronutrients of sunflower presented in table 4 indicated that the shoot and root dry weight $plant^{-1}$ of sunflower and soil pH was positively and significantly (p = 0.01) correlated with leaf macro and micronutrients except Mn, while shoot and root dry weight was non-significantly correlated with leaf Cu. However, the correlation between soil organic matter and leaf nutrients (N, P, K, S, iron and Zn) was negative and non-significant but in case of zinc, the correlation was non-significantly positive. Whereas, Cu was negatively and significantly (p = 0.01) correlated with soil organic matter. Similar correlation was reported by Chen and Tsila (1990) that the humic acid has stimulatory effect on plant growth and was correlated for increasing nutrients uptake.

Table 1. Effect of coal-derived humic acid on shoot & root dry weight plant^{-1}, soil organic matter and soil pH (n = 2).

Treatments HA g l^{-1}	Shoot Dry weight plant^{-1} (g)	Root Dry Weight plant^{-1} (g)	SOM (%)	Soil pH
0.0	#42.85 c	15.05 c	2.11 b	8.02 a
0.5	55.96 b	17.14 b	2.13 a	7.92 b
1.0	58.84 a	18.24 a	2.13 a	7.87 c
5.0	56.69 b	17.11 b	2.14 a	7.79 d
25.0	39.85 d	14.24 d	2.13 a	7.78 d
Mean	50.84	16.48	2.13	7.88
LSD	1.42	0.61	0.01	0.02

#Mean bearing the same letters are statistically alike (P= 0.05); ns = non-significant

Table 2. Effect of coal-derived humic acid on leaf nutrients concentration of sunflower (n = 2).

Treatments (HA g l^{-1})	N	P	K	S	Cu	Fe	Mn	Zn
		(%)				(ppm)		
0.0	#2.22 b	0.23 b	3.34 c	0.12 bc	10.22 a	73.94 b	26.59 a	33.31 b
0.5	2.27 a	0.24 b	3.53 a	0.13 a	10.07 b	78.17 a	27.75 a	34.69 a
1.0	2.27 a	0.26 a	3.45 b	0.13 ab	10.04 b	78.05 a	26.72 a	34.71 a
5.0	2.13 c	0.20 c	3.26 d	0.11 c	9.99 b	66.04 c	26.38 a	33.20 b
25.0	2.03 d	0.17 d	3.14 e	0.08 d	9.86 c	56.24 d	26.45 a	28.81 c
Mean	2.18	0.22	3.34	0.12	10.04	70.49	26.78	32.94
LSD	0.02	0.01	0.04	0.01	0.09	1.74	ns	0.16

#Mean bearing the same letters are statistically alike (P= 0.05); ns = non-significant

Table 3. Range, mean and standard deviation of Shoot & root dry weight, soil organic matter, soil pH and leaf macro and micronutrients of sunflower (n = 2).

Factors under study	Unit	Range	Mean±S.D.
Shoot dry weight plant^{-1}	g	39.43-59.76	50.84±8.08
Root dry weight plant^{-1}	g	14.10-18.88	16.36±1.54
Soil organic matter	%	2.10-2.15	2.13±0.01
Soil pH	-	7.77-8.03	7.88±0.09
Leaf macronutrients			
N	%	2.02-2.28	2.18±0.10
P	%	0.16-0.27	0.22±0.03
K	%	3.13-3.58	3.34±0.14
S	%	0.07-0.14	0.11±0.02
Leaf micronutrients			
Cu	ppm	9.74-10.30	10.04±0.13
Fe	ppm	55.20-79.49	70.09±8.33
Mn	ppm	25.49-28.28	26.78±0.70
Zn	ppm	28.64-35.12	32.94±2.24

Table 4. Pearson correlation between shoot & root dry weight, soil organic matter and soil pH with leaf macro and micronutrients of sunflower (n = 2).

	Shoot dry weight (g)	Root dry weight (g)	Soil organic matter (%)	Soil pH
N	0.6045**	0.6753**	-0.2338ns	0.6675**
P	0.6104**	0.7049**	-0.2043ns	0.5902**
K	0.6515**	0.6998**	-0.1023ns	0.5428**
S	0.6652**	0.7073**	-0.1960ns	0.9065**
Cu	0.0920ns	0.1220ns	-0.5675**	0.8566**
Fe	0.5933**	0.6528**	-0.2537ns	0.6904**
Mn	0.2819ns	0.2175ns	0.1017ns	0.4412ns
Zn	0.7817**	0.8145**	-0.0318ns	0.5079*

** Significant at 1% probability level * Significant at 5% probability level NS Non-significant

Conclusions: The present study in a crystal clear manner outlined the effectiveness of coal-derived humic acid in significantly improving the shoot and root dry weight, soil organic matter and leaf nutrient concentration of sunflower. However higher dozes manifested a suppressive effect on all attributes investigated. Interestingly assigning HA increments favored soil pH decrease leading to enhanced nutrient availability. We, are therefore of the opinion that HA in **s**low concentration under calcareous soil can help not only in improving soil quality but can also positively affect the yield on sustainable basis. Furthermore, the results obtained encourage us to extend the application of HA to other crops of economic importance.

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