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Research Article

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Cotton Seed oil cake as a valuable Source of Plant Nutrients for Sustainable Agriculture

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Abstract Cotton tree is often called silk cotton tree containing non edible oil in its seeds and grows widely in hilly areas in Bangladesh. Oil extracted from the seeds of this plant is used as energy source as well as in tanneries while the cake (a byproduct after extracting oil) was found to be rich in all plant nutrients. In order to reduce the production cost by minimizing or utterly eliminating the use of chemical fertilizers, decreasing environmental hazards, improve soil structure, promote leveraging agriculture and obtain high quality crops, cotton seed oil cake getting importance due its rich mineral contents. Cotton seed oil cake was assessed and it was found to be a potential source of bio-fertilizer due to richness of minerals in the form of plant nutrients. The analysis of valuable elements present in this oil cake was done by Atomic absorption spectrophotometer.

Keywords Cotton seed oil cake, Bio-fertilizer, Atomic Absorption Spectrophotometer, elemental compositions

1. Introduction

Cotton seed oil cake (a byproduct after extracting oil) is obtained from the seeds of cotton plant of various species, mainly *Gossypium hirsutum* and *Gossypium herbaceum*. Cotton grown for oil extraction is one of the big four genetically modified crops grown around the world, next to soy, corn, and rapeseed (canola) [1]. The cotton seed has a similar structure to those of other oilseed such as sunflower seed, having an oil bearing kernel surrounded by a hard outer hull; in processing, the oil is extracted from the kernel.

The cotton plant is a shrub native to tropical and subtropical regions around the world, including the Americas, Africa, and India. In Bangladesh, the trees of Carpash (silk cotton), the *Gossypium herbaceum*, that are found in almost every district of this country. The cotton seed oil cake is a byproduct obtained from cotton seeds with hot pressing and it is used in the production of bio-oil [1]. Although the importance of chemical fertilizers, many constraints have been raised such as their adverse impacts on the public health, environment, increasing the production cost and deterioration of soil fertility [2]. It is very important to find alternative methods for supplying nutrients to the growing plants to confront the previous problems. Now a days, various researchers consider the utilization of organic and bio-fertilizers as promising alternative nutrition especially for developing countries.

Organic fertilization provides the means for stabilizing soil fertility (especially in newly reclaimed soils) converting nitrogen in less soluble form is the main advantage of organic fertilizers compared to chemical fertilizers. The favorable effect of cattle manure on the vegetative growth of some medicinal plant have been reported by numerous investigators. The vegetative growth, fruit as well as oil yields were enhanced by using organic manure [3,4]. Moreover, the photosynthetic pigments and carbohydrate percentage and N, P and K contents [5, 3] were also enhanced. Recent awareness has been offered to reduce pollution practices in sustainable agriculture one of the way to minimize soil pollution is using bio-stimulants compounds without causing any harmful effects on aerial and soil environment to retard nitrification for sufficiently longer time and increase the soil fertility [6]. The effective utilization of bio-fertilizers for crops not only provides economic benefits to the procedures, but also improves, maintains the soil fertility and sustainability in natural soil eco system. The beneficial effects of plant growth promoting rhizobacteria on growth are not only through Nitrogen fixed in the rhizosphere, but also related to the ability of these bacteria to synthesize antibiotics and growth-promoting substances including phytohormones and sometimes the ability to solubilize phosphates; the use of phosphates solubilizing bacteria becomes necessary to minimize the dose of chemical P fertilizer that cause environmental pollution [7, 8].

The mix treatment of nitrogen fixing bacteria and phosphate solubilizing bacteria resulted in the maximum increase in most of the growth and yield parameters of several medicinal and aromatic plants [9, 10, 11, 12, 8, 4]. The essential oil content and its main components of various Apiaceae fruits are increased by using bio-fertilizers. In addition, carbohydrate percentage as well as nitrogen, phosphorus and potassium content in leaves were also promoted [4, 10-13].

The combination of organic manure and bio-fertilizers prove to be the superior treatment compared to the individual application, which reflected in the greatest influence on growth, yield, oil content and chemical constituents of different medicinal and aromatic plants [7, 14- 16].

Although bio-fertilizers are known to play an important role in plant production, little is known about the interactive effects of both of them on growth and secondary metabolites accumulation in medicinal plants. To the best of our knowledge, no systemic studies have been conducted to analyze the mineral contents of cotton seed oil cake so far. So, the aim of this study was to investigate the elemental compositions in cotton seed oil cake and confirm the usability of it as a bio-fertilizer.

2. Materials and Methods

2.1 Collection of plant material

Different varieties of cottons are seen in Bangladesh. They are Shimul, Carpus, Akhondo etc. In this context Carpash (Gossypium herbaceum) seeds have been collected from Nilphamari, the district in Rajshahi, Bangladesh for getting oil cake after extraction of oil from the seeds and then used for subsequent analyses.

2.2. Reagents and Standards

All reagents used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Petroleum ether (b.p 40-60℃, Merck, Germany) of AR grade, under normal atmospheric pressure was employed for extraction of plant material. Solvent from extract were recovered under distillation and the dried extracts were preserved in a refrigerator.

2.3. Extraction & preparation of cotton seed oil cake

The cotton seeds were cleaned to separate from dirt and grounded to obtain powder so that maximum particle exposure was got for extraction of oil. About 100 g of powder were extracted with ANALAR petroleum ether (b.p 40^0C -60^0C) in a soxhlet apparatus for 72h. The extract was first filtered and then vacuum distilled to remove solvent completely. The color of the extracted oil was chocolate maroon. Then the extracted oil was filtered through ANALAR activated charcoal. Finally, cotton seed oil cake (a byproduct after getting oil) was obtained. The yield of cotton seed oil cake was calculated (85%) and stored.

2.4. The proximate Analysis

Cotton seed oil cake was subjected to determine the percentage of moisture, ash, water soluble and insoluble ash, carbohydrate content according to the standard methods.

2.4.1. Moisture and Dry matter contents

The Moisture content was determined by heating the samples in an electric oven at 105̊-110̊C until constant weight (6-10 hours) [17] . The percentage was calculated by

$$Moisture\ content(\%) = \frac{\text{Weight of moisture}}{\text{Weight of sample taken}} \times 100$$

Dry matter (%) = 100-moisture%

2.4.2. Ash Contents

Ash was determined by incineration of the moisture free samples at about 600℃ (about 6-12 hours) in a temperature controlled Muffle furnace until ash becomes almost white or grayish white in color [17]. The percentage of ash was calculated by

$$Ash\ content\ on\ dry\ weight\ basis(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

2.4.3. Water Soluble Ash

Water soluble ash was determined by boiling the ash sample of cotton seed oil cake separately with 25ml distilled water for 5 minutes and then insoluble matter was thus collected [18]. Then they were dried, ignited at 450℃ and weighed. The percentage from the ash taken was calculated by

$$Water\ soluble\ ash\% = \frac{(\text{Weight of ash taken} - \text{Wight of water in soluble ash}\ ) \times \text{Ash Content}(\%)}{\text{Weight of ash taken}}$$

2.4.4. Mineral Compositions

Nitric acid (69%, Merck India) and Percholoric acid (70%, Merck India) was used for the digestion of samples to quantify mineral compositions in dried cotton seed oil cake. For standard calibration of respective elements Na, K, Ca, Mg, Fe, Zn, Ni, Mn, Cu, Pb standard solution (100mg/ml) from were purchased from Hach (Germany). The respective desired standard from the stock solution using lab made double distilled water were prepared.

2.4.5. Ashing and digestion of plant parts

Accurately 2.0g of cotton seed oil cake was taken in a porcelain crucible and heated to about 650℃ and cooled and was weighed. The crucible with sample was placed in the Bunsen burner (at low flow rate gas) until the smoke ceased. Then the crucible was placed in a muffle furnace at 525 °C for about 8-10 hours to obtain carbon free white ash. It was then cooled in desiccators and weighed. This procedure was repeated till the color of the ash was changed to almost white as well as constant weight was obtained. About 1.0g ash sample for cotton seed oil cake was taken separately in 50ml volumetric flask and then 15ml 1M HNO_3 acid was added. Then the flask was placed on magnetic stirrer heater in fume hood for four hours at 250 °C.When the color of the solution was changed to milky solutions, it was cooled for 10 minutes and then 7.5ml concentrated perchloric acid ($HClO_4$) was added. Then it was heated until colorless solution was obtained. For the determination of dissolved elements, the sample was filtered through 0.45 micron filter paper. In all the cases, the pH of the sample was maintained and verified to be less than 2.0 prior to analysis [19]. The standard working solution of interest was prepared to make the standard calibration curve. Absorption for a sample solution used the calibration curve to determine the concentration of particular element in that sample.

2.4.6. Analytical procedure

Among all elements only Sodium (Na) and Potassium (K) were estimated by using flame photometer (Model AnA-135, OSK, Japan). Most of the elements like Calcium, Magnesium, Iron(Fe), Zinc (Zn), Copper (Cu), Nickel (Ni), Lead (Pb) and Manganese [20] in cotton seed oil cake were analyzed by using Atomic Absorption Spectrophotometer (Varian, AA 240FS, Australia) which was equipped with flame and graphite furnace. For the experiment, air acetylene flame mode were used. The condition fixed with acetylene 1.8 l/min and air 15 l/min, argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis.

2.4.7. Carbohydrate Content

Carbohydrate content of cotton seed oil cake was estimated by subtracting the sum of the protein, fat, ash and crude fiber from the dry sample.

Carbohydrate (%)= 100- (Protein+Fat+Ash+Crude Fibre)

Carbohydrate × 4) cal/g

3. Results and Discussion

Proximate composition of cotton seed oil cake was recorded and the results are presented in Table 1 & Figure-1 respectively.

Table 1: Proximate composition (%) cotton seed oil cake

Test parameters	Cotton seed oil cake
	Percent (%) Composition
Moisture	11.88 ±0.23
Organic matter	89.04±0.23
Ash on drying	34.55±0.10
Water soluble part	4.00±0.02
Water insoluble part	90.00±0.02
Carbohydrate	32.65±0.30

Data are expressed as Mean ± SD (n=3)

Cotton seed oil cake has high ash content (34.55%), which indicates the presence of high quality of mineral contents. The moisture content for this oil cake was determined on the fresh weight basis whereas the organic content was calculated on the dry weight basis. Water soluble ash indicates the content of soluble minerals [21] . The present study showed water soluble part in cotton seed oil cake is 4.00% and water insoluble part is 90%.

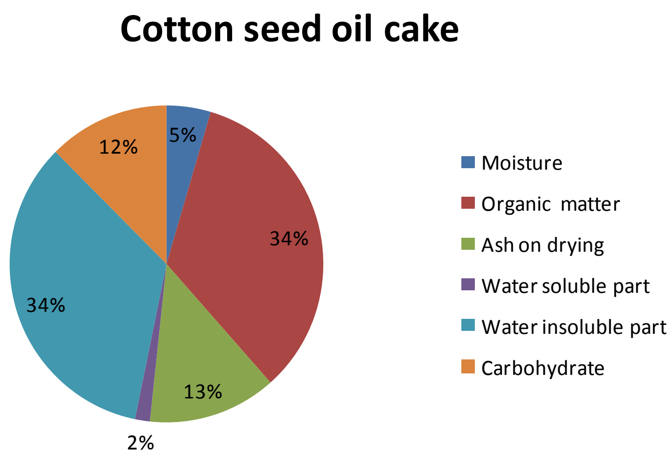

Figure 1: Proximate analysis of Cotton seed oil cake

Plants require energy in chemical form so they can grow and carry out basic life functions like all living organisms. Plants produce, store and burn carbohydrates in the form of sugar to provide themselves with energy [22]. The present study showed the presence of carbohydrate content in the cotton seed oil cake is 32.65%.

A total of 10 elements i.e. Na, K, Ca, Mg, Fe, Zn, Cu, Pb, Mn and Ni were analyzed from cotton seed oil cake by AAS which are accountable for growth of plants in agricultural sector. The result of the analysis is presented in the Table 2 & Figure-2 respectively. It may be noted each result is an average of at least three independent measurements. For the formation of secondary metabolites which are responsible for pharmacological actions these elements play a vital role.

Table 2: Mineral compositions (mg/kg) of cotton seed oil cake

Elements	Cotton seed oil cake (dry weight basis, mg/kg)
Na	184.91±0.021
K	432.01±0.093
Ca	63.87±0.005
Mg	146.70±0.007
Fe	1.27±0.0001
Zn	0.262±<0.001
Cu	0.083±0.01

Ni	0.066±<0.001
Mn	0.122±0.001
Pb	0.003±<0.001

Measured values are mean ± Standard Deviation (SD) of three replicate analysis

From the present study it was observed that Potassium showed higher concentration (432.01 mg/kg) than the other elemental concentration. In phytosynthesis, Potassium regulates the opening and closing of stomata and therefore, regulates CO_2 uptake. Also Potassium triggers activation of enzymes and is essential for production of ATP. Potassium plays a major role in the regulation of water in plants (osmo-regulation) [23].

Although Sodium (Na^+) is not essential for most plants but it can be beneficial to plants in many conditions particularly when Potassium (K^+) is deficient. As such it can be regarded as "functional nutrient". It has been observed many times that during K^+ deficiency many (glycophytic) plants respond positively to Na^+ fertilization for salt-tolerant (halophytic) plants, even high concentration of Na^+ promote growth. From the present study it was found the sodium concentration is 184.91 mg/kg in cotton seed oil cake [24].

The nutrients of plants need are categorized into three different categories, main nutrients, micro nutrients and trace elements. N, P & K are considered primary nutrients. Ca along with Fe, Zn, Mn, Mg, S etc. are micro nutrients and some elements such as Molybdunem, Nickel, Selenium etc. are considered trace elements. Even though the main nutrients are very important for most plants, Calcium is even more important for some plants like tomatoes. In the form of Calcium pectate, Ca holds the cell walls of plants together. It also activates specific plant enzymes, which sends signals to the plant cells that coordinate growth activities. From the present study it was found the Calcium concentration is 63.87 mg/kg in cotton seed oil cake [25].

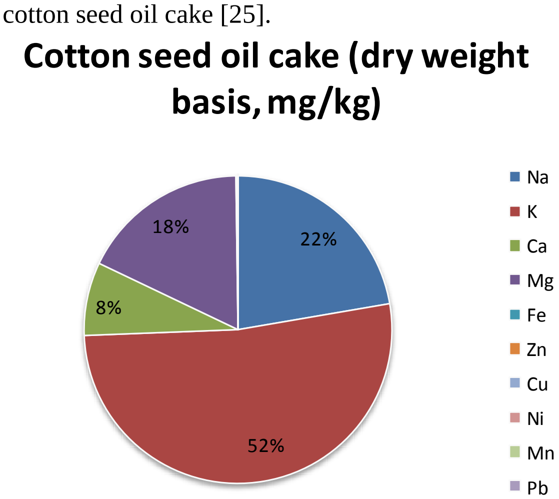

Figure 2: Mineral compositions (mg/kg) of Cotton seed oil cake

Magnesium is involved in many physiological and biochemical processes; it is an essential element for plant growth and development and plays a key role in plant defense mechanisms in abiotic stress solutions. The most commonly known function of Mg in plants is probably its role as the central action of the chlorophyll molecule in the light absorbing complex of chloroplasts and its contribution to photosynthetic fixation of CO_2. From the present study it was found the Magnesium concentration is 146.70 mg/kg in cotton seed oil cake [26].

Zinc is plant micro nutrient which is involved in many physiological functions. Its inadequate supply will reduce crop yields. Zinc deficiencies can affect plant by stunting its growth, decreasing number of tillers, chlorosis and smaller leaves, increasing crop maturity period, spikelet sterility and inferior quality of harvested products. From the present study it was found the Zinc concentration is 0.262 mg/kg in cotton seed oil cake [27].

Iron is an essential micro nutrient for almost all living organisms because it plays critical role in metabolic processes such as DNA synthesis, respiration and photo-synthesis. From the present study it was found the Iron concentration 1.26 mg/kg in cotton seed oil cake [28].

Manganese (Mn) is an essential micro nutrients in most organisms. In plants, it participates in the structure of photo-synthetic proteins and enzymes. Its deficit is dangerous for chloroplast because it affects the water-splitting system of photo-system || (PS ||) which provides the necessary electrons for photo-synthesis. From the present study it was found the Manganese concentration is 0.122 mg/kg in cotton seed oil cake [29].

Lead (Pb) causes a number of toxicity symptoms in plants e.g. stunted growth, chlorosis and backening of root system. Pb inhibits photo-synthesis, upsets mineral nutrition and water balance. From the present study it was found the Lead concentration is 0.003 mg/kg in cotton seed oil cake which lies in the permissible range [30].

Copper (Cu) is an essential metal for normal plant growth and development, although it is potentially toxic. Cu participates in numerous physiological processes and is essential co-factor for many melalloproteins, however problem arises when excess Cu is present in cells. Excess Cu inhibits plant growth and impairs important cellular processes. From the present study it was found the Copper concentration 0.083 mg/kg in cotton seed oil cake [31].

4. Conclusion

From the above study (Proximate analysis & elemental analysis) suggested that the cotton seed oil cake contain sufficient amount of macro and micro elements which justify its suitability in agro-industrial uses. Cotton seed oil cake can be used for the production of biofertilizer in agricultural sectors. However, more detailed analysis of chemical composition of cotton seed oil cake is required.

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