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Effects of distillery effluent and micro alga (*Chroococcus minutus*) treated effluent on germination and seedling growth of *Cicer arietinum* L

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Abstract

The study has been focused on effect of untreated distillery effluent and algal treated effluent (*Chroococcus minutus*) on germination, physiology and biochemical characteristic features of *Cicer arietinum*. The seed treated with different concentration of distillery effluent (25, 50, 75 and 100%) and algal treated effluent. The germination percentage decreased with a rise in distillery effluent concentration. Effluent at 25% concentration induced the growth in, shoot length, leaf area, photosynthetic pigments, carbohydrates, protein, lipids and minerals. While effluent 50% concentration had reduced the following parameters shoot length, leaf area and biochemical attributes as compared to the control and 25% effluent treatment. At 100% effluent concentration growth were completely retarded. When algal treated effluent plant shows increased germination percentage rate from low concentration to high concentration, such concentration was 25 to 100% as compared to that of control and untreated distillery effluent. The results revealed that microalga *Chroococcus minutus* treated effluent influence the better growth and development of *Cicer arietinum*.

Keywords: distillery effluent, *Chroococcus minutus*, *Cicer arietinum*, biochemical

1. Introduction

The industrial revolution is a great boon to mankind, but there is a wide range of environmental impacts created by industries. The majority of these industries are water based. Though the industrial use of water is very low as compared to agricultural use, the disposal of industrial effluents on land surface or water bodies make water resources unsuitable for other uses [1].

Wastewater treatment is an area of fundamental importance in environmental research, due to the ongoing global pollution problems caused by various wastewater streams. It is clear that an increased knowledge concerning waste degrading organisms could assist in the development of new technologies to facilitate better strategies for the treatment of contaminated water courses. The composition of industrial effluents is very variable, with the presence of various recalcitrant compounds making some wastewater streams more difficult to treat. Such is the case for melanoidins, which are complex biopolymers largely recalcitrant to microbial decomposition. They are discharged in huge quantities by cane molasses based distilleries and fermentation industries [2]. This industrial sector could be potentially problematic with respect to environmental pollution, and thus treatment of their effluents is the serious environmental concern.

Distilleries can be categorized among the most polluting industries generating large volume of wastewater, known as spent wash [3]. Distilleries generate wastewater at various stages in the manufacturing process as distillation, condenser cooling, fermenter cooling, fermentation and washing stages. The larger amount of the effluent is produced at distillation and condenser cooling stages [4]. The characteristics of the wastewater generated depend on the feedstock used. Distillery effluents are water pollutants not only as a result of their organic load, but also due to their dark brown color [5]. This color block out sunlight in rivers and other streams, and it is detrimental to aquatic life, as it affects photosynthesis by a reduction in the degree of sunlight penetration into these water courses. It also causes a reduction in soil alkalinity and an inhibition of seed germination.

In this regard, microalgae, and cyanobacteria offer an attractive alternative, based on the high versatility of their metabolic mechanisms which can be used for phycoremediation purposes. Mass cultivation of cyanobacteria is less expensive as well as, more-straight forward when compared to fungi and bacteria, and as has been said have the added advantage of oxygenating to the environment [6, 7]. The present study was to evaluate the extent and impacts of the untreated and algal treated industrial effluents on the germination, growth, physiology and biochemical properties of *Cicer arietinum*.

2. Materials and methods

2.1 collection of Effluent

Distillery effluent was collected from "Midas Golden Distillery Pvt. Ltd," which is located at Padappai, 36 km from Chennai. The geographical location of the Padappai town lies within North Latitude 12°50' and East Longitude 79°42'. The location, of Padappai falls in the South West portion of Tamilnadu, India. Distillery effluent is one such pollutant containing both organic and inorganic matters which are sources of enhanced growth of microalgae.

2.2 Effect on seed germination and seedling growth

Seeds of chickpea (*Cicer arietinum*, L.) were obtained from the Government seed Depot, Chennai-32 and were preserved in an incubator at 19 °C. The seeds were surface sterilized with 0.1% HgCl₂ for 3 to 5 minutes and then thoroughly washed with distilled water. After repeated washings with sterilized distilled water, the 20 sterilized seeds were sowing in each pot. Pots were labeled as per type, concentration of the effluent. Different concentrations distillery effluent (25, 50, 75 and 100%) and algal treated effluent of each effluent were made with distilled water. The seeds were irrigated with equal quantity of different concentration of untreated and algal (*Chroococcus minutus*) treated of effluent samples and the seeds irrigated with distilled water were taken as control. Respective effluent concentration were provided and incubated for three days at 26 ± 2 °C for germination. Daily observations were made for the germinated seeds. The number of seeds germinated in each treatment was recorded and the germination percentage and vigor index was calculated. The growth of the root and shoot length were measured with the help of meter scale. The dry weight of seedlings was taken after keeping them in hot air oven at 80°C for 24 hrs. The moisture content was obtained by the difference between the fresh weight and dry weight. Leaves of control and treated and algal treated effluents plants were used for the estimation of the Chlorophyll [8], carotenoids [9] proteins [10], total free sugars [11] and lipids [12] and minerals.

2.3 Statistical analysis

All data are expressed as mean ± standard deviation (SD). All analyses were carried out in triplicate and the difference between treatments was analyzed by one way ANOVA by using SPSS.17.0.

3. Results

3.1 Changes on Seed germination

The seeds of *Cicer arietinum* (chick pea) were irrigated with water (control), untreated distillery effluent and algal (*C.minutus*) treated effluent (Table.1). The plant *C. arietinum* was allowed to germinate in the control, untreated distillery effluent and effluent treated with *C. minutus* filtrates (Table.1).

The seeds irrigated with untreated effluent shows different rate of seed germination the maximum seed germination was observed in 25%; concentration and minimum seed germination was recorded at 50%. But in 75% and 100% there was no seed germination were observed as compared to that of control. Where-as seeds irrigated with algal treated effluent increased the germination percentage rate from low concentration to high concentration, such concentration was 25 to 100% as compared to that of control and untreated distillery effluent. The significant inhibition on seed germination was observed on untreated distillery effluent treated seed compared to the remaining to treatment.

3.2 Changes on growth parameters

The shoot length and leaf area parameters were analyzed in the plant

C. arietinum after 20 days of seed germination. Length of shoot and leaf area of

C. arietinum was observed after irrigated with 25% of untreated distillery effluent. In lower concentration (25%) of the distillery effluent, the shoot length and leaf area of *C. arietinum* plant was lower than that of control and treated distillery effluent with microalgal filtrates.

The reduced shoot length was observed in 25% concentration of untreated distillery effluent in seedlings of *C. arietinum*. Whereas, in the case of 25% (v/v) concentrations of treated distillery effluent with microalga, the reduction in shoot length was not so obvious. In concentrations of the treated distillery effluent with microalga, the shoot length was increased from 25% (v/v) onwards. The maximum shoot length (27.30 ± 1.0 cm) was observed in effluent treated with 100% concentration of microalga filtrates (*C. minutus*) and the minimum shoot length

(5.60 ± 0.1 cm) was observed in 25% untreated distillery effluent where as in control it showed shoot length (5.60 ± 0.1 cm) and leaf area was (0.60 ± 0.1 cm) (Table.2).

The leaf area parameters were analysed in *C. arietinum* the ranges from 0.50 ± 0.1 to 1.30 ± 0.1 cm. The highest leaf area was observed (1.30 ± 0.1 cm) in 100% distillery effluent treated with microalga (*C. minutus*) and the lowest leaf area was observed (0.50 ± 0.1 cm) in (25%) of untreated distillery effluent (Table.2).

3.3 Changes on Pigments and biochemical composition

The total chlorophyll content of the plant *C. arietinum* was higher in control (1.908 ± 0.05 mg/g) and for the microalga filtrates *C. minutus* the total chlorophyll content was (2.0714 ± 0.05 mg/g) (dry wt) and for the untreated distillery effluent it was 0.924 ± 0.004 mg/g (dry wt) (Table.3). The total chlorophyll content was decreased in the plant treated with untreated distillery effluent it was proved its toxic effect. Statistically significant ($p < 0.05$) increase in chlorophyll content occurs between control, untreated effluent and effluent treated microalga filtrates (*C. minutus*). The carotenoid content of the plant *C. arietinum* in untreated distillery effluent was 0.384 ± 0.07 mg/g (dry wt) and in those treated with microalga filtrates (*C. minutus*) it was 1.1910 ± 0.05 mg/g (dry wt) and in the control, it was 0.849 ± 0.01 mg/g (dry wt). Statistically significant increase in carotenoid content occurs between control, untreated effluent and effluent treated microalga filtrates (*C. minutus*).

The biochemical composition such as carbohydrate, protein and lipid were estimated for the plant *C. arietinum* (Table.4). The carbohydrate content of control was 7.9577 ± 0.10 mg/g (dry wt), in untreated distillery effluent was 4.436 ± 0.12 mg/g (dry wt), when the effluent treated with *C. minutus* it was 12.112 ± 0.12 mg/g (dry wt) (Table.4) respectively.

The protein content of control was 1.9031 ± 0.23 mg/g (dry wt), in untreated distillery effluent it was 0.7593 ± 0.20 mg/g (dry wt), when the effluent treated with *C. minutus* it was 6.384 ± 0.14 mg/g (dry wt) (Table.4) respectively.

The lipid content of control was 0.3732 ± 0.10 mg/g (dry wt), for untreated effluent it was 0.236 ± 0.07 mg/g (dry wt), and effluent treated with *C. minutus* it was 0.5489 ± 0.13 mg/g (dry wt) (Table.4) respectively. There is an increased biochemical composition like chlorophyll, carotenoids, carbohydrate, protein and lipid content and were observed in microalga treated distillery effluent irrigated plants. Statistically significant ($p < 0.05$) increase in carbohydrate, protein and lipid content occurs between control, untreated effluent and effluent treated microalga filtrates (*C. minutus*). When the plant *C. arietinum* was treated with control, the concentration of minerals like nitrogen, phosphorus, potassium, iron and magnesium was 25% higher than that of untreated distillery effluent (Table.5). Whereas, the plant *C. arietinum* was treated with 25% of untreated distillery effluent the concentration of minerals like zinc, copper and sulphur, was higher than the control, whereas, the nitrogen, phosphorus, potassium and iron were lower than control and treated distillery effluent.

The plant *C. arietinum* was irrigated with algal (*C. minutus*) treated distillery effluent the concentration of minerals like nitrogen, phosphorus, potassium, iron, magnesium, zinc, copper and sulphur was higher than that of control and 25% of untreated distillery effluent (Table.5). The results of the present study proved that the algal (*C. minutus*) treated distillery effluent have increased the mineral composition in the plant *C. arietinum*.

4. Discussion

In the present study shows that percentage of seed germination rate was high in 25% concentration of the distillery effluent treated plant than other concentration like 70 and 100%. Because, when the concentration increased it shows more toxicity, it affects germination capacity of the seeds. The inhibitory effects of distillery effluent on seed germination and seedling growth are conclusive and emphasize that various metallic and nonmetallic elements acts as nutrients, but at higher concentration they show toxic effects on seed germination and seedling growth. A similar kind of observation was reported by [13] Ranjeet Singh Baghel (2008) [14]. Veer pratap and Yogesh kumar Sharma (2010) reported that the osmotic pressure, stress is one of the main causes in industrial effluent disorders in *Phaseolus mungo*.

The results of the present study revealed that, algal treated effluent increased germination percentage rate compare to the control. Also, the algal treated effluent increased shoot length of the plant compare to the control and untreated effluent. The untreated effluent concentration was increased germination capacity of the seeds also decreased, that shows more toxicity of untreated effluents when compared to algal treated distillery effluent and control. The percentage of germination and rate of germination of seeds are highly

affected by the pH of the effluent. A similar kind of observation was observed by Bhaumik and Mondal (2012) [15]. This is quite possible because the seed physiology is greatly influenced by the acidity of the medium [16]. The reduction in germination and growth may be due to the interference of pollutants present in the distillery effluent. A similar inhibition of germination was observed by [17] Chien and Kao, (1995) in rice; [18] Boussama *et al.*, (1999) in barely; [19] Mishra *et al.*, (1995) in maize. There is a gradual decrease in seedling growth with increase in different concentration of the distillery effluent. In the present study, germination was significantly reduced in higher concentration, but in lower concentration germination was although affected but not completely suppressed. The slow and poor germination under water stress is obviously due to decreased water potential of the germination medium, which restricts the water availability to the seeds [20].

The root and shoot length are also related to the fact that some of the nutrients present in the effluent are essentials, but at above a particular concentration, they become hazardous. [21] Prabhakar *et al.*, (2006) reported the growth promotion in terms of root and shoot length at higher concentrations of the fertilizer effluent which was too toxic to the plants. Similar observations have also reported by [22] Sundramoorthy *et al.*, (2001) in groundnut varieties; [23] Mall *et al.*, (2005) in *Phaseolus aureus* and [24] Deka *et al.*, (2011) in *Pisum sativum*.

The inhibitory effect of seed germination by untreated distillery effluent may be due to excess of total nitrogen, phosphate, potassium, sulphate and chloride present in the effluent which inhibits plant growth by affecting water absorption and other metabolic processes in the plants. In this present study, some agricultural crops, a maximum percentage of germination was recorded in treated effluents. The increase in germination and seedling growth in treating effluent are due to the presence of sufficient nutrient in the effluent. The excess minerals promote the germination and growth.

It is concluded that the growth characters (shoot length and leaf area) are positively affected by untreated distillery effluent concentration of 25 to 100%. The increase in such characters was insignificant when effluent treated microalgal filtrates were used at concentrations higher than 50%. These results are in coincidence with those obtained by [25] Omran *et al.*, (2003).

The properly treated distillery effluent used for agricultural purpose, provides not only water to the plants, but also increases the nutrient availability of the plants and efficiency of the fertilizer applied. These results indicate that the treated distillery effluent can be successfully utilized for irrigating agricultural purpose, if proper combinations of management techniques are employed to ensure effectiveness and environmental safety.

The use of recycled water for the irrigation of crops has benefits in using a resource that would otherwise be discarded and wasted. Using recycled water also reduces the pressures on the environment by reducing the use of groundwater. Recycling and reuse of water in agriculture is not only helpful in conserving the water for irrigation but also the plant nutrients. So, it is essential that the implication of the use of treated industrial effluents in the crop fields and their effects should be assessed before they are recommended for use in irrigation. The present research and development will also improve the process of recycled water

for irrigation purposes as well as increasing public confidence. The challenge facing wastewater reuse is to minimize such risks so as to maximize the net environmental gain.

Biochemical analysis of the plant chick pea (*C. arietinum*) treated with microalgal filtrates results in increased chlorophyll, carotenoids, carbohydrates, protein and lipid contents. In the present study, the total chlorophyll and carotenoid contents of *C. arietinum* was found to be higher in the effluent treated with microalga filtrates *C. minutus* than the control and untreated distillery effluent. Enhancement of chlorophyll could be due to high nutrient uptake, synthesis and translocation, probably, facilitated by optimum availability of iron and magnesium in the treated distillery effluent. Similar kind of observation was made by ^[26] Behra *et al.*, (1982). In the untreated distillery effluent, an overall decrease in chlorophyll content was recorded as compared to control ^[27]. Kamlesh Nath *et al.*, (2007) reported that the total chlorophyll content was found to be diminished from lower to higher concentration of the treated effluent.

In the present study the total chlorophyll and carotenoid contents *C. arietinum* were found to be higher in the effluent treated with microalgal filtrates than the control and untreated distillery effluent. Similar kind of observations was carried out by ^[28] Kotteswari (2012) in *Phaseolus mungo*; ^[29] Tharakeshwari and Shoba Jagannath (2011) in *Vigna unguiculata*.

The changes in total chlorophyll content indicated that the chlorophyll synthesizing capacity of the crop has diminished affecting the overall photosynthetic process. The significant fall in the chlorophyll content under the higher percentage of effluent concentration might have been due to the inhibitory effect of toxicants of effluent on chlorophyll synthesis in exposed plant. ^[30] Nagda *et al.*, (2006) have reported that the enrichment of chlorophyll could be due to the high nutrient uptake, synthesis and translocation most likely facilitated by optimal availability of iron and magnesium and also due to the reduction in phenolic content in the treated distillery effluent. Similar observations have been reported by ^[31] Sheela *et al.*, (2013) in *Solanum nigrum*. The increased in carotenoid content might be due to enhanced influence of nitrogen and other inorganic element present in the effluent. The decline in the levels of chlorophyll and carotenoids by untreated distillery effluent treated plant of chick pea suggests that the inhibition of chlorophyll by the heavy metals is present in distillery effluent.

4.3 Biochemical composition of the plant *C. arietinum*

The carbohydrate content was very high in algal treated effluent as compared to control and untreated effluent. A similar kind of observation was reported by ^[31] Sheela *et al.*, (2013) have reported that the rise in carbohydrate content in *Solanum nigrum* when the textile effluent was treated with *Chroococcus minutus*. Carbohydrate content increases by CO₂ enrichment have been observed previously in a number of species (Loehle, 1995) ^[32]. The increase in carbohydrate content at lower concentrations was mainly due to the availability of more nutrients in the effluent ^[33]. In the present study, a significant increase in carbohydrate content of *C. arietinum* when it was treated with microalga filtrates *C. minutus* may be due to the nutrients in their optimum quantity.

Similar to the carbohydrate the protein content also significantly increased in microalga treated effluent when compared to the control and untreated distillery effluent. Under the environmental stress conditions, the energy forming molecules may be troubled and subsequently carbohydrates and protein metabolites of the membrane were altered. Studies have shown that stress induced the decline in protein contents in plants and raised in soluble sugar content ^[34]. Protein content under heavy metal treatment may be affected due to enhanced protein hydrolysis resulting in decreased concentration of soluble protein ^[35] Melnichuk *et al.*, (1982) and Catalytic activity of lead ^[36].

The protein content was found to be decreased in untreated distillery effluent of chickpea because of toxicity. Due to this toxicity the protein is get denatured it reduce the protein content in untreated effluent plant. This was reported by ^[36] Tripathi and Gautham, 2007. The decreased photosynthesis and/or breakdown of existing protein or due to reduced *de novo* synthesis ^[37]. The enhanced protein denaturation and breakdown of existing protein to amino acid is the main cause of the reduction in the protein content ^[38]. The present study showed that the microalga *C. minutus* treated distillery effluent can be successfully utilized for agricultural purposes and also increased the nutrients present in the plants.

The algal treated effluent provoke the lipid content (*C. minutus*) when compared to the control and untreated distillery effluent. Similar kind of observation was observed by ^[31] Sheela *et al.*, (2013) and they have reported the rise in lipid content in *Solanum nigrum*, when the dye effluent was treated with *Chroococcus minutus*. An increase in lipid content at lower concentrations was mainly due to the availability of nutrients in the effluent ^[33]. The present study showed that the microalga *C. minutus* treated distillery effluent can be effectively used for various purposes and that it is safe for our environment.

Mineral plays a major role to improve the growth of the plants. When the

C. arietinum was treated with algal treated effluent it increased the amount of minerals in the plants when compared to control and untreated distillery effluent. Similarly ^[39], Singh (2009) had reported that soil in India is affected with deficiency of micronutrients. It is supposed that potassium does not participate directly in the formation of organic compounds present in plants, but plays an important role in respiration. The microalgal filtrate has the capacity to increase the potassium content in the leaf of plants as reported by ^[40] Stino *et al.*, (2009).

Similarly, nitrogen and phosphorus are another macro-nutrient essential for growth, which are taken up by microalgae as inorganic orthophosphate. Microalgae are able to assimilate phosphorus in excess, which is stored within the cells in the form of polyphosphate (volutin) granules. The phosphorus and nitrogen content also increased when the plant was treated with algal extracts when compare to control and untreated effluent.

The magnesium concentration was increased when the plant *C. arietinum* was irrigated with algal treated effluent. Magnesium is a main constituent of the chlorophyll molecule, and it is therefore energetically involved in photosynthesis. The sulphur uptake by *C. minutus* was significantly higher over control. This might be attributed to increase in growth. The benefits from sulphur fertilization of

crops can be traced to its role in protein development, to improvement of nitrogen, etc. [41]. Zinc is present in the soil in the form of divalent ions which are released by the weathering of minerals like magnetite, biotic and hornblende. They are gradually increased in the algal treated effluent than in the control and untreated effluent. Iron is absorbed in the form of ferrous ions from the soil solution. These results are supported by the findings of [42] Khandagave *et al.*, (1996) [43].

Microalgae biomass recovered from such systems has a variety of potential on and off-farm uses. Combining conventional cropping systems with an algal treatment system could facilitate more efficient crop production and farm nutrient management, allowing distillery operations to be environmentally sustainable on fewer areas. Yet, the potential for high protein yields and nutrient uptake rates justifies consideration of microalgal production system for nutrient recovery from distillery effluent. The present study, clearly proves that the microalga filtrates *C. minutus* significantly increases the mineral contents in leaf tissues of the plant *C. arietinum* when compared to that of control and untreated distillery effluent. The microalgal filtrates *C.*

minutus have triggered effects on growth and minerals in *C. arietinum*.

From the above results, it can be concluded that microalga (*C. minutus*) utilizes the organic and inorganic matters present in the effluent as nutrients. After nutrient depletion, microalga start undergoing degeneration, leading to the release of several substances back into the soil. As already pointed out, the changes in biochemical contents of microalga might be due to the distillery effluent which affects the microalgae metabolism at multiple sites [43].

5. Conclusion

The decrease of pollution in effluent treated seedlings suggests pollution injury. The present study proved that the microalga *C. minutus* treated distillery effluent can be successfully utilized for irrigating agricultural crops if good combinations of phycoremediation method are prudently worked to make sure effectiveness and an ecological safety. These kinds of economic considerations must be dealt with if distillery effluent treatment is to be mandated in the future.

Table 1: Effect of distillery effluent on seed germination in *C. arietinum*.

Effluent Concentration	No. of Seeds	Germination Percentage		
		Control	Untreated effluent	Effluent treated with <i>C. minutus</i>
25%	25	92	36	40
50%	25	92	32	52
75%	25	92	0	72
100%	25	92	0	96

Table 2: Effect of untreated and treated distillery effluent on early in seedling growth *C. arietinum*.

Irrigation Period	Treatment	Shoot length (cm)	Leaf area (cm)
5 th day	Control	5.60 ± 0.01 ^d	0.60 ± 0.1 ^a
	Untreated distillery effluent	5.60 ± 0.1 ^b	0.50 ± 0.1 ^{aa}
	Effluent treated with <i>C. minutus</i>	7.30 ± 0.1 ^d	0.70 ± 0.1 ^a
10 th day	Control	12.20 ± 0.1 ^e	0.70 ± 0.1 ^{ab}
	Untreated distillery effluent	11.30 ± 0.1 ^c	0.50 ± 0.1 ^{aa}
	Effluent treated with <i>C. minutus</i>	15.80 ± 0.1 ^e	0.80 ± 0.1 ^{ab}
15 th day	Control	16.60 ± 0.1 ^f	0.90 ± 0.1 ^{bc}
	Untreated distillery effluent	12.80 ± 0.1 ^d	0.60 ± 0.1 ^{aa}
	Effluent treated with <i>C. minutus</i>	23.90 ± 1.0 ^f	1.0 ± 0.1 ^b
20 th day	Control	21.70 ± 0.1 ^g	1.00 ± 0.1 ^c
	Untreated distillery effluent	14.60 ± 1.0 ^e	0.60 ± 0.1 ^{aa}
	Effluent treated with <i>C. minutus</i>	27.30 ± 1.0 ^g	1.30 ± 0.1 ^c

Dry wt (mg/g) Values are expressed as mean ± SD as Anova at $p < 0.05\%$ level of triplicates

Table 3: Effect of microalgae filtrates on photosynthetic pigments of *C. arietinum*.

S. No	Pigment composition	Control	Untreated Effluent	Effluent treated with <i>C. minutus</i>
1	Chlorophyll	1.908 ± 0.05 ^c	0.924 ± 0.004 ^b	2.0714 ± 0.05 ^c
2	Carotenoids	0.849 ± 0.01 ^b	0.384 ± 0.07 ^c	1.1910 ± 0.05 ^b
	F-Value	1538.11	871.28	1097.49
	P-Value	0.000	0.000	0.000

Dry wt (mg/g)

Values are expressed as mean ± SD as Anova at $p < 0.05\%$ level of triplicates.

Means in each column with different superscripts(s) are significant different ($p < 0.05$)

Table 4: Effect of microalgal filtrates on Biochemical composition of *C. arietinum*

S. No	Biochemical composition	Control	Untreated Effluent	Effluent treated with <i>C. minutus</i>
1	Carbohydrates	7.9577 ± 0.10 ^c	4.436 ± 0.12 ^c	12.112 ± 0.12 ^c
2	Proteins	1.9031 ± 0.23 ^b	0.759 ± 0.20 ^b	6.384 ± 0.14 ^b
3	Lipids	0.3732 ± 0.10 ^a	0.236 ± 0.07 ^a	0.5489 ± 0.13 ^a
	F-Value	5766.49	3003.88	10186.42
	P-Value	0.000	0.000	0.000

Dry wt (mg/g)

Values are expressed as mean ± SD as Anova at $p < 0.05\%$ level of triplicates.

Table 5: Mineral composition of leaves treated with distillery effluent in *C. arietinum*.

S. No.	Minerals	Control	Untreated effluent	Effluent treated with <i>C. minutus</i>
1	Nitrogen	256.30 ± 0.08 ^h	73.63 ± 0.25 ^h	302.40 ± 0.08 ^h
2	Phosphorus	149.20 ± 1.01 ^g	48.46 ± 0.15 ^g	164.20 ± 0.06 ^g
3	Potassium	89.45 ± 0.86 ^f	46.14 ± 0.12 ^f	103.61 ± 0.51 ^f
4	Iron	11.84 ± 0.13 ^d	9.74 ± 0.02 ^c	14.58 ± 0.51 ^d
5	Zinc	6.34 ± 0.20 ^c	9.20 ± 0.02 ^b	8.45 ± 0.30 ^c
6	Magnesium	27.45 ± 0.07 ^e	15.22 ± 0.02 ^d	43.40 ± 0.18 ^e
7	Copper	4.93 ± 0.06 ^b	19.76 ± 0.05 ^e	6.39 ± 0.17 ^b
8	Sulphur	0.94 ± 0.07 ^a	2.32 ± 0.01 ^a	2.71 ± 0.08 ^a
	F value	110176.9	142162.3	388888.1
	P value	0.000	0.000	0.000

Dry wt (mg/g) Values are expressed as mean ± Standard deviation as Anova at $p < 0.05\%$ level of triplicates.

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