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# Evaluation of termiticidal activity and phytochemical anaylsis of *Crotalaria burhia* (Buch-Ham) and *Anacardium occidentale* (L.)

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**Abstract**
*Crotalaria burhia* (Buch-Ham) a member of fabaceae family and *Anacardium occidentale* (L.) belonging to family anacardaceae were tested for their termiticidal activity against *Odontotermes obesus* (Rambur) a major pest in wheat, sugarcane, maize and groundnut under laboratory conditions. Different concentration of aqueous root extracts of *C. burhia* and leaf dust of *A. occidentale* were prepared using standard methods. Aqueous root extracts of
*C. burhia* at (10%) concentration resulted in 73.33 per cent mortality which was on par with higher concentration of (20%) which resulted in 76.67 per cent mortality. Powdered leaf dust of *A. occidentale* at (5%) resulted in 53.33 per cent mortality which was on par with (10 %) concentration. In both the experiments chlropyriphos maintained as a standard check gave cent per cent mortality 24 hours after treatment. Lower doses of *C. burhia* and *A. occidentale* were less effective against *O. obesus* and the phytochemical analysis of aqueous root extract of *C. burhia* and leaf dust of *A. occidentale* were found to possess carbohydrates, proteins, amino acids, flavonoids, saponins, wax, terpenoids, mucilage and gum. The termiticidal property of both the botanicals may be due to the presence of secondary metabolites which were already proved lethal to insects.

**Keywords:** *Crotalaria burhia, Anacardium occidentale,* Termiticidal, Phytochemicals and Secondary metabolites

## Introduction
Termites are the most troublesome pest of plants, trees and wooden structures. They severely damage agricultural crops and urban infrastructure. Out of 2,500 termites species in the world only 10 per cent have attained pest status. In India out of 300 species, about 35 have been reported as damaging agricultural crops and timber in buildings. Approximately, 15 to 20 per cent yield loss is reported in maize and it accounts for 1,478 million Rupees. Rajagopal (2002) [24] suggested that severe loss has been recorded by termites on highly susceptible crops such as wheat and sugarcane in Northern India. In South India, the crops that suffer maximum damage are maize, groundnut, sunflower and sugarcane while the victims in North Eastern and Western India are tea and cotton, respectively.
In the past, the control of termites has been totally based on chemicals especially synthetic insecticides such as persistent organochlorine (OC) and organophosphate (OP) insecticides (Venkateswara *et al.*, 2005) [25]. Replacement of synthetic chemicals by bio-rational insecticides is universally acceptable and approved worldwide (Logan *et al.*, 1990) [15]. In this regard, bioactive compounds of plant origin are considered as ecologically safe alternatives. The plant extracts with complex mixtures of such compounds have been investigated for their insecticidal, repellent and anti-feedant properties (Zhu *et al.*, 2001; Isman *et al.*, 2006) [30, 9]. Because of the defense chemicals present, these plants can be used for the development of effective insecticides against termites and thus these plant chemicals would be able to replace the persistent synthetic insecticides (Ahmed and Qasim, 2011) [1]. Green pesticides despite their moderate efficacy are the latest global trend in the management of agricultural pests. Among the various botanicals, neem and pyrethrum have so far dominated the scene. Nevertheless considerable scope exists for the exploration of huge plant biodiversity for new plant based crop protectants (Mahapatro, 2011) [16].
*Crotalaria burhia* Buch- Ham belonging to Leguminaceae is an herb found in North- West India (Punjab, Rajasthan and Gujarat). It is known as Shinio in Rajasthan, its hindi name is bhip and in Punjabi it is known as Bhata and in Gujarat as Ghugato. The genus *Crotalaria* has 300 species worldwide and out of these, about 18 species are reported in India. Phytochemical studies have revealed that pyrrollizidine alkaloids are the main compounds in this plant.

Anticancer, antimicrobial and antibacterial properties have been reported for these pyrollizidine alkaloids. (Kataria *et al.*, 2010) [12]. Choudhary and Rajamani (2010) [3] has observed that the mixture of cow dung, aak (*Calotropis* spp.), kheip or khip (*Crotalaria burhia*) and local xerophic plant foliage when allowed to rot in a pit for about two months and then applied in chillies and tomato fields as a manure is very effective for the control of root-knot nematodes, termites and it also aids in good growth of the plants.

*Anacardium occidentale* L. commonly known as cashew belonging to the family Anacardiaceae is a native of Brazil and has great economic and medicinal value. Laboratory studies conducted by Ileke, 2012 [8] revealed that the powder and oil extracts of *A. occidentale* seeds proved effective against the cowpea bruchid, *Callosobruchus maculatus* (Fab.) in cowpea seeds. Research studies revealed that Cashew Nut Shell Liquid (CNSL) and leaf extracts of *A. occidentale* effectively repelled termites in Nigeria (Osipitami and Oseyemi, 2012) [22].

Phtyochemical analysis of leaves of *C. burhia* proved the presence of alkaloids, tannins, proteins and amino acids, terpenoids (Gautam *et al*., 2011) [5] and *A. occidentale* leaves were found to possess proteins, carbohydrates, saponins, phenols, terpenoids and flavonoids (Jaiswal *et al.*, 2012) [10], The insecticidal properties of various plant derived bio-pesticides are due to the presence of secondary metabolites and various chemical groups.

In this regard the present study was undertaken to explore the bioefficacy of root extracts of *C. burhia* and leaf extracts of *A. occidentale* against *Odontotermes obesus* (Rambur) under laboratory conditions and to analyze the chemical groups present in roots of *C. burhia* and leaves of *A. occidentale.*

## Materials and Methods

### Preparation of plant extracts

#### *A. occidentale* leaf dust

Cashew leaves collected from Agricultural college and Research Institute, Killikulam, Tamil Nadu were air dried till the leaves are devoid of moisture. The dried leaves were crushed and then powdered using mixer. Different concentrations of leaf dust *viz*., 0.5, 1, 2.5, 5, 7.5 and 10 per cent were prepared by mixing determined amount of powdered leaf with china clay, which acts as a carrier material.

#### *C. burhia* root extract

Roots of *C. burhia* were collected from Udaipur, Rajasthan and then sun dried to remove the moisture content in roots. The roots were then hammered well and the hammered roots were collected in a tray. Different concentrations of root extracts *viz.*, 0.5, 1, 2.5, 5, 10 and 20 per cent were prepared by dissolving 0.5, 1, 2.5, 5, 10 and 20 g of root powder in 100 ml of distilled water. The supernatant were filtered after 24 hours and used for testing their efficacy against *O. obesus.*

### Laboratory bioassay for the efficacy of botanicals against *O. obesus*

The efficacy of different concentrations of aqueous root extracts of *C. burhia* and leaf dust of

*A. occidentale* were tested for their efficacy against termites using the following methods.

### Efficacy of Cashew leaf dust against *O. obesus*

Laboratory bioassay of cashew leaf dust at different concentrations *viz.*, 0.5, 1, 2.5, 5, 7.5 and 10 per cent were tested against termites to fix the effective concentration. Toxicity of cashew leaf dust to *O. obesus* was determined using methods described by Grace and Abdally, (1990) [7]. Worker termites and termite nymphs were collected from termite mound near wheat field at IPFT, Gurgaon. Thirty termites were used for each replication and laboratory bioassay was carried out. The termites were placed in a Petri dish containing a thin layer of powder, gently shaking the disc for 10 seconds. Then the termites are poured out onto a small weighing paper placed in the center of a larger 9cm Whatman No.1 filter paper in a glass Petri dish. After the termites walked off the weighing paper, it was removed and the dish placed in a plastic box lined with damp paper toweling to maintain humidity. Mortality was observed 6, 12 and 24 hours after treatment.

### No-choice bioassay method

The "no-choice" bioassay method adopted by Kang *et al.* (1990) [11] was followed to evaluate the anti-termitic activity of *C.burhia*. Six treatments of aqueous root extract *viz.*, 0.5, 1, 2.5, 5, 10 and 20 per cent were applied to 1 g filter paper discs (Whatman no. 1, 90 mm in diameter and thickness 1.5 mm), individually. Discs of filter paper treated with water alone were used as a control. The treated filter papers were air dried at room temperature and a total of 10 active adult worker termites were randomly introduced on each filter paper impregnated with the test materials housed in an empty Petri dish (90 mm in diameter with 12mm in height). The Petri dishes with the test samples were then placed in an incubator maintained at 25 to 26˚C and 80% RH in incubator. Surviving termites were counted every 6 hours to determine the mortality rates (Nobuhiro *et al.*, 2009) [21]. Only those termites were considered to be dead if appendages did not move when prodded with a probe. Three replicates were made for each test sample.

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Number of initial termites in the test}} \times 100$$

$$\text{Corrected per cent mortality} = \frac{\text{Treatment} - \text{Control}}{100 - \text{Control}} \times 100$$

### Phytochemical analysis of aqueous extracts of roots of *C. burhia* and leaves of *A. occidentale*.

#### Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

Wagner's reagent - Reddish brown ppt

Mayer's reagent - Cream color ppt

#### Tests for carbohydrates and glycosides

#### Molisch's test

Sample was treated with 2-3 drops of 1% alcoholic- napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

#### Borntrager's test

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

**Tests for fixed oils and fats**

**Spot test**

A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolphthalein, the mixture was heated on a water bath for 1h, format ion of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**Test for tannins**

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

Dilute Ferric chloride solution (5%) - Violet color.

Lead acetate solution (10%) - White precipitate

**Test for proteins and free amino acids**

Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

**Test for flavonoids**

**Alkaline reagent test**

To the test solution a few drops of magnesium hydroxide solution was added, intense yellow color was formed which turned to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

**Tests for steroids and triterpenoids**

**Salkowski test**

Few drops of conc. Sulphuric acid was added to the sample, red color at lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

**Test for mucilage's and gums**

Small quantities of sample was added separately to 25 ml. of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

**Test for waxes**

To the test solution alcoholic alkali solution was added and waxes got saponified.

**Results and Discussion**

Laboratory studies revealed that lower concentration of leaf dust of *A. occidentale* at 0.5, 1 and 2.5 per cent were least effective causing a mortality per cent ranging from 16.67 to 26.67 (Table 1). Meanwhile 5 % leaf dust caused more than fifty per cent mortality and was on par with higher doses *viz*., 7.5 and 10 % which resulted in 56.67 per cent and 60.00 per cent respectively. Studies conducted by Osipitan and Oseyemi (2012) [22] revealed that extracts of *A. occidentale* resulted in 80.00 per cent mortality to *Macrotermes* spp. which falls in line with our study. Aqueous root extract of *C. burhia* @ 10 % and 20 % were found effective against *O. obesus* resulting in 73.33 and 76.67 per cent mortality 24 HAT (Table 2). Lower doses of aqueous root extracts resulted in less than 40 per cent mortality. Standard check chlorpyrifos 20 EC in both the experiments resulted in 100 per cent mortality 24 HAT.

Phytochemical analysis on the aqueous extracts of roots of *C. burhia* revealed the presence of tannins, sterols, triterpenoids, mucilage and gum (Table 3). The findings were further supported by the results obtained by Gautam *et al.* (2011) [5] and Sandeep *et al.* (2011) [25] who revealed the presence of saponins and glycosides in the whole plant extracts of *C. burhia*. On the other hand aqueous leaf extracts of *A. occidentale* were found to possess alkaloids, carbohydrates, tannins, proteins, sterols, triterpenoids, waxes and saponins. The results on the phytochemical analysis of *C. burhia* concurred with those of Kumar *et al*. (2011) [14], who revealed the presence of steroids and triterpenoids in aqueous extract of *C. burhia* with the help of Salkowski's test and the presence of mucilage and gum. Aqueous root extracts of *C. burhia* was found to possess tannins which was not reported before. Further, Naseem *et al*. (2006) [19] and Rao *et al*. (1975) [23] revealed the presence of pyrrolizidine alkaloids like crosemperine and flavonoid quercetin. Findings on the presence of secondary metabolites in aqueous leaf extract of *A. occidentale* was well supported by the findings of Jaiswal *et al*. (2012) [10] and Fazali *et al*. (2011) [4]. Standard quantitative method for the phytochemical screening of extracts of leaf, bark and nutshell liquid of *A. occidentale* by Nnamani *et al*. (2011) [20], revealed the presence of tannin, oxalate, stearic acid, glucoronic acid and glutamic acid

The insecticidal property of *C. burhia* and *A. occidentale* against *O. obesus* might be due to the presence of secondary metabolites and the role of secondary metabolites in insect pest management has been greatly studied. Alkaloid nicotine and dihydronicotyrine from *Nicotiana tabacum* L., anabasine form *Anabasis aphylla* L., Guinesine-A from the bark of the Brazilian plant *Cassipourea guianensis* Aubl. were proved for their insecticidal properties against whiteflies, cockroach and maize stem borer (Brossi and Pei,1988, Mukhamedzhanov *et al*, 1968, Kato *et al*, 1989) [2, 18, 13]. Unsaturated amide pyrethrin from *Anacyclus prythrum* L. was lethal to wide range of insect pests (Su, 1985) [26], solanum alkaloids, physostigmine, ryanodine, rocaglamide, cocaine, methylxanthines, isoquinoline, dioncophyllines, erythrina alkaloids, stemona alkaloids, trypterygium alkaloids, halophyton alkaloids and polyhydroxy alkaloids from various plants and trees have proved their insecticidal properties against numerous pests (Ujvary, 1999) [28]. Most of the insecticidal properties of botanicals are due to the presence of alkaloids, terpenoids, phenols and essential oils (Tripathi *et al.,* 2009 and Mann and Kaufman, 2012) [9, 17].

Both the botanicals *A. occidentale* and *C. burhia* caused more than fifty per cent mortality in *O. obesus* under controlled conditions which proves they are effective against termites. The termiticidal activity of

*A. occidentale* and *C. burhia* may be due to the presence of secondary metabolites in the leaf and roots of these plants.

**Table 1:** Efficacy of powdered leaf dust *A. occidentale* against *O. obesus*

| Treatments | 6 HAT | 12 HAT | 24 HAT |
|---|---|---|---|
| | Mortality (%)* | Mortality (%)* | Mortality (%)* |
| *A. occidentale* 0.5 % | 0.00 (0.00)$^{c}$ | 3.33 (10.52)$^{de}$ | 16.67 (24.09)$^{d}$ |
| *A. occidentale* 1% | 0.00 (0.00)$^{c}$ | 10.00 (18.43)$^{de}$ | 23.33 (28.88)$^{cd}$ |
| *A. occidentale* 2.5 % | 0.00 (0.00)$^{c}$ | 13.33 (21.42)$^{cd}$ | 26.67 (31.09)$^{c}$ |
| *A. occidentale* 5% | 13.33 (21.42)$^{c}$ | 33.33 (35.26)$^{bc}$ | 53.33 (46.91)$^{b}$ |
| *A. occidentale* 7.5% | 16.67 (24.09)$^{b}$ | 36.67 (37.27)$^{b}$ | 56.67 (48.83)$^{b}$ |

| | | | |
|---|---|---|---|
| *A. occidentale* 10 % | 23.33 (28.88)[b] | 43.33 (41.17)[b] | 60.00 (50.77)[b] |
| Chlorpyrifos 20 EC | 66.67 (54.74)[a] | 90.00 (71.57)[a] | 100.00 (90.00)[a] |
| Control | 0.00 (0.00)[c] | 10.00 (18.43)[de] | 16.67 (24.09)[d] |

*Mean of three replications; HAT – Hour after treatment
In a column means followed by a common letter are not significantly different at P = 0.05 by LSD
Figures in parentheses are arcsine √ P transformed values

**Table 2:** Efficacy of aqueous root extract of *C. burhia* against *O. obesus*

| Treatments | 6 HAT | 12 HAT | 24 HAT |
|---|---|---|---|
| | Mortality (%)* | Mortality (%)* | Mortality (%)* |
| *C. burhia* 0.5% | 0.00 (0.00)[d] | 6.67 (14.96)[de] | 16.67 (24.09)[d] |
| *C. burhia* 1% | 0.00 (0.00)[d] | 6.67 (14.96)[de] | 20.00 (26.57)[d] |
| *C. burhia* 2.5% | 0.00 (0.00)[d] | 16.67 (24.09)[cd] | 26.67 (31.09)[cd] |
| *C. burhia* 5% | 6.67 (14.96)[c] | 33.33 (35.26)[bc] | 40.00 (29.23)[c] |
| *C. burhia* 10% | 16.67 (24.09)[b] | 46.67 (43.09)[b] | 73.33 (58.91)[b] |
| *C. burhia* 20% | 23.33 (28.88)[b] | 53.33 (46.91)[b] | 76.67 (61.12)[ab] |
| Chlorpyrifos 20 EC | 66.67 (54.74)[a] | 90.00 (71.57)[a] | 100.00 (90.00)[a] |
| Control | 0.00 (0.00)[d] | 3.33 (10.52)[e] | 3.33 (10.52)[e] |

*Mean of three replications; HAT – Hour after treatment
In a column means followed by a common letter are not significantly different at *P* = 0.05 by LSD
Figures in parentheses are arcsine √ P transformed values

**Table 3:** Phytochemical analysis of aqueous root extract of *C. burhia* (10%) and leaf extract of *A. occidentale* (5%)

| S. No | Constituents | Chemical Tests | *C. burhia* | *A. occidentale* |
|---|---|---|---|---|
| 1. | Alkaloids | Mayers Test<br>Wangers Test | –<br>– | –<br>++ |
| 2. | Carbohydrates | Molisch Test | – | ++ |
| 3. | Glycosides | Bortner reagents Test | – | – |
| 4. | Fixed oils and fats | Spot test | – | – |
| 5. | Tannins | Ferric chloride<br>Lead acetate | ++<br>++ | ++<br>++ |
| 6. | Proteins and Aminoacids | Biuret Test | – | ++ |
| 7. | Flavanoids | Alkaline reagents Test | – | – |
| 8. | Sterols and Triterpenoids | Con. Sulphuric acid | ++ | ++ |
| 9. | Mucilage and Gum | Absolute alcohol | ++ | – |
| 10. | Waxes | Alcoholic alkali | – | ++ |
| 11. | Saponins | Saponification | – | ++ |

## Conclusion

The botanicals tested proved to be effective against *O. obesus* under laboratory conditions and further detailed studies on the efficacy of these botanicals against termites have to be carried out under field conditions. If proved effective they can be used as an effective botanical pesticides for the management of termites.

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