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Studies on use of Cashew Nut Shell Liquid (CNSL) in Biopesticide and Biofertilizer

C. Vasanth Pandiyan*†, Gunasekaran Shylaja*, Gokul Raghavendra Srinivasan and Sujatha Saravanan***

*Department of Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai-600073, Tamil Nadu, India

**Department of Mechanical Engineering, Bharath Institute of Higher Education and Research, Selaiyur, Chennai-600073, Tamil Nadu, India

†Corresponding author: C. Vasanth Pandiyan

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ABSTRACT

Cashew is an immigrant tree from eastern Brazil and is regarded as one of the top three commercial crops of India. In general, Cashew nut shell contains 25-34% oil and was not much used earlier; however, commercial and industrial applications are being developed in the recent decade. In this present study, 100 kg of dried cashew shells yielded around 18.5 to 22.6% of cashew nut shell liquid (CNSL) while 58-63% of waste dark solid oil cake retained in extraction chamber. Eventually, these oil cakes were rich in fibre, protein and energy contents and offered as good alternative to traditional applications in the production of environmentally friendly organic fertilizer for vegetable crops. Also, this study investigated the safety of using CNSL cake as fertilizer for leaf & vegetable; and also noted improvised physico-chemical properties of soil which enhanced the soil fertility thereby leading to high crop yield and nutritional quality. Furthermore, CNSL was also proven to be a phytopesticide for eradication of serious pests, Odontopus varicornis, on cotton plant. Interestingly, toxicity study proved that the applications of CNSL was not only limited to biofertilizer but was also proven to be an effective biopesticide for the control of pest and insects.

INTRODUCTION

The developing and developed countries are seeing the rapid growth of specific wastes, such as electrical and electronics equipment waste, agricultural biomass waste, and plastic waste. These specific wastes require proper management, as most of the materials could be reduced, recycled or re-used with the help of environmentally friendly methods and technologies.

Cashew nut is one of the greatest yielded item on the planet, with production capacity more than 3.35 million tonnes per year and yet expanding in coming years. It is likewise considered as a standout amongst the most critical nuts in universal market because of its well acknowledged demand (MacLeod et al. 1982, Maia et al. 2000). Crude cashew nut contains both edible kernel with health significance; and a shell, which has been considered as waste and may bring an ecological issue if not dealt with appropriately.

The primary component of cashew plant is its cashew nuts, while the least regarded product was cashew nut shell liquid (CNSL). At a level of about 50% of cashews weight (Patel et al. 2006), a heavy liquid called cashew nut shell liquid (CNSL) is produced, which is considered an important material in view of the high centrality of unsaturated long-chain phenols, for example, cardanol, cardol and isomers (Rodriguez et al. 2006). Both anacardic and cardol corrosion were included as corrosion inhibitors (Kozubek et al. 2001, Kubo et al. 1993), antimicrobial inhibitory (Kubo et al. 1993) and lipoxygenase activities (Kubo et al. 1993). On the other hand, cardanol, because of its remarkable protection against the photolytic activity of mineral oils and the high protection of acids, alkalis, microorganisms, termites and creepers widely used in the production of caps and fabrics.

Cashew nut shell fluid or CNSL or CNSL oil is an adaptable by-product of the cashew business. CNSL is widely used in polymer-based businesses, for example, contact linings, paints and varnishes, overlaying tars, rubber aggravating saps, cashew bonds, polyurethane based polymers, surfactants, epoxy tars, foundry synthetics and intermediates for concoction industry and automobiles. In its existing form, CNSL is accounted as an effective chemical against termites and has water repellency (Lepage & Delelis l980). With the end goal of using the cashew nutshell, we have made an endeavour to change over the CNSL oils and waste cake to bio-insecticide against and bio-fertilizer. We hypothesize that if not the neat form, some particular blend may actually be good enough for commercial use. The objective of the study was to characterize the physicochemical quality of oil from cashew nut shell and to evaluate the potential of cashew nut shell cake as natural insecticide against termites.

MATERIALS AND METHODS

Collection of Cashew Nut Shells

Cashew nut shells crude and unshelled cashew nuts were collected from the trees grown in the Panruti, Cuddalore area in Tamil Nadu (India) amid August to September 2012. Cuddalore region was chosen for the examination since this locale has the most extreme territory of cashew generation than different areas of the state. Towns from Panruti and Virudhachalam obstructs in Cuddalore locale were chosen for this examination.

Preparation of samples

The piece nuts were broken, the nuts were collected, washed, dried in a stove at 40°C. The dried, clean nuts were processed using manual blender on account of the hardness of the nut (Figs. 1 & 2). During the timeframe of August to September, 2018, four samples were gathered and CNSL oil was separated.

Extraction of Cashew Nut Shell Liquid (CNSL)

This is the most well-known technique for extraction of CNSL these days. The cashew nut shells are gathered in the chamber, where steam warming is connected at temperatures around 200-250°C for 2-3 minutes, CNSL is then discharged out of shells. Figs. 3, 4A & 4B show the small-scale extraction units at Panruti, Cuddalore area in Tamil Nadu (India).

CNSL as Biopesticide Studies

Adult insects of *Odontopus varicornis*, collected from the Annamalai University campus, were brought to the laboratory and reared in wooden cage (45 × 32 × 30 cm dimension), at laboratory temperature of 29°±1°C with 12 hours of light and dark cycle. The sides of the insect cages were fabricated with wire meshes to provide sufficient aeration and light also easy for observation. The cage surface was covered with fine sand with moderate humidity maintained by adding a little amount of water frequently. The soaked cotton seeds of *Bombax ceiba* as well as seeds of its host plant, *Stericulia foetida* and *Gossypium* sp. were fed daily to the insects. Additionally, the pieces of *Sechium edule* (Chow chow) were also fed to the insects. The cages were maintained properly by cleaning on alternative days by eliminating the excreta and other waste substances. The continuous culture was maintained by transferring the laid eggs to other cages.

The nuts *A. Occidentale* were collected from cashew plantation area of Cuddalore district, Tamil Nadu, India. Cashew nut shell liquid (CNSL) has a structure of soft honey comb with dark brownish red viscose liquid. It is a one of the by-products of the cashew production which is the pericarp fluid of the nut.

To evaluate the acute toxicity in the atmosphere, the LD_{50} values (medium tolerance limit) are positive measures to test the toxicity of the test toxicant, under definite environmental conditions. The application of LD_{50} value has significantly gained more importance among toxicologists and it is the most consistent measure to evaluate potential hazards of aquatic and terrestrial life. The LD_{50} value for the same toxicant varies due to the mode of action and animal responses. In this viewpoint, the study has been done to evaluate the LD_{50} values for the male *Odontopus varicornis* on exposure to the phytopesticide, cashew shell oil. To provide a basis for evaluating and comparing the acute toxicity with other, 24, 48, 72 and 96 hours median lethal dose (LD_{50}) values are determined for the cashew nut shell oil commercial product. The LD_{50} is a statistical estimate of the concentration of the

Fig. 1: Manually separating activity for cashew kernel and shells.

Fig. 2: Picture of cashew nut shells.

Fig. 3: Top view - Input loading cylinder with cover.

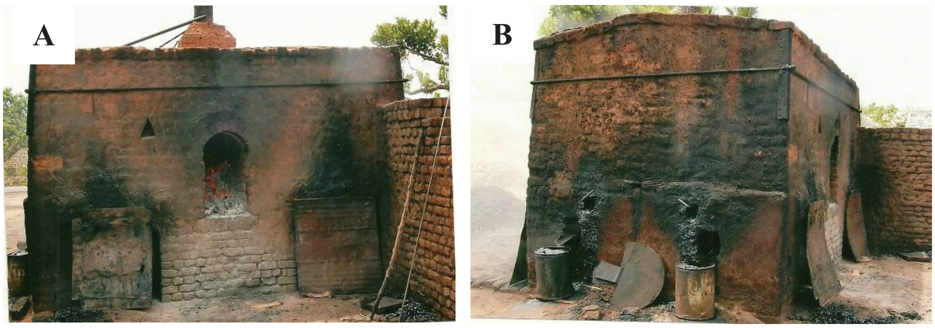

Fig. 4A: Input loading cylinder with cover. 4B: Collection container of small-scale extraction.

toxic material that kills 50 percent of the test species under experimental conditions during a specific time interval. The LD_{50} values are used because of the concentration required to affect a response in 50 percent of the test insects is more reproducible than any other values.

Range Finding Test

The solutions were made at varying range of concentrations. The tests were performed in adult insects, by injecting 0.025 mL, to each insect with a 2.6-gauge syringe needle. Ten treated insects were moved into a little insect cage (29 × 25 × 11cm) and their mortality was noted from 24 hours thereafter up to 96 hours. The results observed from the exploratory test indicated the range of the insecticide concentration.

Full Scale Test of Toxicity Evaluation

Different concentration of cashew nut shell oil was prepared. Insecticide treated and control insect were maintained for each bioassay. One hundred insects were administered with each concentration and allowed in separate insect cages. The observations on mortality are made at 25, 48, 72 and 96 hours. Insects showing no activities or response to physical stimulus were noted as dead and taken out instantly. The recorded values were used for calculating LD_{50} values.

Calculation of LD_{50}

The test was done to find the series of concentration for mortality. The mortality was recorded for *Odontopus varicornis* at 24, 48, 72 and 96 hours of exposure by injecting the insecticide of various concentrations. The LD_{50} value was determined by the profit analysis method depending on the measured percentages of test animal existing at concentrated lethal to more than half and less than half of the test subject (Huson method). This method described a quick and exact method of determining the median lethal dose (LD_{50} or ED_{50}). The method can be used to drive point and interval estimates or percentage points other than the LD_{50}. It is claimed that the method is less accurate for points other than the medium but equal to other approximate techniques. Following the probit analysis procedure, the LD_{50} values of 24, 48, 72 and 96 hours were evaluated. By using the LD_{50} values, a toxicity curve was constructed on a centimetre graph sheet (Fig. 10).

The LD_{50} values were derived by probit regression, i.e. taking test concentration and the respective mortality percentages on log values and probit scales, respectively. From the line drawn to the concentration ordinates, LD_{50} values were fixed and also calculated their 95% upper and lower confidence limits. The method of calculation is as below:

Step 1: The doses (d_1); number of insects tested (n_1); number of insects died (r_1) were wrote down; then the natural log for the doses (x_1) were found out.

Step 2: The method of finding y_1 is as follows.

$y_1 = In\frac{r_1}{n_1 - r_1}$, if number of insects ($r_1$) died is less than number of insects tested (n_1).

$y_1 = (2n_1 - 1)$, if number of insects died is equal to number of insects tested.

$y_1 = In\frac{1}{(2n - 1)}$, if number of insects died is 'zero'.

Step 3: The transformed data were divided and then set into upper lower halves.

Step 4: The total amount of transformed doses and the responses were calculated and the values were denoted as y_u, y_l, x_u, x_l where subscript U and 1 refer upper and lower halves of the data set.

Step 5: The mean of all transformed doses and the responses included in Step 3 were calculated. The means denoted as $\overline{X}$ and $\overline{Y}$ respectively.

Step 6: The slope (b) was calculated as:

$$b = \frac{(yu - y1)}{(Xu - X1)}$$

Step 7: The intercept (a) was calculated as:

$$a = \overline{y} - \overline{bx}$$

Step 8: LD_{50} was found as follows:

$$LD_{50} = e^c$$

Where, $c = \frac{(F - a)}{b}$ F is the log of LD_{50}

$$F = In\left[\frac{50}{(100 - 50)}\right] = 0$$

Step 9: The total number of insects tested (N) in the data included in Step 3 was calculated.

Step10: Sc was found using the formula:

$$Sc = \sqrt{\frac{5}{(b^2 \times N)}}$$

Step 11: The standard error (S) of the desired percentage was estimated as follows:

$$S(LD_{50}) = LD_{50} \text{ x } Sc$$

Step 12: The approximate 95% confidence interval was calculated by using the following formula:

$$LD_{50} \pm 1.96 \times S(LD_{50})$$

Bionomics

The details of its systematic position, distribution, habitat, morphology, feeding habits, cannibalism, longevity, sexual dimorphism, mating habits, oviposition, eggs, metamorphosis and adult characters are briefly presented in Table 1 and Table 2.

CNSL as Biofertilizer

Farm site: All the green leaves crops were grown in the farm house at Vallambadugai nearer to Chidambaram town. The plantation of green leaves and ornamental plants located in the region of plant research farm nearer to Annamalai University. This region is located in the central plain of Chidambaram town and characterized as clay loan soil.

De-oiled CNSL seed cake: The de-oiled CNSL cake was generously processed. The seedcake was kept back at room temperature for 90 days before using as a fertilizer.

Fig. 5: *Odontopus varicornis* in mating condition.

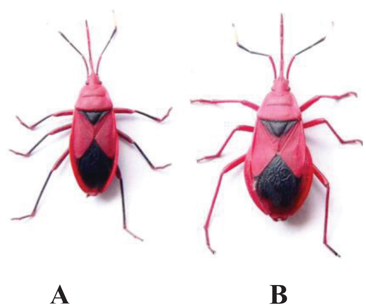

Fig. 6A: Adult male *Odontopus varicornis;* 6B: Adult female *Odontopus varicornis.*

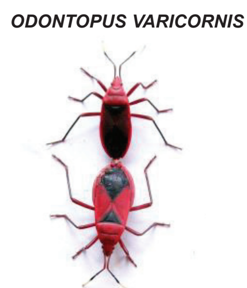

Fig. 7: Adult male and female *Odontopus varicornis* in mating condition.

Table 1: Systematic position of *O. varicornis.*

Systematic Position	
Phylum	Arthropoda
Class	Insecta
Order	Hemiptera
Suborder	Heteroptera
Series	Geocorisae
Super family	Pyrrhocoroidea
Family	Pyrrhocoridae
Genus	*Odontopus*
Species	*varicornis*

Table 2: Details of *O. varicornis.*

Distribution	Ethiopiean and Oriental regions, *Odontopus cultellaris*, O. *nigricornis*, *O. varicornis*, and *O. sanguinolens* are found in different parts of India. *O. varicornis* and *O. sanguinolens* are found in the State of Tamil Nadu and Puducherry.
Habitat	Fallen leaves of host plants such as *Stericulia foetida* and *Gossypium* sp.
Feeding Habits	Phytophagus insect with piercing and sucking type of mouth parts. The bug exhibits minimum feeding during hot hours of the day.
Cannibalism	Cannibalism is exhibited by these insects. The eggs laid by them as well as newly moulted insects are found to be susceptible to this activity. Field observations have shown that these insects are Cannibalistic occasionally and moreover feed on snails and dead animals.
Longevity	Adult male lives for about 75 days and female for about 70 days under laboratory conditions of 282±^{o}C and 80 * 5% RH
Sexual dimorphism	Adult male is smaller in size than the female with pointed aedeagus female with broader abdomen (Figs. 6A and 6B).
Mating habits	Exactly on 7th day after last imaginable moult; it exhibits mating behaviour (Fig. 5); Male crawls over the body of the female, after gripping the male inserts its aedeagus into the gonopore and remains in end to end position in natural and laboratory condition (Fig. 7), all usual activities like roaring, feeding, etc. are carried out by the pair in copula conditions (Ranganathan 1984), it mates continuously for three days without any separation.
Oviposition	At the time of oviposition, the female separates from the male and scoops at the sand and finally lays its eggs. The first oviposition occurs at about 25 days after the last moult, depositing 80 to 120 eggs in 2 to 3 hours; second oviposition occurs a week after the first oviposition producing 50 to 70 eggs.
Meta Morphosis	Incomplete metamorphosis with five nymphal instars; to reach its final moulting, it takes about 42 to 45 days.
Adults	The fifth nymphal instar on its final moulting becomes an adult with attains sexual maturity on the 7th day of its imaginal moult.

Plant material and propagation: All the leaves and ornamental plants need different agricultural practices such as the ratio of chemical fertilizers for each plant varies based on the recommendation by the Department of Agriculture, Annamalai University, India. The development of the plant and yield is independent of each other. However, the intention of this study is to trace the growth of leaves bushiness height as well as ornamental plant height, growth etc. Different rates of CNSL cake would have the variation in influencing the growth of each plant, but propagation methods were varied between these plants. The addition of CNSL cake at land preparation would exacerbate the phorbol ester content in the soil which was reported to decompose at time of harvesting (Joshi et al. 2011).

Plant Experiments

Amaranthus seeds were planted in control medium and allowed to grow for 4 weeks (Figs. 11 & 12). seedlings were transplanted individually into different test potting media and placed in a greenhouse maintained at 28°C, 16-h day/20°C 8-h night utilizing both natural light and supplemental artificial lighting to maintain an average light intensity of approximately 600 µmol m^{-2} s^{-1}. Change in plant heights were recorded from transplanting, when further growth ceased.

RESULTS AND DISCUSSION

Extraction of CNSL

The shell is about 0.3 inches thick having a sensitive cush-

Fig. 8: Raw cashew nut shell liquid

Fig. 9: End products like oil cake

Table 3: Process and recovery rate of cashew shells.

Sample	1	2	3	4
Processing quantity of Cashew shells (kg)	100	100	100	100
Recovery of cashew nut shell oil (mL)	18.8	22.6	21	21.5
Oil cake (kg)	58.8	62.4	57.4	60.4

ioned outside skin and a pitiful hard internal skin. CNSL inside the shell is the segment wrapped by a pitiful skin known as the test. 20 to 25% partition liquid, 20 to 25% test, 2% others, rest being the shell. The raw material for the creation of CNSL is the cashew shells. Cashew nuts contain 35-45% of seeds and 55-65% of shells. The shells contain 15-30% oil. CNSL is used as oil in industries. In extraction process of 100kg of dried cashew shells, oil range between 18.5 to 22.6% was extracted (Table 3). The colour of the solution became reddish brown or dark brown as shown in Fig. 8. The end products or waste like dark solid is shown in Fig 9. It was observed that 58-63% of end product like oil cake retains in chamber.

The most generally utilized technique, the hot oil shower, removes about half of the CNSL (7-15% of nut weight) from the shell (Ohler 1966, Gedam et al.1986). A few unique medications before simmering can convey the rate up to 90-95% and with solvents 100% can be accomplished, however these strategies are costly and their utilization relies upon the CNSL value (Ohler 1966).

Toxicity Studies

The present mortality values of *Odontopus varicornis* when injected with different concentrations of cashew nut shell oil for different are presented in Table 4. Detailed probit analysis of cashew nut shell oil concentrations and present mortality of *Odontopus varicornis* for 24, 48, 72 and 96 hours of exposure were determined. The LD_{50} values were calculated for 24, 48, 72 and 96 hours as 3.3, 3.5, 3.6, and 3.8 respectively. The mortality rate showed the mode of action and toxicity of for different concentrations. Toxicity test revealed that the rates of mortality increased with the increased concentrations.

Plant Growth and Yield

To measure the growth of *Amaranthus tricolour*, five parameters were subjected to be analysed, namely leaf length, leaf width, plant height, canopy height, canopy width. The trends toward these five characters were not similar. Among all, the one with biofertilizer provided the best outcome as an appearance of the largest of the plant growth characters than control. Whereas, the control plant resulted in a medium-sized growth of the same plant. The highest yield was obtained from the treatment of biofertilizer. Therefore, it could be implied that the treatment of CNSL oil cake would be able to partly replace the usage of chemical fertilizer. The usage of CNSL oil cake as fertilizer could, therefore alleviate the problem of high price of chemical fertilizer. Among these treatments, the control without adding biofertilizer gave the poor yield of *Amaranthus* plants.

Peace lily plant planted in a pot (Fig. 13) showed comparatively more growth in height of about 6.2 cm and increased the leaf surface area of about 4.8% compared to control when the soil was mixed with CNSL cake. Further, the same trail was showed for sword fern Nephrolepis obliterate in both height and leaf area. Plant height exhibited around1.2cm increase and height surface area comparatively increased about 7.6% when compared to control.

Table 4: LD_{50} value and their 95% confidence limit (μg/insect) of cashew nut shell liquid and the difference between upper and lower confidence limits for male *Odontopus varicornis*.

Exposure time intervals (in hours)	Number of animal introduction	Number of animals died in exposure concentration (%)						LD_{50}	95% Confidence limits (upper- lower)
		0.26	0.28	0.30	0.32	0.34	0.36		
24	100	-	-	10	20	30	40	3.3	3.6-3.1
48	100	5	10	20	30	40	50	3.5	3.6-3.4
72	100	10	25	40	55	70	85	3.6	3.4-3.1
96	100	15	30	50	65	80	100	3.8	3.4-3.9

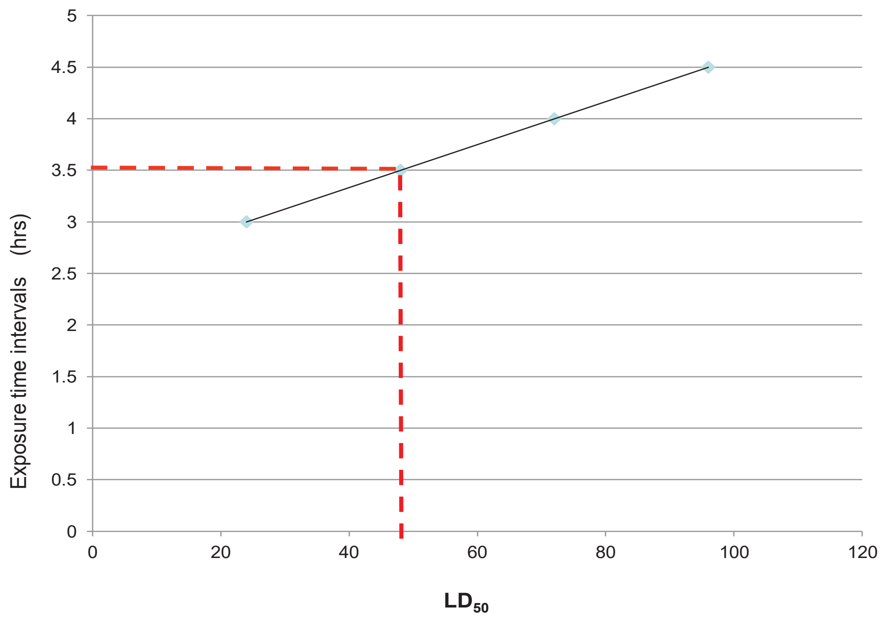

Fig. 10: LD50 values for 24h, 48h, 72h and 96h of O. varicornis (Toxicity Curve).

Nursery trial on oil extracted CNSL cake act as biofertilizer when peace lily, cobra plant, *Spathiphyllum wallisii* was planted in pot and observed that in the oil cake treated plant, the height increased by 6.2cm and leaf surface area was also increased as compared to control plant (Fig. 14).

CNSL Used as Biopesticide

The toxicity tests were ideally suited for assessing the relative impact of cashew nut shell liquid concentration on the insect for different time intervals. The toxicity level was also influenced by the size and age of the insect under study. The toxicity level also is influenced by sex; similarly, the nutrient supply to the assaying insect (Arunachalam et al. 1980) and the pH. It can be seen that the toxic action is specific for a particular insecticide in a particular insect which has been substantiated by the researchers in their studies.

The toxicity values denote the nature of cashew nut shell oil on the organism. It indicates whether the action is regular or irregular or cumulative in its effect. In the present investigation on *Odontopus varicornis* treated with cashew nut shell oil of phytopesticide, the acute toxicity level was expressed in terms of LD_{50}. The LD_{50} values of the cashew nut shell oil of *Odontopus varicornis* were 3.3, 3.5, 3.6, 3.8 at 24, 48, 72 and 96 hours, respectively.

The present experimental results were obtained with increased concentration of phytopesticide cashew nut shell liquid with decreased time of exposure, to bring about 50% mortality of insects. This trend was showing a risk in mortality rate with increasing concentration and period of administration sub-cutaneously, revealing the regular mode

Fig. 11: Control Plant *Amaranthus tricolour.*

Fig. 12: Treated plant *Amaranthus tricolour***.**

Fig. 13: Nursery trail on Spathiphyllum wallisii using oil extracted cashew nut shells (OECNS) as biofertilizers.

Fig.14: Nursery trail on Nephrolepis obliterata using oil extracted cashew nut shells (OECNS) as biofertilizers.

of action due to the toxicant. Cause of death may be attributed to the lethal action of cashew nut shell oil that inhibited biochemical processes and cellular metabolic pathways and other inclusions. These results are correlated with the previous reports which exposed Laccotrephes ruber, *Gryllotalpa africana*, *Spherodema ruticum*, *Odontopus varicornis* to monocrotophos, endosulfan, heavy metal mercury, pygidial secretion and nimbicidine, respectively. (Ha & Kubo 2005, Selvisabhanayakam et al. 2002, Lousia et al. 2010.

CNSL Cake Application as Biofertilizer

Organic manure, apart from supplying nutrient to the soil, conserves soil moisture, moderates soil pH, improves bulk density, increase carbon dioxide level in plant canopy, alleviates the toxicity of A^{3+}, improves aeration and activity of beneficial soil microbes, increase cation exchange capacity and retards nitrification for longer time. The increased yield resulting from the application of fertilizer was as a result of the improved soil fertility. It has been shown that crops respond more to fertilizer application. The improved nutritional value of the crop resulting from the application of the formulations may be attributed to the increased soil fertility. Increase in yield attributes and nutritional value of crops arising from the use of combination of organic and chemical fertilizers has also been reported. It has been suggested from the present study that CNSL oil cake helps to improve the growth and canopy of both test plant. Further, the present study revealed that the use of CNSL oil cake used for both plants increased the height and leaf surface area, especially in sword fern, which absorbed the air pollutants and *Amaranthus tricolor*, which produce pure air, in addition to controlling environmental contamination.

CONCLUSIONS

Oil cakes are rich in fibre, protein and energy contents. They offer potential benefits when used as substrates in developing bioprocesses for the production of organic chemicals and bio-molecules. Also, the use of oil cakes offers good alternative to traditional applications by their exploitation in the production of environmentally friendly green biofuel. Another key point to be noted is that the bioprocess utilizing oil cakes is attractive due to relatively cheaper availability of the oil cakes throughout the year, making it even more favourable when economics is considered. The utilization of de-oiled CNSL oil cake was proven to have potential for being used as an organic fertilizer for vegetable crops. These studies implied the safety of an application of CNSL cake as fertilizer in leaf and vegetable. It has also been proved that CNSL acted as phytopesticide for the eradication of one of the serious pests on cotton plant, *Odontopus varicornis.* Toxicity study also proved the hypothesis that CNSL has an application not only as biofertilizer but also as biopesticide for the control of pest and insects.

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