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Research Article

Bioaccumulation Potential of *Cynodon dactylon* Linn. in Crude Oil Contaminated Soil.

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Abstract

A greenhouse experiment was conducted to investigate the growth of *Cynodon dactylon* Linn. in soils contaminated by various concentrations of crude oil with a view to assessing its phytoremediating potential. The crude oil prepared at different concentrations of 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 (w/v) acted as contaminants on 3kg each of air-dried soil collected from the Obafemi Awolowo University Biological garden which is rich in organic matter. Each treatment was replicated 10 times in complete randomized design. Significantly ($p<0.05$) highest residual total petroleum hydrocarbon content (THC) uptake (39.26 ppm) was obtained in the plant at 12.5 ml contamination with the least residual total petroleum hydrocarbon content uptake (12.79 ppm) obtained at 2.5 ml contamination. With increased contamination, there was positive correlation with residual total petroleum hydrocarbon content uptake in the plants. No detectable amount of petroleum hydrocarbon content was found in the soil at the end of the experiment. The study concluded that *C. dactylon* plant could be effectively used in the phytoremediation of crude oil contaminated soil without addition of soil amendment.

Keywords: Crude Oil, *Cynodon Dactylon*, Oil Spillage, Petroleum Hydrocarbon, Phytoremediation,Soil Contamination

Introduction

Oil exploration and exploitation in Nigeria has evolved through a long history (Olujimi et al., 2011). However, the exploration and exploitation of this glossy façade of financial benefits have created serious environmental pollution for host communities. Nigeria has the largest natural gas reserve and the second largest oil reserve in Africa. The environmental impacts of exploration and exploitation have been of serious concern to Nigerian government regulatory agencies and other environmental outfits. Ghosh and Singh (2005) reported oil exploration and exploitation, accidental and process spillage among others, as major factors

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responsible for the migration of pollutants into the soil and water ecosystem. Okoloko (1974) reported the main causes of oil pollution in Nigeria to include discharge from sludge, production tests, drilling mud, spills from pipelines, well-blow outs, gas flaring and sabotage.

The consequences of the aforementioned factors that lead to environmental hazards need to be remedied. Doelman (1994) defined remediation as the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health, or the environment. Remediation techniques include: (i) ex-situ (excavation) or in-situ (on-site) soil washing/leaching/flushing with chemical agents, (ii) chemical immobilization/stabilization method to reduce the solubility of heavy metals by adding some non-toxic materials into the soils, (iii) electrokinetics (electromigration), (iv) covering the original polluted soil surface with clean soils, (v) dilution method (mixing polluted soils with surface and subsurface clean soils to reduce the concentration of heavy metals), (vi) phytoremediation by plants (GOC, 2003; Fawzy, 2008). These techniques, however, have their various merits and demerits. Detailed assessment of the advantages and disadvantages of various remediation techniques are reported by USEPA (2001).

Phytoremediation is the in-situ use of plants and their associated microorganisms to degrade, contain or render harmless contaminants in soil or groundwater (Cunningham et al., 1996). It is a non-destructive and economical in-situ process that uses plants to remove, degrade or stabilize contaminants in soil. This remediation technique is especially promising due to favourable climatic conditions of the tropics. Karenlampi et al. (2000) reported four characteristics that makes a plant suitable for phytoremediation, these include: (i) the plant's ability to accumulate extracted pollutant; (ii) plants should have enough tolerance to be able to not only survive in polluted soils, but to carry pollutants within their shoots; (iii) the species should be fast growing with an amplified ability to accumulate toxins; and (iv) the plant should be easily harvestable for simple disposal. Prasad & Freitas 2003 reported that about 400 species of plants have been found with the potential of remedying crude oil polluted sites. Among the families documented by Hameed et al. (2010) were Asteraceae, Brassiaceae, Caryophylaceae, Cyperaceae, Cunouniaceae, Fabaceae, Poaceae, Violaceae, Lamiaceae, Euphorbiaceae and Flacourtiaceae. In their study, the Poaceae family was reported to be the most abundant, frequent and most tolerant to crude oil contamination. Hameed et al. (2010) reported that *Cynodon dactylon* Linn., a member of the family Poaceae showed specific root and stem anatomical adaptation for its better survival under harsh environments.

Studies on the phytoremediation of soils contaminated with petroleum hydrocarbon using different plants in Nigeria and other parts of the world are well documented. However, there is little or no work on the remediation of soils contaminated with crude oil using *C. dactylon*. This study therefore investigated the growth of *C. dactylon* under different concentrations of crude oil and also determined the residual hydrocarbon content in the soil at plant maturity with a view to assessing its phytoremediating potential.

Materials and Methods

Soil preparation and planting of *C. dactylon*

This was a greenhouse experiment carried out in the Faculty of Agriculture, Obafemi Awolowo University (OAU), Ile-Ife. Surface soil from the OAU Biological Garden was collected and air-dried for 7 days and then sieved using a 2-mm mesh to remove debris. The surface soil belongs to the Iwo series soil classification. The typical vegetation of the surface soil used for the experiment included trees like *Hildergadia barteri* Mast., *Voancanga Africana* Stapf., *Azadiractha indica* A. Juss., *Antiaris toxicaria* Lesch., *Pycanthus angolensis* Welw., *Terminalia catapa* Linn. among other tree species. The physical and chemical characteristic of the soil prior to planting was carried out using standard methods. The soil analysis (physical properties) was done using the method described by Gee & Bauder (1986). Air-dried and sieved soil (3 kg) was weighed into each of 60 plastic bowls measuring 30 cm by 12 cm (diameter by depth) and perforated at the base to drain water and increase soil aeration. Crude oil obtained from the Nigerian National Petroleum Corporation (NNPC), Eleme, Rivers State was used as the contaminant.

There were six treatments and each was replicated 10 times. The treatments included: 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 ml of crude oil and were randomly assigned to bowls in a completely randomized design (CRD). The contaminated soil was watered regularly for a week to allow penetration of the crude oil. Five rhizomes of *C. dactylon*, each measuring 2 cm long collected from the OAU main-bowl sports complex were planted in each bowl one week after contaminating the soil with crude oil. The experiment was left for four weeks to establish before the commencement of data collection. Plants were watered regularly and the pots were maintained to be weed free.

Plant growth measurement

The leaf size was measured weekly by measuring the length and breadth of the leaves per bowl using a metre ruler while grass cover percentage in each bowl was measured using the pin-point method (Canfield, 1941). The experiment was terminated 12 weeks after planting. The plants were uprooted and fresh weights

were determined. The plants were dried in a Binder FED 400 model oven at a temperature of 80° C to constant weights and thereafter weighed.

Analyses of soil samples

Soil pH was measured by glass electrode pH meter using soil to water ratio of 1:1 (w/v) and the particle size analysis was determined using the hydrometer method outlined by Bouyoucos, (1951). Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) were determined using 1M NH_4OAc (Ammonium acetate) buffered at pH 7.0 as extractant (Thomas, 1982). The Sodium ion concentration in the soil extracts was determined using flame photometer while Ca^{2+}, Mg^{2+} and K^{+} were obtained using atomic absorption spectrophotometer (Perkin–Elmer Model 403, Shelton, Connecticut, USA). Exchangeable acidity in the soil samples was extracted with 1M KCl (Thomas, 1982). Solution of the extract was titrated with 0.05M NaOH to a permanent pink endpoint using phenolphthalein as indicator. The amount of base (NaOH) used is equivalent to the total amount of exchangeable acidity in the aliquot taken (Odu et al., 1986).

Total organic carbon was determined following the wet digestion method as described by Walkley & Black (1934) and the micro Kjeldahl procedure was used for the determination of total nitrogen (Bremner & Mulvaney, 1982). The available phosphorus was determined using the Bray P_1 method (Olsen & Sommers, 1982).

Determination of total hydrocarbon content

Ten grammes of each of the soil samples was weighed into a 250 ml conical flask and 20 ml of Xylene was added and then placed on a reciprocating shaker for 30 minutes. The soil solution was later filtered using No. 1 Whatman 11 cm filter paper. The concentration of each soil sample was determined using a 21D spectrophotometer (CECIL 3041) at a wavelength of 650 nm. A set of standards: 0.00, 5.00, 10.00, 15.00, 20.00 and 25.00 ppm was prepared and read on the spectrophotometer to calibrate the equipment before the final result was calculated. However, 0.5 g of each of the oven-dried and ground plant sample was used for the determination of THC in the plant samples using the same procedure (Greenberg et al., 1981). The initial crude oil concentration was 155.88 ppm.

Statistical analysis

A statistical comparison of means of different treatments was carried out using analysis of variance and treatment means were separated using the Duncan Multiple Range Test. Significance level was set at $p<0.05$. The data analysis was done using SPSS version 13.0 for Windows.

Results and Discussion

Soil physico-chemical properties

The physical and chemical characteristics of the pre-soil used in the greenhouse before contamination is presented in Table 1. The texture of the soil was sandy loam with high organic matter content. The soil pH was 5.40 indicating a strongly acidic soil condition. The organic carbon content of the soil was 41.58g/kg while the nitrogen and available phosphorus values were 1.62 g/kg and 22.36 mg/kg respectively. The cation exchange capacity (CEC) of the soil was 30.27 cmol/kg while the exchangeable acidity was 0.95 cmol/kg.

Table 1. Physical and chemical characteristics of soil used in the study before experiment

Parameter	Value
pH in water (H_2O)	5.4±0.12
Organic carbon (g/kg)	41.58±0.57
Nitrogen (g/kg)	1.62±0.52
Phosphorus (mg/kg)	22.36±0.73
Exchangeable acidity	0.95±0.01
Exchangeable cations (cmol/kg)	
K^+	0.72±0.01
Na^+	0.41±0.01
Ca^{2+}	26.20±0.13
Mg^{2+}	2.94±0.04
Particle size distribution (soil texture)	
Sand (%)	72.22±0.81
Silt (%)	16.20±0.11
Clay (%)	11.58±0.41
Textural class	Sandy loam

Mean±S.D

Growth characteristics of *Cynodon dactylon*

The effect of crude oil soil contaminations on the growth response of *C. dactylon* are presented in Figures 1 to 4. The growth characteristics, namely, leaf length, leaf width, number of leaves and percentage cover showed statistical significance ($p<0.05$) difference between the plants in the control bowls and plants in the crude oil treated bowls. The leaf length was significantly ($p<0.05$) higher in all the crude oil treated bowls than in the control bowls while not much difference was observed among all the treated bowls and the crude oil in terms of leaf width. Crude oil is known to have varying effects on plant growth. The leaf length was longer and the width, wider than those earlier reported on *C. dactylon* by Clayton & Harlan (1974). They described the plant as a stoutly stoloniferous perennial grass, culms robust or somewhat slender. At 12 WAP, 2.5 ml gave the significantly ($p<0.05$) highest leaf length while control treatment gave significantly ($p<0.05$) highest leaf count. Also, percentage cover for the test plant was highest in the control and significantly ($p<0.05$) different from all other treatments except 2.5

ml. Growth performance of *C. dactylon* was however negatively affected but without plant mortality. The finding in this study is at variance with Lin & Mendelssohn (1998) where mortality of *Spartina alterniflora* and *Spartina patens* were reported as a result of petroleum hydrocarbon contamination. These grass species are of the same Poaceae family with the test plant.

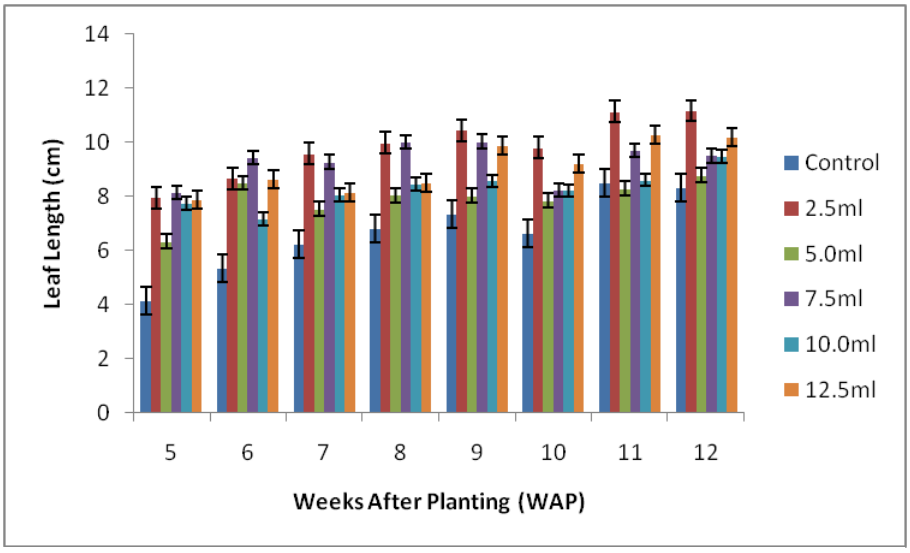

Figure 1. Effects of different concentrations of crude oil on the leaf length of *Cynodon dactylon*. Bars represent standard error.

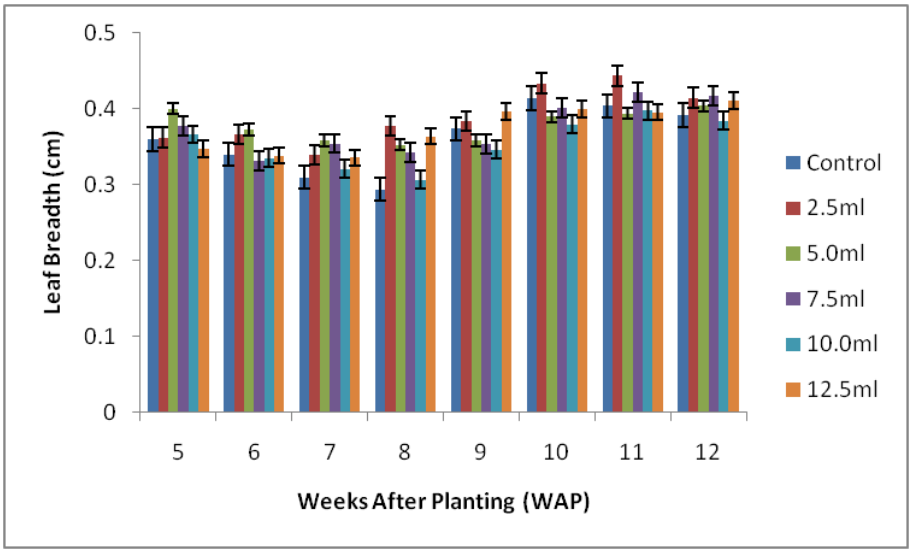

Figure 2. Effects of different concentrations of crude oil on the leaf breadth of *Cynodon dactylon*. Bars represent standard error.

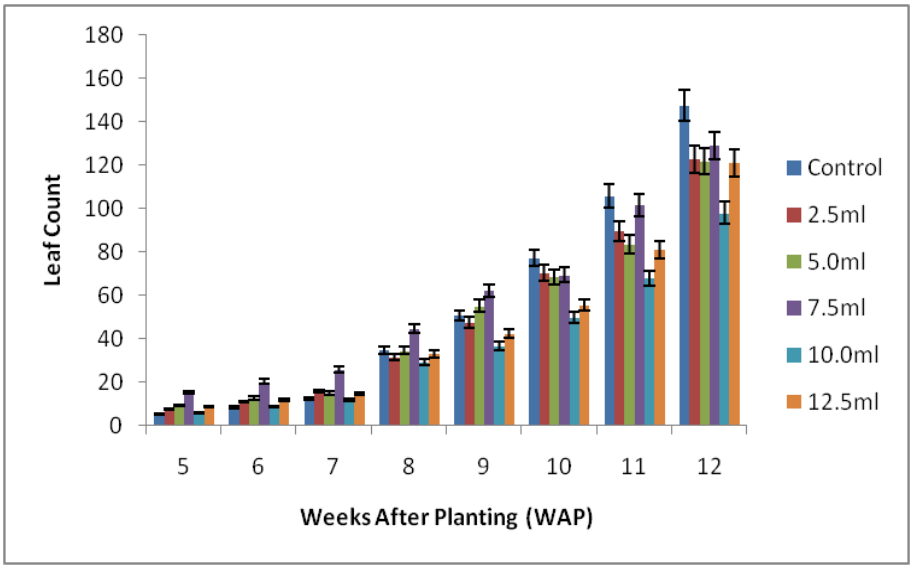

Figure 3. Effects of different concentrations of crude oil on the leaf count of *Cynodon dactylon*. Bars represent standard error

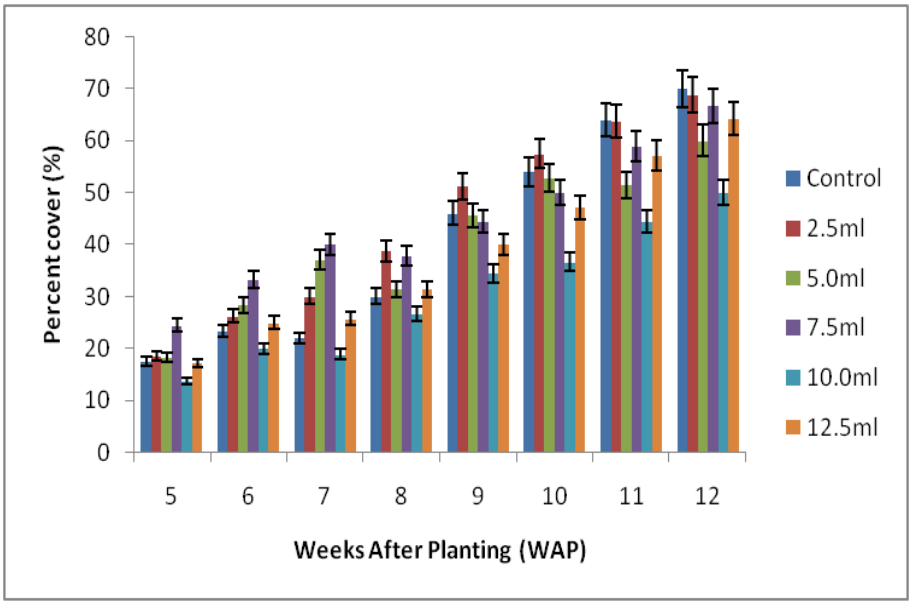

Figure 4. Effects of different concentrations of crude oil on the percentage of cover of *Cynodon dactylon*. Bars represent standard error.

Yield of *Cynodon dactylon*

The yield (fresh and dry weight) of *C. dactylon* at different crude oil concentrations is presented in Table 2. The fresh weight of plants per bowl after harvesting ranged from 41.00 ± 10.52 g to 51.00 ± 14.43 g in the 10.0 ml treatment and the control bowls respectively. The trend observed in the growth yield of the test crop revealed that the control bowls had the highest mean weight while the least mean weight was observed in the bowls with highest crude oil contamination. This is in agreement with the findings of Udo & Fayemi (1975) where poor growth of maize in polluted fields was attributed to the suffocation of the plants by contaminants which reduce air passage through the soil pores.

Table 2. Fresh and dry weight (in grammes/bowl) of *Cynodon dactylon* after harvest in different crude oil concentrations [Values shown are mean ± standard deviation (n = 10)].

Treatment	Fresh weight (g)	Dry weight (g)
Control	50.60 ±13.14	10.40 ± 3.07
2.5 ml	51.00 ± 14.43	13.00 ± 5.23
5.0 ml	47.71 ± 14.15	10.14 ± 3.39
7.5 ml	46.00 ± 12.16	10.33 ± 3.04
10.0 ml	41.00 ± 10.52	8.33 ± 2.61
12.5 ml	48.75 ± 14.19	8.63 ± 2.59

Nexus between soil properties and growth behaviour of *C. dactylon*

The post-soil pH ranged from 5.54 (10.0 ml of contaminant) to 5.72 (0 ml of contaminant), indicating a marginal increase in soil acidity (Table 3). This observation agreed with the findings of Lawlor et al., 1997 where decrease in pH was reported to be as a result of production of organic acids as intermediates of petroleum hydrocarbon degradation. Also, Andrade et al.(2004), Ayotamuno et al.(2004) and Njoku et al.(2009) reported positive correlation between soil acidity and the amount of crude oil. Previous studies had indicated negative impact of crude oil on the physical, chemical and biological properties of the soil (Abbey & Anthony, 1994). The Poaceae family has been reported by Halvin et al., 1999 to perform optimally at soil pH of 5.5 to 7.0. However, in Merkl & Schultze-Kraft, 2005, it was reported that leguminous grasses died within 6 to 8 weeks after these were planted in heavily crude oil contaminated soil, whilst non-leguminous grasses only showed reduced biomass production.

The *C. dactylon* exhibited tolerance and luxuriance to strong acidic soil. The finding of this work where reduced biomass production was observed at higher crude oil pollution is in line with the work of (Marcum, 1999 and Qian et al., 2001). The reduction in the amount of organic carbon with increase in hydrocarbon contamination could be due to formation of organic acids. The total weight (root and shoot) of *C. dactylon* also decreased with increase in contaminants concentration.

Table 3. Soil physicochemical properties and total hydrocarbon content (THC) in plant and soil after harvest of *C. dactylon*

	Control	2.5ml	5.0 ml	7.5 ml	10.0 ml	12.5 ml
pH	5.72^{a}	5.62^{a}	5.64^{a}	5.56^{a}	5.54^{a}	5.58^{a}
Organic carbon (g/kg)	40.08^{b}	46.45^{a}	29.29^{b}	39.95^{b}	39.07^{b}	39.08^{b}
N (g/kg)	1.52^{b}	2.03^{a}	1.36^{b}	1.39^{b}	1.33^{b}	1.25^{b}
P (mg/kg)	23.76^{a}	23.30^{a}	23.29^{a}	24.77^{a}	23.65^{a}	25.99^{a}
Exchangeable acidity	0.89^{a}	0.35^{b}	0.41^{b}	0.39^{b}	0.38^{b}	0.33^{b}
Exchangeable cations (cmol/kg)						
K^{+}	0.45^{ab}	0.34^{b}	0.38^{ab}	0.31^{b}	0.38^{ab}	0.50^{a}
Na^{+}	0.79^{ab}	0.81^{a}	0.67^{bc}	0.59^{c}	0.63^{c}	0.68^{bc}
Ca^{2+}	24.47^{ab}	24.73^{ab}	22.68^{ab}	21.52^{b}	25.21^{a}	22.04^{ab}
Mg^{2+}	2.54^{a}	2.59^{a}	2.43^{a}	2.29^{a}	2.44^{a}	1.29^{b}
Cation exchange capacity	28.04^{a}	28.46^{a}	26.16^{ab}	24.71^{b}	28.66^{a}	24.51^{b}
Total hydrocarbon content (ppm/L)						
Plant	5.05^{c}	12.79^{b}	16.46^{b}	13.49^{b}	16.33^{b}	39.26^{a}
Soil	0.00^{a}	0.00^{a}	0.00^{a}	0.12^{a}	0.02^{a}	0.00^{a}
Initial crude oil concentration (155.88 ppm/L)						

*Means with the same letter(s) across the rows are not significantly different ($p < 0.05$).

Bioaccumulation of THC by *C. dactylon*

Total hydrocarbon content observed in the test crop at the end of the study showed the extent to which *C. dactylon* can grow and accumulate hydrocarbon in its tissues (Table 3). Remediating plants readily absorb, volatilize and/or metabolize compounds such as tetrachloroethane, trichloroethylene and various petroleum hydrocarbons (Kim, 1996). The effectiveness of *C. dactylon* to accumulate hydrocarbon in its tissues as observed in this experiment was due to its ability to survive in a harsh environment. Similar results were obtained by Hameed et al., (2010) where it was reported that *C. dactylon* showed specific root and stem anatomical adaptations for its better survival under a harsh saline environment.

Conclusion

Phytoremediation is a non-destructive and economical in situ process that uses plants to remove, degrade or stabilize contaminants in soil. This technique is especially promising for the tropics due to climatic conditions that favour plant growth and microbial activity. *Cynodon dactylon* stored hydrocarbon in its tissue on maturity and the largest amount was observed in the highest crude oil contamination treatment. Furthermore, *C. dactylon* proved to be highly effective in the remediation of crude oil contaminated soils through its bioaccumulation ability by roots and shoots as shown in this study. This study has shown that *Cynodon dactylon* is useful for soil remediation and further research should be carried out to help identify more plants that can be used for this purpose.

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