P-ISSN 2382-4583 E-ISSN 2964-3648

International Journal of Agricultural Research and Educational Management
Vol. 8 No. 2 (June, 2025) | pp 1-10
Available online at https://iigdpublishers.com/journals/27

Open Access | **Original Article**

Response of Nasutitermes Luzonicus and Macrotermes Gilvus (Isoptera: Termitidae) to Allamanda Cathartica (Apocynaceae) Leaf Extract Under Laboratory Conditions

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Keywords
Allamandin, Biotermiticides, Macrotermes Gilvus, Nasutitermes Luzonicus, Pest Control.

Volume 8 | Issue 2
June, 2025

P-ISSN 2382-4583
E-ISSN 2964-3648

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Abstract
Termites eat cellulose-based materials and become economically important pests due to substantial mutilation on anthropogenic structures. It is imperative, therefore, to look for economical, natural, and effective solution without environmental and human hazards of chemical termiticides. Allamanda cathartica possesses toxic iridoid lactones, such as allamandin, plumieride, plumericin, and isoplumiricin, which create a possible termite control. A. cathartica leaf decoction, crude leaf extract, 40% and 80% methanolic leaf extract (% v/v), distilled water (negative control) and Solignum® (standard check) were sprayed on one hundred soldiers and workers of Nasutitermes luzonicus and Macrotermes gilvus. The experiment was arranged in a Completely Randomized Design (CRD) with three replications for each treatment. Results showed that the experimental treatments exhibited termiticidal activity against N. luzonicus and M. gilvus. Across castes, significant differences ($P < 0.05$) on mean mortality of leaf extracts were observed. In terms of N. luzonicus, the maximum mean mortality was recorded by the 80% methanolic leaf extract which registered 96.67% ± 2.03% in soldiers and 100.00% in workers, with an overall mean mortality of 98.34%. In terms of M. gilvus, it is the 40% and 80% methanolic leaf extract which measured the maximum mean mortality in soldiers (60.00 ± 0.58% and 91.00 ± 7.09%) and workers (87.00 ± 2.08% and 85.33 ± 3.48%) and made an overall mean mortality of 88.17%. Generally, the greatest potency was delivered by the 80% methanolic leaf extracts which displayed comparable effects with standard check and can be used as a biotermiticide for pest control and management.

INTRODUCTION

Termites (Isoptera: Termitidae) commonly occur in tropical soils, like the Philippines, where they play an important part in soil ecology by reprocessing wood and decomposing plant materials (Lee and Wood 1971). Sadly, they become economic pests when they feast on anthropological structures and agricultural harvests. In the Philippines, there are 55 known species of termites from 18 genera (Acda, 2004, 2007; Snyder & Francia, 1962). Of the 55 species, four subterranean species are considered serious structural pests: Coptotermes vastator Light, Nasutitermes luzonicus Oshima, Macrotermes gilvus Hagen, and Microcerotermes losbanosensis Oshima (Valino, 1967; Acda, 2004). They infest cellulose containing debris in soil and attack wood structures through direct soil contact (Zabel and Morrell, 1992).

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The costs of damage around the world by termites are reported annually to be approximately in billions of dollars. However, the total cost due to termite damage in the Philippines is unknown; however considering the abundance and level of activity of these insects, financial losses due to infestations are massive (Acda, 2004). Most well-known and extensively used pro-active measure to reduce the incursion of termites is the use of synthetic termiticides. These chemicals pose threats not only to the environment but more so to the other organisms including man due to high residual toxicity of these products. Termiticides are termite control products come with different chemical names and brand names like spinosad, disodium octaborate tetrahydrate (DOT), calcium arsenate, and chlorpyriphos (Scheffrahn et al., 1997). Though proven effective, excessive use may harm the environment and results are unsustainable. Researches are continuously being done to develop new options of termite control. One option is the utilization of plant-derived pesticidal products.

Over the past 50 years, more than 2000 plant species belonging to different families and genera have been reported to contain toxic chemicals and bioactive compounds possessing varied and fresh types of structural patterns (Adityachaudhury et al., 1985); Apocynaceae can be credited for such qualities. A list of readings (Abdel-Kader et al., 1997; Ahirwar and Jain, 2011; Prabhadevi et al., 2012; Kadir et al., 2013) attested plant species under the said family markedly indicate different types of chemical elements associated to lethal ability against pests; Allamanda cathartica Linnaeus was pointed out as a good entrant of this manifestation.

Apocynaceae consists of various genera of plants with significant chemical constituents having wide range of biological activities. Because of its swift growth, shearing is often important, which may expose gardeners to toxic sap that causes dermatitis symptoms such as rashes, blisters, and itches (Prabhadevi et al., 2012). Even though the number of incidences is less, all plant parts are toxic if ingested in large amount. The entire parts contain a toxic iridoid lactone, allamandin, and other poisonous bioactive compounds such as alkaloids and cyanogenic glycosides (Abdel-Kader et al., 1997; Ahirwar and Jain 2011). Prabhadevi et al. (2012) mentioned that the ethanolic leaf extract contains ethyl caprinate (Retention time: 6.67; Peak Area: 0.07), ethyl caprylate (RT: 9.41; PA: 0.09), and squalene (RT: 31.27; PA: 6.60), which are known natural pesticides. Moreover, one significant plant associated with this family is Thevetia peruviana (Pers.) Schum. Many researchers have been doing experiment using T. peruviana as plant sample. One of which was the production of an oil-based paint containing the seed oil extract of T. peruviana. The study showed that the oil-based paint possesses an anti-termite activity on Microtermes spp. (Kadir et al., 2013).

In these contexts, there is a concrete point to the conceivable characteristics of A. cathartica as an evident termite control preference. Chemical elements and its properties, therefore, become the foundation of the termiticidal activity of A. cathartica against 2 economically important subterranean termites (Termitidae). Further, other plant species within Apocynaceae prove an activity against termites. It is in this background that the present study was conducted. This study scrutinized the vulnerability of soldiers and workers of Nasutitermes luzonicus and Macrotermes gilvus (Isoptera: Termitidae) towards A. cathartica leaf extract. It precisely attempted to determine whether there was a significant difference on the effects of leaf extract on the soldiers and workers of both termite species and to identify which amongst the treatment was highly effective in carrying out the effect of A. cathartica.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Disease-free, and mature A. cathartica leaves were collected, using a dirt-free polythene bag, from field Fatima Village, Tacloban City, Philippines (11o12’47.982” N latitude and 125o0’19.698” E longitude, at an elevation of 4.00 meters). The leaves were washed thoroughly

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with running tap water to eliminate surface dirt, traces of fertilizers, and other contaminants. Leaves were drained, air dried for 2 days until crisp and stored in polythene bag at room temperature in the laboratory.

Extraction and Preparation of Test Materials

Nine hundred (900) grams of dried leaves were weighed and placed in an individual sterilized aluminum tray. Three methods of leaf extractions were made namely; (1) leaf decoction, (2) crude leaf extract, (3) methanolic leaf extract.

Leaf Decoction

One 300-gram dried leaves were cut into pieces and boiled in a clay pot containing 475-mL distilled water and stirred frequently with wooden spoon for 10 minutes in a temperature range of 57 oC to 65 oC. The pot was removed from direct fire and allowed to cool off for 30 minutes. The mixture was filtered using 4" x 4" cheesecloth and stored in dark-brown bottles labeled appropriately.

Crude Leaf Extract

One 300-gram dried leaves were cut into tiny pieces and homogenized, with 475-mL distilled water. Resultant product was percolated using 4" x 4" cheesecloth and stored the same manner as above.

Methanolic Leaf Extract

Three hundred (300) grams of homogenized dried leaves were soaked in 1050-mL methanol (Sigma-Aldrich; AR Grade) for 1 ½ day with occasional stirring. The suspension was filtered through 4" x 4" cheesecloth and stored in reagent bottle, transferred to a distilling flask, heated up to 64.0 oC to 65.2 oC to remove the methanol content from the solution recovered through simple distillation method. To confirm the absence of methanol in the leaf extract, a flame test was done. Only 93.8 -mL concentrated methanolic extract was obtained after distillation with a stock solution concentration of 69500 ppm (6.95% v/v). Two (2) methanolic leaf extract concentrations, 40% and 80%, were prepared from the stock solution. The concentrates were stored in a light-protected bottle and labeled appropriately until use.

Collection of Termites

Two species of economically significant subterranean termites were used as test insects composed of the soldiers and workers. To avoid extreme temperature exposure, test samples were collected early morning between 6:00 AM to 8:00 AM, using soft-bristled brush to gently place them into 26 x 18.5 x 15 inches (L-W-H) plastic containers ventilated lids and covered by dark polyethylene plastic sheets. Moisture level inside the container was maintained by introducing wet filter papers (Whatman No. 1; 9 cm in diameter) with laboratory conditions at 26 ± 2 oC and 80 ± 5% relative humidity (RH) in total darkness (Tamashiro et al., 1973). Alternatively, a few moist pieces of wood or soil from their natural habitat are introduced to provide some familiar environment for stress reduction. To prevent desiccation, hydration is maintained every 24 to 48 hours wherein the moisture level of substrates (damp filter paper, piece of wood, or soil) was checked regularly and re-moistened as needed (Woodrow et al., 2008).

Macrotermes Gilvus

The M. gilvus soldiers and workers were collected from a mound, located in a forest near Tacloban-Palo Bypass Road, Palo, Leyte, Philippines, by digging up several pieces of it, including the soil and its nest (termitaria).

Nasutitermes Luzonicus

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Bait system was used in this type of species. Coconut flower sheaths were used as lure. A number of sheaths were cut into 12" long and 7" wide and were rolled like a pancake, then tied with plastic ribbons to secure. The baits were placed near a dead tree with N. luzonicus infestations and were left undisturbed for 2 weeks. After the duration, lures with termite infestations were collected.

Notably, the proportion of soldiers and workers in a termitarium was importantly considered. To maintain social cohesion within the foraging group, the worker-soldier ratio of 10:1 was adapted (Su & Scheffrahn, 1988; Acda, 2007). An excess number of soldiers would burden the termitaria because the soldiers must be fed by the termite workers or food-producing members of the colony (Haverty, 1977). Disproportionate population may disrupt the social structure of the termitaria and could affect the foraging behavior of the workers.

Treatment of Samples

In this study, a Completely Randomized Design (CRD) with three replications was implemented. The M. gilvus soldiers and workers from the mound were separated from soil particles and were placed on a 90 x 15 mm in diameter Petri plate (Sigma -Aldrich). The baits for N. luzonicus were untied and both castes were separated as stated previously.

Using 36 Petri plates for each termite species and a dissecting needle, samples were selected and distributed randomly, sorting out 100 soldiers and 100 workers in 2 plates (each plate contains 50 caste individuals). Each group was treated with treatments using direct-spray method, yet not flooding the plate. After 20 minutes of treatment exposure, the number of dead soldiers and workers on each termite species were recorded. A verification of status of dead and undead termites was done using a simple compound microscope to increase the reliability of the results.

Data Analysis

In this study, the collected data were subjected to One-way Analysis of Variance (ANOVA) using SPSS Statistics version 22 software. To complement a significant ANOVA result and determine which treatments statistically differ while maintaining family-wise error rate, a comparison of treatment means was performed using Turkey's post-hoc test (HSD; $p < 0.05$) to identify which treatments statistically differ at significant levels, supporting further insights into its effects. This post-hoc analysis is suitable as it adjusts all pairwise comparisons, ensuring that observed mean differences are statistically meaningful.

RESULTS AND DISCUSSION

Results of the Study

The mean mortality (20-minute post-treatment exposure) of dead soldiers and workers of M. gilvus and N. luzonicus, due to different concentrations of A. cathartica leaf extracts, showed significant effect ($p < 0.05$) in terms of its termiticidal activity as shown on Table 1 and 2. Evidently, no mortality of castes in both termite species occurred in using the distilled water (negative control) over the length of exposure. On the other hand, Solignum® (standard check) recorded a maximum termiticidal effect (100% mortality) across the 2 castes and species. Additionally, a non-significant difference on the termiticidal effects of leaf decoction, crude leaf extract, 40% and 80% methanolic leaf extract occurred at N. luzonicus soldiers and M. gilvus workers.

As seen on Table 1, the positive control (Solignum®) measured a maximum mean mortality of 100% against N. luzonicus species. Contrariwise, the negative control (distilled water) recorded zero mortality. All leaf extracts have shown relatively good mortality level, varying from 30.33% to 100.00%, which is statistically different from the negative control. In terms of soldiers, the

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40% and 80% methanolic leaf extract showed similar effects to that of the positive control which registered 81.00 ± 11.79% and 96.67 ± 2.03% mortality, separately.

In terms of workers, the positive control and 80% methanolic leaf extract have shown the greatest mean mortality (100%). A statistically similar termiticidal effect was observed between leaf decoction-40% methanolic leaf extract and 80% methanolic leaf extract-Solignum pairs. But, most of the experimental treatments were significantly different from each other in this termite caste. In general, the 80% methanolic leaf extract was the most potent leaf extract against N. luzonicus species with overall mean mortality of 98.34% (both soldiers and workers) and had a comparable termiticidal potency with Solignum® (standard check), followed by 40% methanolic leaf extract.

Table 1: Mean mortality of soldiers and workers of N. luzonicus due to the potency (termiticidal activity) of A. cathartica leaf extracts.

Treatments	Mean mortality (in %; 95% CI) *	
	Soldier	Worker
Leaf decoction	56.67a	57.67a
Crude leaf extract	56.00a	30.33b
40% Methanolic leaf extract	81.00a,b	55.00a
80% Methanolic leaf extract	96.67a,b	100.00c
Negative control (Distilled water)	0c	0d
Solignum (standard check)	100.00b	100.00c
CV	0.57	0.65
HSD	4.52	4.52

*Means along a column with similar superscripts are not significantly different; $p < 0.05$; Tukey post-hoc test (HSD); 20-minute post-treatment exposure)

Table 2: Mean mortality of soldiers and workers of M. gilvus due to the potency (termiticidal activity) of A. cathartica leaf extracts.

Treatments	Mean mortality (in %; 95% CI) *	
	Soldier	Worker
Leaf decoction	36.67a	72.33a
Crude leaf extract	36.33a	62.33a
40% Methanolic leaf extract	60.00b	87.00a,b
80% Methanolic leaf extract	91.00c	85.33a,b
Negative control (Distilled water)	0d	0c
Solignum (standard check)	100.00c	100.00b
CV	0.66	0.51
HSD	4.52	4.52

*Means along a column with similar superscripts are not significantly different; $p < 0.05$; Turkey post-hoc test (HSD); 20-minute post-treatment exposure).

Correspondingly, a significant difference in termiticidal potency with M. gilvus occurred in various concentrations as shown in Table 2. The standard check (Solignum®) measured the maximum mean mortality (100%) while no mortality has been recorded by the negative control in both cases. All leaf extracts have shown good mortality effect, ranging from 36.33% to

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91.00%, which is statistically different from that of the negative control. In terms of soldiers, the leaf decoction-crude leaf extract and 80% methanolic leaf extract-Solignum pairs have shown statistically comparable effect to each other. It is the 40% methanolic leaf extract which revealed a significant difference against all treatments. In terms of workers, the 40% and 80% methanolic leaf extract were statistically similar to that of the positive control which documented 87.00 ± 2.08% and 85.333.48% mortality, respectively. Unlike all other castes and termite species, it is the 40% methanolic leaf extract which was the most potent experimental treatment against M. gilvus workers which rendered a slightly higher mean mortality of 87.00%. At large, it is the 80% methanolic leaf extract which was the most effective treatments within this termite species which recorded an overall mean mortality of 88.17%, followed by the 40% methanolic leaf extract.

Discussion of the Results

The present study is in agreement to several researches performed to assess anti -termite activities of plant extract to various termite species. A corpus of studies (Ahmed et al., 2006; Djenontin Tindo et al., 2012; Jembere et al., 2002; Kareru et al., 2010; Manzoor et al., 2011; Rupal et al., 2011; Serit et al., 1991) corroborates the anti-termite potential of Azadirachta indica, Ocimum sanctum extract against H. indicola; leaf extract of Calotropis procera, Ricinus communis, Parthenium hysterophorus and Prosopis juliflora; seed oil-based paint of Thevetia peruviana versus Microtermes spp.; 2% leaf extract of Cymbopogon nardus against Macrotermes gilvus; leaf and seed extract of Whitania somnifera, Croton tiglium and Hydgrophilla auriculata against Microtermes obesi; leaf extract of Milletia ferruginea, Croton macrostachys, and Datura stramonium; methanol extract of Citrus netsudaidai seed (Limonoids: active ingredient) versus nymphs of Reticuliteranes speratus. Researches on essential oils from Chrisopogon zizanioides (Vetiver grass), Eucalyptus spp. (Lemon scented gum), Eugenia spp. (Eugenia), Tagetes erecta (Mexican marigold), Nepeta cataria (Catnip), Lepidium meyenii (Maca), Calocedrus formosana (Taiwan incense-cedar), Melaleuca cajuputi (Gelam), caryophyllata (Clove bud) and Allium sativum (Garlic) have also shown biological activities as feeding deterrents, repellants, or toxicants against termites (Cheng et al., 2004; Park and Shin, 2005; Peterson and Ems-Wilson, 2003; Sakasegawa et al., 2003; Singh et al., 2002; Tellez et al,. 2002; Zhu et al., 2001).

The principle toxicant and potential biotermiticidal substance of A. cathartica is the presence of a toxic iridoid lactone in all plant parts called allamandin (Abdel-Kader et al., 1997). Allamandin is known for its potent biological effects, including insecticidal properties that may impact termte physiology in several ways. his compound could disrupt termite cellular processes due to its toxicity, potentially interfering with their metabolism, neural function, and digestion (Al - Rajhy et al., 2005) Specifically, as an iridoid lactone, allamandin may inhibit or disrupt key enzymes or receptors within termites, leading to oxidative stress and cellular damage, which can impair essential physiological functions like feeding, movement, and reproduction. Additionally, allamandin's effects on termite gut microbiota—vital for digesting cellulose—could further weaken termites by disrupting nutrient absorption and energy production, leading to lethargy or death (Rattan, 2010; Duke & Dayan, 2015). Thus, its broad-spectrum toxicity could make allamandin a promising biotermiticidal agent, capable of reducing termite populations by affecting various physiological pathways critical for survival.

Other iridoid substances include plumieride, plumericin, isoplumiricin (Amaral et al. 2013). Ethyl caprinate, ethyl caprylate, squalene (Prabhadevi et al., 2012), alkaloids, and cyanogenic glycosides (Ahirwar and Jain, 2011) were other bioactive compounds recorded on previous studies which exhibit insecticidal potentials. Biological activities of these compounds were catalogued on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

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The persistence and degradation of allamandin in natural environments depend on factors like soil composition, temperature, and microbial activity. Iridoid lactones like allamandin are often relatively stable in the environment, breaking down slowly through microbial metabolism or exposure to sunlight (Petricevich & Abarca-Vargas, 2019). This slow degradation means that allamandin could remain effective for longer periods in pest management, providing sustained protection against termites (Shang et al., 2024). Understanding its environmental persistence helps in designing targeted pest control strategies, ensuring it remains potent without excessive accumulation or environmental harm. Effective application in pest management would balance persistence with biodegradability to minimize ecological impact.

CONCLUSION AND RECOMMENDATION

Based on the study's findings, Allamanda cathartica L. leaf extracts, particularly at an 80% methanolic concentration, show significant potential as a natural termite control option. With mean mortality rates reaching 98.34% for Nasutitermes luzonicus and 88.17% for Macrotermes gilvus, these extracts demonstrate a high level of effectiveness in managing termite populations. This promising result suggests that A. cathartica extracts could provide a viable alternative to chemical termiticides, particularly since their efficacy nearly matches that of Solignum®, a widely used synthetic product. Notably, in most cases, the 80% methanolic extract matched Solignum® in effectiveness, except for M. gilvus workers, where the 40% extract slightly outperformed it.

Given these encouraging outcomes, further study is recommended to advance A. cathartica L. as a sustainable option for termite management. Exploring its formulation for field application and assessing its long-term environmental effects could make it feasible for broader use. Based on these promising results, further research into A. cathartica L. extracts is highly encouraged, particularly exploring different formulations and concentrations. While the 80% methanolic extract has shown strong effectiveness, testing alternative concentrations and solvent mixtures could uncover even better results or lead to more practical, cost-effective solutions. Adjusting extract strengths or preparation methods may reveal optimal treatments for specific termite species or infestation levels, as well as adaptability across different environmental conditions. Field trials would also help to confirm its effectiveness across different environments, providing a more comprehensive understanding of its utility in real-world scenarios. Moreover, examining other active compounds within the plant and with other termite species may reveal additional benefits or ways to optimize its termiticidal action.

Incorporating A. cathartica extracts into termite management practices aligns with sustainable pest control efforts, potentially reducing reliance on conventional chemicals and their ecological impacts. With the appropriate development, an 80% methanolic formulation could be made into a user-friendly spray or coating, offering a natural, eco-friendly solution to termite problems. This alternative could benefit agriculture, forestry, and construction sectors by providing a safer option for termite control that minimizes environmental harm while addressing pest issues effectively.

ACKNOWLEDGMENT

This research project was supported by the faculty, administration, Department of Teacher Education, and the Office of the Director of Research, Extension, Innovation, and Commercialization of Visayas State University Tolosa and the Science Unit of Leyte N ormal University. More importantly, the invaluable contribution of Prof. Facundo Rey Ladiao was deeply appreciated in terms of technical content and data analysis. Furthermore, the researchers extend their gratitude to the technical support provided by Mr. and Mrs. Cesar de los Reyes (Taxonomist and Biologist), and Engr. Myrna Ambe (Chemist).

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In addition, the author declares that he has no conflicts of interest, financial or otherwise, that might be remarked as affecting the research stated in this article.

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