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June 2020 | Vol. 1 Issue. 1
www.uijir.com

# BIOTERMICIDAL ACTIVITY OF *AZADIRACHTA INDICA* (NEEM) AND *JATROPHA CURCAS* (TUBA-TUBA) LEAF ETHANOLIC EXTRACTS

**LESTER GENE V. AREVALO, M.A.Ed.**
*Mote Elementary School, Malita West District, Division of Davao Occidental*
*Mote, Pinalpalan, Malita, Davao Occidental, Philippines, 8012*
*Email: villegaslestergene@gmail.com*

**ABSTRACT**

***The study aimed to evaluate the biotermicidal activity of the leaf ethanolic extracts from Neem (Azadiractha indica) and Tuba-Tuba (Jatropha curcas) as biotermicides against termites. General types of compounds that in the leaf extracts were Flavonoids, Saponins, and Anthraquinones as determined through phytochemical screening. There were five (5) treatments in the three (3) replications namely: Pure extract, 500 ppm, 100 ppm, Solignum as the positive control and distilled water as the negative control tested for the study. The mortality of the test animals was recorded per treatment and subjected to statistical analysis using the Kruskal-Wallis Test.***

***There was a significant difference in the mortality of termites where the leaf ethanolic extracts and its mixture was observed more potent compound treatments tested concentration of 500 ppm and 100 ppm, respectively. The mortality recorded from extract was comparable to the positive control. This study demonstrated that the potency of the leaf ethanolic extracts from A. indica and J. curcas is affected by its concentration.***

***Keywords:*** *Biotermidical, Ethanolic, Extract*

## INTRODUCTION

Termites are soil or wood inhabiting insects which generally have soft, white bodies and secretive habits. Termites are known to cause tremendous losses to finish and unfinished wooden structures in buildings, besides loss in agriculture and forestry crops (Sen-Sarma *et al.,* 1975). They mostly feed on dead plant material and cellulose in the form of wood, leaf litter, soil, or animal dung.

However, for controlling termite population in the field, various synthetic pesticides such as chlorodane, cypermethrin, hydroquinone, and indoxacarb have been used. But all such synthetic pesticides are highly poisonous and kill non target organism. Due to their longer residual persistence in the environment, these have been banned and new alternatives are investigated in the form of natural pesticides.

In this regard, bioactive compounds of plant origin are considered as ecologically safe alternatives. In an attempt to provide an additional option to plant-derived termiticide, this study was conducted. This study aims to evaluate the biotermicidal activity of the leaf ethanolic crude extracts from *A. indica* and *J. curcas* leaf extracts against termites. These plant species are sufficiently available within the general locality and is also used for its medicinal property. This study may serve as a cost-saving and eco-saving option for people who cannot afford to use synthetic termiticides in eradicating termites in their homes. This study may also serve as a

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June 2020 | Vol. 1 Issue. 1
www.uijir.com

scientific basis that *A. indica* and *J. curcas* leaf extracts can be used as an effective alternative biotermicides. Furthermore, the result of this study may provide an additional support towards the discovery of potentially new natural foundation of insecticides from plants. The present research work highlights the Biotermicidal activity of *A. indica* and *J. curcas* leaf ethanolic extracts in different concentrations against termites under laboratory conditions.

## MATERIALS AND METHODS

This study composed of three phases: Phase I – Plant Extract Preparation, Phase II – Termite Experimentation, and Phase III – Data Collection and Analysis. All experimental procedures were done in the Southern Philippines Agri-Business and Marine and Aquatic School of Technology (SPAMAST) Research and Laboratory Services Center, Malita Campus, Malita, Davao Occiental. The units of analysis in this study were the biotermicidal activity of leaf ethanolic extracts from *A. indica* and *J. curcas* to termites.

## PHASE I – PLANT EXTRACT PREPARATION

- ***Collection and Preparation of Samples***

The leaf samples of *A. indica* and *J. curcas* were collected within the locality of Malita, Davao Occidental. Samples of
the leaf were packed separately in plastic bags for transport to the SPAMAST Research and Laboratory Services Center, Malita, Davao Occidental. In the laboratory, the samples were ground using the electrical grinder. Ground samples were transferred to an air-tight glass container to prevent oxidation and mold formation and stocked at room temperature.

- ***Preparation of leaf Extraction***

Extraction was done using the ethyl alcohol. The collected ground samples were soaked in a sufficient amount of 95% ethyl alcohol for 48 hours at room temperature, with occasional shaking and stirring, and were then filtered. All the filtrates were pooled and evaporated under vacuum using a rotary evaporator under reduced pressure of 60 mm Hg (~ 1.16 psi) at a temperature range of 40 – 50˚ C.

## PHYTOCHEMICAL ANALYSIS

To test what phytochemical compounds may be present in the samples; screening was carried out following the method described by (Gul, et al.,2017). This was to detect the presence of general compounds such as saponins, flavonoid, and anthraquinones in the leaf ethanolic extracts of J. curcas and A. indica. These compounds have been reported for their termiticidal effect.

### *A.TEST FOR SAPONINS*

The presence of saponins was determined through froth test described by Gul, *et al.,* (2017). A 5.0 ml of distilled water
was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously.

### *B.TEST FOR ANTHRAQUINONES*

The presence of anthraquinones was detected following the method described by Gul *et al.,* (2017). A 10 ml of benzene
was added in 6 grams of the leaf extract sample on conical flasks and immediately soaked for 10 minutes and filtered. Further, 10 ml of 10% NOH solution was added to the filtrate and shaken vigorously for 30 seconds and observed the colors pink, violet or red indicating the presence of anthraquinones.

### *C.TEST FOR FLAVONOIDS*

The presence of flavonoids was determined by the Alkaline Reagent Test. A 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when added 2 drops of diluted acid to the mixture. This showed the

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June 2020 | Vol. 1 Issue. 1
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presence of flavonoids (Gul, *et al.,* 2017).

**PHASE II – TERMITE EXPERIMENTATION**

**COLLECTION OF TEST ANIMALS**

Termites were collected from their natural habitats which can be found on the school premises of Mote Elementary School, Pinalpalan, Malita, Davao Occidental. Depending on the colony activity, the termites were kept in the container at ambient temperature within 24-25 degree Celsius**.**

**TOXICITY TEST**

Toxicity test was carried out to evaluate the potential biotermicidal activity of the various concentrations representing the treatments (pure extracts, 500 ppm, 100 ppm, Solignum, and distilled water) and its fraction was placed in 1-L capacity beakers in three replicates. Ten termites were placed in each concentration and sprayed 10 times. Its mortality was monitored up to 6 hours.

**PHASE III – DATA COLLECTION AND ANALYSIS**

**WASTE DISPOSAL**

Proper protocol for waste disposal was observed after the activity. Excess leaf extracts were disposed of in the sink after conducting the experiment. Since the solution is organic, it will not pose harm to the environment. However, termites were placed in hot cement and poured with boiling water to ensure no termites are left that may cause problems in the future.

**RISK AND SAFETY**

The experimental procedures were done at SPAMAST Research and Laboratory Services Center, Malita Campus, Malita, Davao Occidental. The researcher made use of any hazardous chemical or biological agents, thus avoiding possible hazards. Prior to the actual laboratory manipulation, the researcher were oriented on the laboratory procedures and precautionary measures by the laboratory in-charge. Personal Protective Equipment (PPE) such as laboratory gown, hair net, goggles, face mask and hand gloves were worn to minimize possible risks.

**STATISTICAL ANALYSIS**

The Kruskal-Wallis, a non-parametric test, was used to determine whether or not the termiticidal effect of leaf extracts using different treatments were significantly different from each other *(P<0.05)*. All statistical calculations were performed using JASP software.

**RESEARCH DESIGN**

This study used a Complete Randomize Design wherein the quantitative analysis of the difference between the mortality of termites from different leaf ethanolic extracts.

**RESULTS AND DISCUSSIONS PHYTOCHEMICAL ANALYSIS**

The extracts were found to be positive in saponins when froth test method used (Table 1). The presence of flavonoids was confirmed when the extract was subjected to Alkaline Reagent Test and Anthraquinones test showed that the extracts were positive to anthraquinones.

**Table 1.*Phytochemical Screening Result from the crude leaf ethanolic extracts of J. curcas and A. indica***

| COMPOUNDS | METHODS USED | RESULT | INDICATORS |
|---|---|---|---|
| Saponins | Froth Test | Positive | Presence of persisting froth |

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June 2020 | Vol. 1 Issue. 1
www.uijir.com

| | | | |
|---|---|---|---|
| Flavonoids | Alkaline Reagent Test | Positive | Appearance of magenta and green color |
| Anthraquinones | Colored Test | Positive | Appearance of pink color |

Table 1 shows the bioactive compounds from the organic leaf of *J. curcas* and *A. indica*phytochemical screening. The samples were found to contain saponins, flavonoids, and anthraquinones. According to Weimann and Heinrich (1997) that plants are rich in a wide variety of phytochemicals like saponins, athraquinones, flavonoids, etc., that have been found to have antimicrobial activities.

**Effects of the leaf ethanolic extract from *J. curcas* against termites**

The mortality for termites treated with different concentrations was recorded every 2 hours of 6 hours. Within 6 hours, higher mortality of termites was recorded from pure leaf extract was in Treatment 1 (pure extract) followed by Treatment 2 (500 ppm) and Treatment 3 (100 ppm) shown in Table 2. In the positive control test, all test organisms died while in the negative control test, all test organisms were found alive.

Table 2. *The mortality of termites from the leaf ethanolic extract of J. curcas.*

| TREATMENT | NO. OF TERMITES | MORTALITY | | | TOTAL | MEAN |
|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | |
| T1-Pure Extract | 30 | 10 | 10 | 10 | 30 | 10 |
| T2 - 500 ppm | 30 | 7 | 8 | 8 | 23 | 7.66 |
| T3 - 100 ppm | 30 | 6 | 6 | 5 | 17 | 5.66 |
| T4 – Positive | 30 | 10 | 10 | 10 | 30 | 10 |
| T5 – Negative | 30 | 0 | 0 | 0 | 0 | 0 |

***Mean Mortality (Total No. of dead termites/No. of replicates per treatment)***

Based on the findings, the *J. curcas*pure leaf extract was more potent and showed the highest mortality compared to the diluted concentrations. There was a significant difference observed in the mortality of termites between the concentrations of the extract. Reports confirmed phytochemical screening of *J. curcas* leaf extract contains saponin, anthraquinones, and flavonoids (Onuh, 2008). The leaf extracts were found to significantly reduce fungal pathogen growth in cowpea and against a range of Lepidopteran species (Phowichit *et al.,* 2008).

**EFFECTS OF THE LEAF ETHANOLIC EXTRACT FROM *A. INDICA* AGAINST TERMITES**

Table 3 shows the result of the mortality of termites exposed to the leaf ethanolic extracts from *A. indica.* The highest mean mortality was found using the pure extract (Treatment 1=10.00) followed by (Treatment 2=7.33). Whereas, the lowest mortality was observed in (Treatment 3=5.00). These findings indicated that diluted concentrations reduced the biotermicidal activity of extracts. Evidently, the significant difference was observed in the mortality of the test organism exposed to the concentrations of extracts.

Table 3. *The mortality of termites from the leaf ethanolic extract of A. indica.*

| TREATMENT | NO. OF TERMITES | MORTALITY | | | TOTAL | MEAN |
|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | |
| T1-Pure Extract | 30 | 10 | 10 | 10 | 30 | 10 |
| T2 - 500 ppm | 30 | 7 | 7 | 8 | 22 | 7.33 |
| T3 - 100 ppm | 30 | 4 | 5 | 6 | 15 | 5 |
| T4 – Positive | 30 | 10 | 10 | 10 | 30 | 10 |
| T5 – Negative | 30 | 0 | 0 | 0 | 0 | 0 |

***Mean Mortality (Total No. of dead termites/No. of replicates per treatment)***

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June 2020 | Vol. 1 Issue. 1
www.uijir.com

This finding indicates that higher concentration (pure extract) showed the best result than in lower concentration. The anti-termite activity of the extract tested increased with increase in concentration (100 ppm – 500 ppm). Phytochemical screening confirmed that *A. indica* contains flavonoids, anthraquinones, and saponins. Meepagala *et, al.* (2006) reported that most plant contains the large number of phytochemicals such as quinones, di-terpines, flavonoids, amides, anthraquinones, etc. which are well known for their anti-feedant, repellant and toxic nature against termites.

**EFFECTS OF THE LEAF ETHANOLIC EXTRACTS FROM *J. CURCAS* AND *A. INDICA* ETHANOLIC AGAINST TERMITES**

There was higher mean mortality in all the treatments of the pure mixture of *A. indica* and *J. curcas* leaf ethanol extracts that was similar to the positive control treatment (Table 2). The concentrations (pure extract, 500 ppm, 100 ppm, and positive control) display highest mean mortality (10.00) while the negative control displayed no mortality. It suggested that *J. curcas* and *A. indica* leaf ethanolic extracts contain toxins that could cause mortality.

**Table 4.** ***The mortality of termites from the mixed leaf ethanolic extracts of A. indica and J. Curcas***

| TREATMENT | NO. OF TERMITES | MORTALITY | | | TOTAL | MEAN |
|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | | |
| T1-Pure Extract | 30 | 10 | 10 | 10 | 30 | 10 |
| T2 - 500 ppm | 30 | 10 | 10 | 10 | 30 | 10 |
| T3 - 100 ppm | 30 | 10 | 10 | 10 | 30 | 10 |
| T4 – Positive | 30 | 10 | 10 | 10 | 30 | 10 |
| T5 – Negative | 30 | 0 | 0 | 0 | 0 | 0 |

***Mean Mortality (Total No. of dead termites/No. of replicates per treatment)**

The effect of the mixture from *A. indica* and *J. curcas*leaf ethanolic extracts was perhaps due to the combined potency of the active compound which plays role in plant defense. There was a significant difference in the mortality of termites. The *A. indica* and *J. curcas* leaf ethanolic extracts contained flavonoids, anthraquinones, and saponins which repel termites or interfere with their gut flora (Verma, 2009).

**CONCLUSION**

From the data gathered, the researcher concluded that *J. curcas* and *A. indica* leaf ethanolic extracts were potential alternative biotermicides in killing termites. The bioactive compounds that were present *J. curcas* and *A. indica* leaf extracts by phytochemical screening (flavonoids, saponins, and athraquinones) revealed that the termites were sensitive to the compounds since the results show that there was mortality observed even at lower concentrations. The highest mortality mean was shown by the treatment having a pure concentration of the leaf extracts and the treatment with solignum. The obtained mean mortality per treatment confirmed that the leaf extracts of *A. indica* and *J. curcas* have a deleterious effect on the termites, collected within the school campus. The finding also indicated that prolonged exposure to high concentration (pure extracts) of *A. indica* and *J. curcas* leaf extracts had resulted in high mortality of termites. Therefore, the most effective concentration of the best extracts that can kill termites were on the pure or mixed leaf ethanolic extracts of *J. curcas* and *A. indica.*

The termiticidal activity exhibited by *A. indica* and *J. curcas* leaf ethanolic extracts in this study can be used as a scientific basis in using *A. indica* and *J. curcas* leaf ethanolic extracts at high concentration as an alternative, eco-friendly termiticide, though proper processing is still needed to ensure its appropriateness as the substitute.

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June 2020 | Vol. 1 Issue. 1
www.uijir.com

**RECOMMENDATIONS**

Since it was proven that *J. curcas* and *A. indica* ethanolic leaf extracts were potential alternative biotermicides, the researcher would like to offer the following recommendations:

1. More intensive investigation of the toxicological effect of saponins, flavonoids, anthraquinones found in the leaf extracts of *J. curcas* and *A. indica*.
2. Advance procedure on phytochemical screening is suggested to identify other bioactive compounds found in young leaves of *A. indica* and *J. curcas* which contributed to its termiticidal action.
3. Field trials also needed to recommend the development of an eco-friendly termiticide for termite control.

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