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BIOLOGICAL AND CHEMICAL EFFECT OF SOIL CONDITIONER- LIGNITE HUMIC ACID ON SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANT GROWTH AND FATTY ACID CONSTITUENTS

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ABSTRACT

Field trials of sunflower (*Helianthus annuus* L.) were conducted with different concentrations of Pakistani lignite derived humic acid. The present study included seed treatment with 0.050, 0.075, 0.125 and 0.200 % aqueous ammonium humate along with control crop for data evaluation. Furthermore, oil content of seeds obtained from treated and untreated plant seeds using n-hexane and their physicochemical properties were compared with standard values. The fatty acid analysis of sunflower seeds oil was performed by gas chromatography after successive extractions. Encouraging results were obtained for seed oil content, unsaturated hydrocarbons and plant yield. The physicochemical properties (proximate analysis) of oil did not show any significant change in the oil composition. The oils extracted from seeds obtained from treated plants showed relatively higher content (47.2 %) of linoleic acid ($C_{18:2}$).

Keywords: Lignite, Humic acid, Soil-conditioner, Sunflower Oil yield, fatty acid analysis.

INTRODUCTION

The importance of humic acid (HA) in agriculture has been acknowledged for over 150 years. The extension of organic farming and sustainable agriculture has led to increasing applications of organic fertilizers. Humic acid is a complex organic supra molecules formed by the breakdown of organic matter in lignite deposits abundant in low-rank coal like one’s found in Pakistan. Humic acid constitutes stable fraction of carbon, thus regulating the carbon cycle and the release of nutrients, including nitrogen, phosphorus and sulphur in the soil. Additionally, the presence of HA improves water holding capacity, pH buffering and thermal insulation (Stevenson, 1994). Humic acid contains a variety of functional groups, including carboxylic acid (COOH), phenolic hydroxy (OH), enolic (OH), alcoholic (OH), quinone, hydroxylquinone, lactone, and ether (Sposito, 1986). Humic acids are extensively versatile. They provide a concentrated economical form of organic matter as a soil-conditioner to replace humus depletion caused by conventional fertilization farming as well as being used in biological programs throughout the world.

The commercial sunflower of today is believed to have originated in America. Sunflower is the fourth largest source of vegetable oil which reflects the influence of climate, temperature, genetic variability and position of seed location in the flower head so significantly in their composition as does sunflower oil. Generally, sunflower grown towards North in USA is high in linoleic acid and towards below will be high in oleic acid. The differences also vary with temperature. A hot summer will lower the linoleic content of northern sunflower oils (Morrison, 1975), therefore fatty acid compositions will vary from year to year and region to region However today in the Russia and Eastern Europe together account for over 80 % of the world sunflower output. The sunflower is the leading oil seed in Europe. Sunflower oil with 60-75 % oleic ($C_{18:1}$) fatty acid in contrast to 20 % in regular sunflower oil would provide a superior quality oil and it will be trans-acid free (Gupta, 2001).

Fertilizers may also affect seed charactertics, phosphate can increase seed-oil content and nitrogen may reduce it. There is an optimum level for both to achieve highest oil content viability and subsequently seedling vigour can also be affected by the nutrient status under which the parent plant was grown. Initially the most important aim of plant breeds was to increase seed-oil content, reduce height and introduce pest and disease resistant (Klocking, 2002)

Unfortunately, so far no detailed research study was reported for humic acid applications to valuable and economically important sunflower crop and correlates this information with its oil yield and fatty acid analysis. Sunflower being a short duration and day neutral plant is quite suitable to fit in our existing cropping system. Pakistan is producing about one third of its edible oil requirements and the rest is met through import at a cost of about 40 billion rupees each year. Sunflower is grown on an area of 256 thousand hectares in Pakistan with a production of 359 thousand tonnes sunflower seed and 1402 kg per hectare yield (Anon, 2004). This per hectare yield is very low as compared to major sunflower growing countries (Argentina 1698, China 1539 and USA 1339 kg/ha).

In the present work, effect of humic acid with variable dosage on the oil content of *Helianthus annuus* L. has been studied. The study was focused on comparing the results of control and treated crops with reference to plant yield, fatty acid compositional changes and other industrial physicochemical parameters. Research studies have shown (Hai and Surriayia, 1998, Mir and Khan, 2002, Nasir et.al., (In press) that the Pakistan's lignitic low-rank coal contain sizable amount of humic acid. A wide spread deficiency of micro-nutrients is observed in Pakistani soil (Kalyal and Randhawa., 1982). Thus to enhance agricultural growth and to overcome humic acid and micro-nutrients deficiency large quantity of humic acid can be easily produced from huge coal deposits of country and may be incorporated in the soil for increasing the soil fertility and per acre yield of crop. The objective of this study is to evaluate the effect of humic acids (ammonium humate) without using any chemical fertilizer (like phosphorous or nitrogen fertilizers) and in turn observe the yield components and fatty acids composition of treated sunflower crop.

MATERIALS AND METHODS

Lignite humic acid was prepared from coal and characterized by standard methods (Mir and Khan 2002). It was used as ammonium humate. All chemical used were of the analytical grade and products of Fluka and BDH, England. During an oil authenticity check, one of the analyses performed is the determination of the fatty acid composition. This is affected by transesterification of oil triglycerides and analysis of the produced fatty acid methyl esters by gas chromatography using capillary columns. Capillary columns and the integration facilities of modern gas chromatographs can provide accurate information on the percentages of different fatty acid methyl esters.

INSTRUMENTATION

The fatty acid methyl esters were analyzed by Carlo Erba HRGC model 5300 gas chromatography equipped with flame ionization detector (FID). The components were separated in 10 % carbowex packed column (10 % DEGS on CW-AW, 80/100, 3×1/8" s.s) column length; 30 mm. The following chromatographic conditions were observed; column temperature was program from initial 110˚C hold for 1 min to final 190˚C at a rate of 5.0 ˚C/min: injector temperature; 230˚C: detector temperature; 250˚C: carrier gas; Nitrogen: carrier gas flow; 30 ml/min: volume of injected sample; 0.5 µl. Fatty acids were identified by comparing of retention time with standard data and expressed as percentage of total fatty acids (area/area), including minor fatty acids.

SEED TREATMENT

The seed of variety Hysine 33 (ICI Agrochemicals, Pakistan) was purchased from the local market. All experimental/field trials were accomplished on the soil that has slit loam texture with pH 7.8 to 8.05 at Fuel Research Centre (FRC), PCSIR Karachi in mid of December 2007. The layout of the experimental work was randomized complete block with three replicates. The entire field of area 9×9 feet (3×9 feet each plot) was divided at a spacing of 30 cm between row and 15 cm between plant in the row. The required quantity of ammonium humic acid as humate was dissolved in water and diluted solution was sprayed evenly with the help of a hand sprayer before sowing seeds (pre-sowing spray). For comparison and determine effect of soil conditioner on oil content of seeds, four concentration of ammonium humate (0.05, 0.075, 0.125, 0.2 %) were selected for present study. Crop received normal irrigation and production practices were kept free of pest through scheduled spray during the growth season. Oil seed content/plant growth measurement was taken at weekly intervals beginning from plat maturity. Seed yield and its components were determined by study whole plot and the measurement was made on area basis.

OIL EXTRACTION PROCEDURE

The dried seeds (100 grams) of the sunflower were ground with an electrical blender into fine powder. Extraction of the seeds was carried out in a Soxhelt apparatus using n-hexane (200 ml) in the Soxhelt till it became colorless. The oil obtained after distilling off the hexane was stored in a labeled flask for further analysis.

PREPARATION OF METHYL ESTER OF EXTRACTED OIL

To accurately weighed 4 grams of extracted oil into dry transesterification flask add 0.5 ml 1 N methanolic potassium hydroxide and 40 ml methanol and reflux for 1 hour. Cool and add 20 ml distilled water and extract with 20 ml distilled water and extract with 20 ml hexane twice. Hexane layer were combined washed with water, dried over anhydrous sodium sulfate. The solvent is evaporated under the stream of nitrogen gas atmosphere. The fatty acid methyl ester obtained was diluted with hexane, and inject 0.5 µL into gas chromatography (Figure 1) (AOAC, 2002).

Table 1. Agronomic efficiency of ammonium humate for plant growth

Sample	Treatment	Length of Plant Root (cm)	Length of Plant Shoot (cm)	Wt. of Plant Flower (Capitatum) (g)	Wt. of Dry Plant Seed (g)
E-01	Control	19.325 ± 6.20	171.150 ± 17.32	172.86 ± 44.21	14.76 ± 0.57
E-02	0.050 % AH	26.325 ± 3.40	170.900 ± 3.60	227.63 ± 27.26	21.06 ± 1.37
E-03	0.075 % AH	26.650 ± 0.27	177.500 ± 1.12	209.83 ± 35.97	20.46 ± 2.37
E-04	0.125 % AH	30.500 ± 2.27	174.375 ± 3.60	216.76 ± 43.16	21.33 ± 0.90
E-05	0.200 % AH	26.900 ± 0.12	185.750 ± 7.22	222.93 ± 47.92	22.60 ± 1.06

Table 2. Physicochemical properties of extracted seed oil

Sample	Specific gravity (g/ml)	Color	State at 29˚C	Refractive Index	Iodine Value (gm/0.5 g of sample)	Saponification Value (g/2 gm of sample)
E-01	0.917	Yellow	Liquid	1.47	32	189
E-02	0.920	Yellow	Liquid	1.47	28	188
E-03	0.922	Yellow	Liquid	1.47	28	187
E-04	0.917	Yellow	Liquid	1.47	32	188
E-05	0.918	Yellow	Liquid	1.47	32	187

Table 3. Fatty acid profile of extracted seed oil.

Sample	Oil Content (%)	Palimitic acid ($C_{16:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Eicosanoic acid ($C_{20:0}$)	Docosanoic acid ($C_{22:0}$)
E-01	19.70	7.12	49.89	41.83	Trace	1.15
E-02	28.40	5.65	47.99	46.30	Trace	Trace
E-03	28.80	5.93	46.90	47.20	Trace	Trace
E-04	24.60	5.15	50.17	44.68	Trace	Trace
E-05	30.30	7.22	49.89	41.83	Trace	1.15

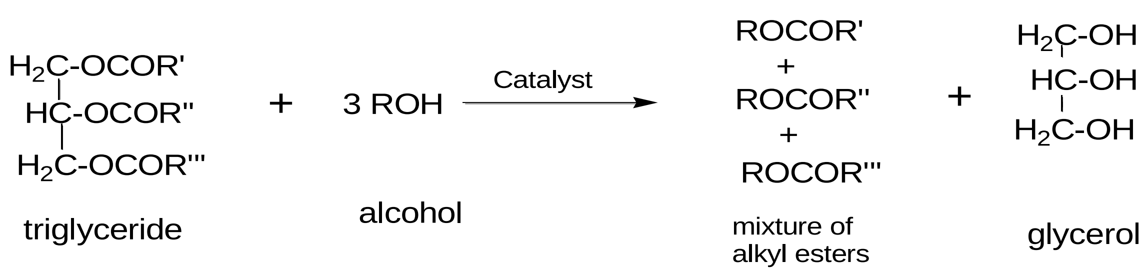

Figure-1: Transesterification reaction of sunflower seed oil

PHYSICOCHEMICAL PROPERTIES

Iodine value (Wiji's method) and saponification number values were determined as by recommended standard methods (AOAC, 2000).

RESULT AND DISCUSSION

The treatment of seeds with various percentages of ammonium humate solution and the effect thus resulted is reported in Table 1. Optimum effect was observed in case of 0.200 % humic acid on plant length, weight of plants and yield of seeds. However, root length was maximum at 0.125 % humic acid salt.

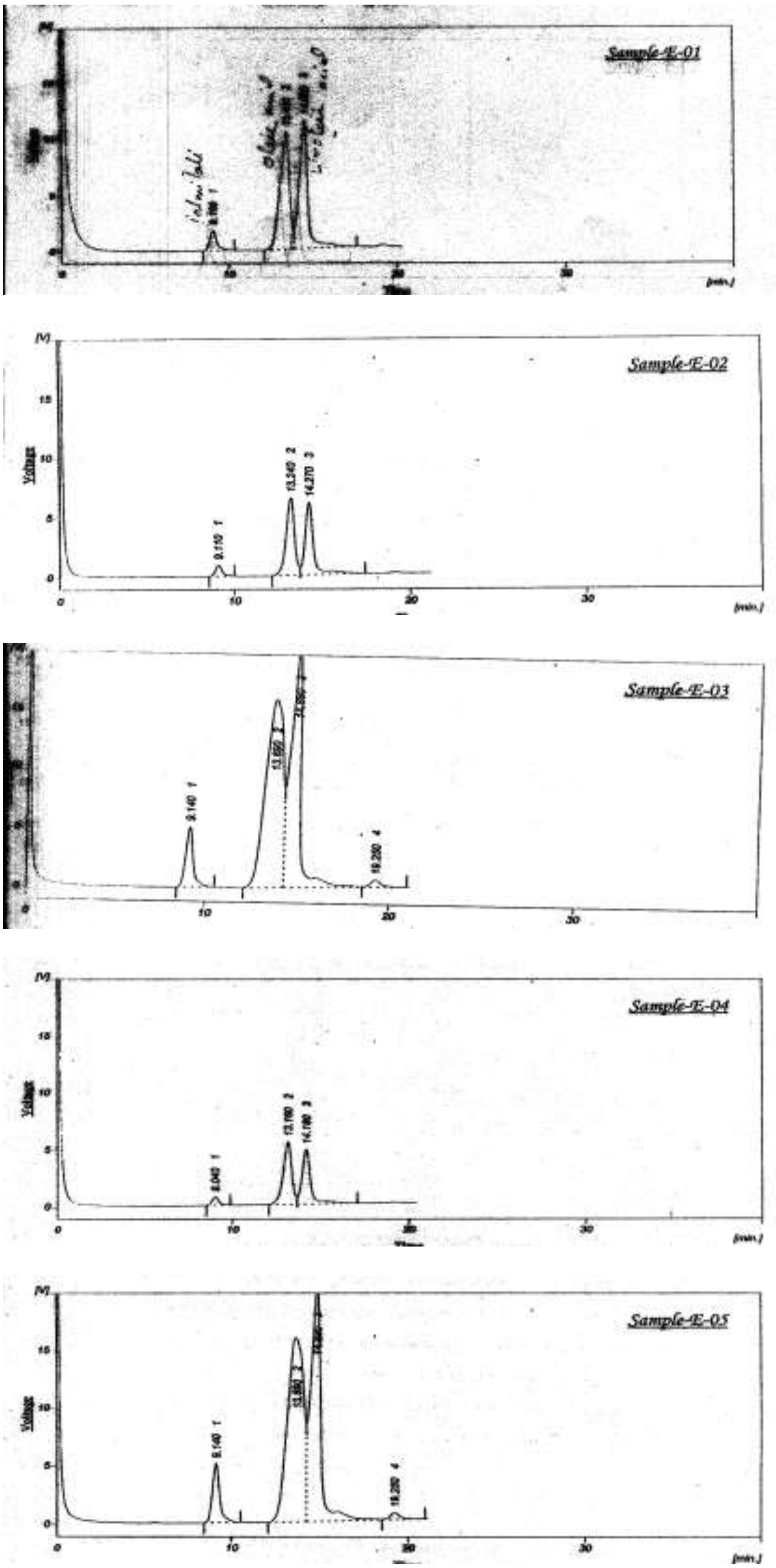

Fig. 2. Chromatograms of control & treated plant seed oil

As far as proximate analysis is considered (Table 2) no significant effect was observed as compared to untreated plant. The variety of sunflower seeds under study showed the oil content and composition of oil as described in Table 3. Percentage of oil content showed an increase in the yield of oil content upon treatment with humic acid, 0.2 % treated seeds showed optimum yield. However, oleic acid which is desirable in high percentage in commercial preparations is present in E-03 as well as in E-05. Chromatographic analysis of methylated esters showed (Fig. 2). Oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) are present in about 50:50 ratio. In reported literature data usually oleic acid content is less in untreated plant. Palmitic acid content is 7.22 % with 0.200 % humic acid was observed in maximum yield consistent with the reported values. (Gupta, 2001).

In brief when the diluted solution of humate is sprayed on the soil it complexes with the essential mineral ions (macro and micro nutrients) present in the soil and release these ions slowly to crop required. It makes good soil structure and saves the soil from leaching effect which in turn increases the yield of oil content and dry seed weight. Furthermore, the addition of humate improves seed germination and stimulates beneficial microbial activity. Hence humic acid is a good soil conditioner/plant hormone for edible oil (sunflower) cultivation and may be used without addition of any synthetic fertilizer.

CONCLUSION

Present study showed promising effect of humic acid as indigenous, cheap organic fertilizer derived from Pakistani lignite on yield of oil content of sunflower. In a recent economical report, to meet bulk of the edible oil national demand Government of Pakistan (GoP) has decided to increase the area under sunflower cultivation by 10 % for on-going financial year (FY 2008-09) by growing the crop over 1.1 million acres against one million last year to produce 2,23,000 tons of edible oil. Therefore, it is suggested that some more varieties in other environment should to be tried for oil production after treatment with the organic fertilizer.

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