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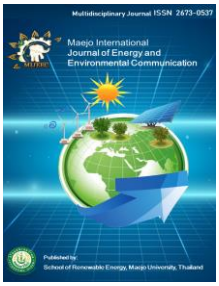

ARTICLE

A comparative study on the insecticidal property of *Bambusa vulgaris* and *Bambusa malingensis* shoot and leaf extract against green leafhopper (*Nephotettix virescens*)

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ABSTRACT

Rice is considered one of the staple foods in Southeast Asian countries, most especially in the Philippines. There are many factors needed to be considered in growing rice crops: the soil nutrient, the climate, the water supply, and the fertilizer used as a supplement to crop growth. But one important thing that farmers should consider is the infestation of pests, specifically the green leafhoppers, which is the primary carrier of tungro virus. This virus affects the growth and development of rice crops. This study aims to investigate the insecticidal effect of *Bambusa vulgaris* and *Bambusa malingensis* shoot and leaves extract against green leafhoppers (*Nephotettix virescens*). The extract was obtained by the decoction method. Phytochemical analysis of the two bamboo species has been carried out, as it was found out that both of them contains alkaloids, flavonoids, and glycosides. The results showed that both the shoot and leaves extract of *Bambusa vulgaris* and the shoot extract of *Bambusa malingensis* have potential insecticidal activity against *Nephotettix virescens*. Further research is needed to identify other factors that could strengthen the effectiveness of applying the extract to the insect specimen.

1. Introduction

Farmers described tungro as a significant problem in rice fields. The cause of the disease and its spreading was not clearly understood. They often thought it is transmitted through soil, water, air, and even in other insects. Over the years of thorough studies, it showed that the primary carrier of the disease is the green leafhopper *Nephotettix virescens* (Warburton et al., 1997; Bhuyar et al., 2018, 2021; Bhuyar et al., 2019a). The *Nephotettix virescens* is the primary vector of tungro-related disease in rice crops (Hibino and Cabunagan, 1986). Tungro is one of the disease-causing viruses which greatly affected the rice crops in Southeast Asian countries (Tantera, et al., 1973). It is associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) which causes the leaf yellowing and stunting of rice crops (Hibino et al., 1978; Omura et al., 1983). It causes great yield loss in rice crops production. The occurrence of such disease is associated with the density of vector present which determines the

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amount of virus-carrying organism present in a rice field (Suzuki et al., 1992). The population growth of vector and tungro outbreaks are caused by large-scale planting of high-yielding cultivars and the exhaustive use of fertilizers (Ling, 1972; Suzuki et al., 1989; Tantera, 1986). *Nephotettix virescens* is usually seen in wide areas of tropical and sub-tropical regions (Hokyo et al., 1967; Mejica et al., 2020a). Pattern of its population growth is significantly higher in temperate regions than other species of leaf hoppers (Cook et al., 1989). Initially, there is a low growth density of *N. virescens* and the reproductive rate is high during the growing season of rice crops (Widiarta, et al., 1990). Virus-causing insects can be reduced by effectively restraining the feeding activity of *N. virescens*.

Chemicals, such as insecticides have the ability to restrain the activity of this vector (Bhuyar et al., 2020b; Bhuyar et al., 2020c; Mejica et al., 2021b). Such activity is found in alkaloids which is considered as one of the most significant group of naturally occurring substances in insecticidal application (Rattan, 2010; Ramli et al., 2021a,b). Bioactive plant extracts such as alkaloids act as an insecticide and performed a significant role in the management of pests in agriculture (Bhuyar et al., 2020a, Bhuyar et al., 2019b; Mejica et al., 2021a). It is also proven to provide chemical protection against predatory insects and pathogens, damaging plants through multiple biological mechanisms (Brown & Trigo, 1995). Effectiveness is observed against the malaria vector *Anopheles gambiae* from the pyridine alkaloids extracted from *Ricinus communis*. Additionally, larvicidal and antifeedant activities have been showing against *Spodoptera littoralis* larvae from the extracted furocoumarin and quinolone alkaloids of *Ruta chapensis* leaves (Emam et al., 2009). Alkaloids from *Pergularia tomentosa* also caused antifeeding and larvicidal effects based on the study of Acheuk and Doumandji-Mitiche (2013). Extract from *Arachis hypogea* also exhibited alkaloid compounds and proven to have a larvicidal effect on the chikungunya and malarial vectors (Velu et al., 2015). It has also been deduced from a thorough study of Lee (2000) that piperidine and pipernonaline alkaloids have larvicidal activity on mosquitos. Moreover, flavonoids are also effective against eliminating pests (Bhuyar et al., 2020d; Mejica et al., 2020b).

In Acheuk & Doumanji-Mitchie (2013) study, flavonoids safeguard the plants against the plant-feeding herbivores and insects. Proanthocyanidins and isoflavonoids are some of the classes of flavonoids responsible for protecting plants from insects (Santos et al., 2016; Ramli et aal., 2020a,b). Golawska et al. (2014) isolated two polyphenolic flavonoids: flavonol quercetin and flavonone naringenin and used them as effective insecticides against *Acyrthosiphon pisum*. Morimoto et al. (2000) concluded that flavonoids could act as feeding deterrents. In a study by Maliang et al. (2015), they investigated the insecticide and acaricide activity of bamboo charcoal by-products by distilling the crude bamboo vinegar collected from bamboo charcoal under reduced pressure. They have proven that bamboo tar could be used as a new natural insecticide and acaricide in the agricultural field instead of synthetic pesticides, which could harm humans and other animals. Jia Sun et al. (2016) examined the chemical constituents of bamboo shoots by HPLC-UV analysis, where 12 significant components were quantitatively analyzed. 2,400 mg/kg of L-phenylalanine is considered a precursor to the alkaloid, and 80 mg/kg of uridine has been found in bamboo shoots. Previous reports of the chemical composition have found out that the bamboo shoots contain aromatic amino acids and nucleotides for flavonoids and phenolic acids. Furthermore, the pathogenic effect of alkaloid compounds can also be applied to the emergence of tungro virus brought by *N. virescens*. Hence, this paper aims to investigate the bioactive phytochemicals in *Bambusa vulgaris* and *Bambusa malingensis* shoot and leaves extract based on the mortality rate of *N. virescens*.

2. Materials and Methods

2.1. Sample preparation and extraction

The study was conducted using the completely randomized design for experimental research, with three trials and five treatments. Figure 1, displays the collection of a mature *B. vulgaris* and *B. malingensis* from a bamboo farm in San Miguel, Iloilo. Each of the treatments has varying concentrations of leaf and shoot extract. Treatment A was extracted from the leaves of *B. vulgaris* (BVLE) with 50% volume concentration and 100% volume concentration. Then, treatment B was extracted from the shoots of *B. vulgaris* (BVSE) with 50% volume concentration and 100% volume concentration, respectively.

On the other hand, Treatment C was extracted from B. malingensis (BMLE) leaves with 50% volume concentration and 100% volume concentration, respectively. Treatment D was extracted from the shoots of *B. malingensis* (BMSE) with 50% volume concentration and 100% volume concentration. Each bamboo species extract was tested against a positive control Azundra 5EC, a commercial insecticide used in rice crops.

Figure 1 Sample collection.

The crude extracts were obtained using the decoction method. This method is preferred for more complex herbs like roots, barks, and seeds. The shoots and leaves were ground first before the decoction process to ensure effective extraction. A 500 mL of distilled water was pre-boiled for 10 mins. At boiling temperature on a hot plate. 150 grams of the raw material was weighed and was added to the boiling water. The heating temperature was lowered to 60 ºC. The solution was heated for 30 mins. And maintained a constant temperature of 60 ºC until 50% of the water was vaporized. The boiling temperature was maintained to prevent any damage to its chemical constituents. The vessel was maintained in a closed condition to prevent any loss through evaporation. The extract was removed from the heating source and strained using a filter paper at the end of boiling.

The extracts were prepared at varying volume concentrations of 50% and 100% bamboo leaf and shoot. A volume of 80.0 mL of the solution was used. For 50% v/v concentration, 40.0 mL of the extract was added to 40.0 mL distilled water for dilution. A pure extract was used in 100% v/v concentration.

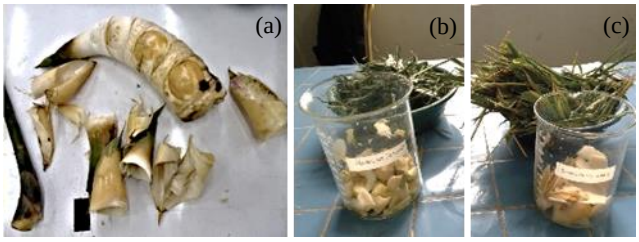

Figure 2 Bamboo shoots (a), *B. vulgaris* (b), and *B. Malingensis* (c).

2.2. Phytochemical Analysis

Different phytochemical screening tests were carried out for all the extracts based on the standard methods. Small quantities of aqueous, ethanol, acetone and methanol (Mejica et al., 2020a; Nasution et al., 2021) extracts of leaves and shoots of *B. vulgaris* and *B. malingensis* were dissolved and used to detect phytochemicals such as glycosides, phytosterols, proteins, alkaloids, flavonoids, tannins, saponins.

Figure 3 Phytochemical analysis.

2.2.1. Test for Glycosides

To the solution of extract in glacial acetic acid, few drops of ferric chloride and concentrated H_2SO_4 were added and observed for reddish-brown colouration at the junction of 2 layers and bluish-green colour in the upper layer.

2.2.2. Test for flavonoids

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed, and metal magnesium was added to this solution 5-6 drops of con. HCl was added. Red colouration was observed for flavonoids and orange for flavones.

2.2.3. Test for Tannins

To 0.5 ml of extract solution, 1 ml water and 1-2 drops of ferric chloride solution was added. The blue colour was observed for garlic tannins and greenish-black for catecholic tannin.

2.2.4. Reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling solution was added and observed for brick red colored ppt.

2.2.5. Test for Alkaloids

Most alkaloids are precipitated from neutral or slightly acidic solutions by Mayer's reagent. The alcoholic extract was evaporated to dryness, and the residue was heated on a boiling water bath with 2% HCl. After cooling, the mixture was filtered and treated with few drops of Mayer's reagent. The sample was then observed for turbidity or yellow ppt.

2.2.6. Test for Saponins

Froth Test was used to test saponins in the extract. 10 mL of extracts diluted with distilled water to 20 mL, and this shaken in a test tube for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

2.3. Preparation of Collection Test Tubes of N. virescens

Thirty vials of the same size were prepared to store *N. virescens* with an alaskin net as a cover sealed with a rubber band. Each test tube has the same number of *N. virescens*. Three sweep nets were prepared to collect *N. virescens* made of alaskin net, wire, and stick for catching the green leafhoppers.

Random *N. virescens* was collected at Sitio Binaobao, Brgy. Cali, Dumangas. Pests were collected, and vials and bottles were prepared and sealed with alaskin net and rubber band, which was used to store the collected pests. The transfer of *N. virescens* was carried out through siphon method with the aid of straw.

2.4. Application of Leaf Extract to N. virescens

The spray method (figure 4) was used to apply the alternative pesticide with different leaf and shoot extract concentrations using a sprayer. Approximately one mL of each treatment was sprayed into each test tube which contains ten green leafhoppers. Three trials of the experiment were performed, which made up a total of 500 green leafhoppers used in the experiment. The effectiveness of

the alternative pesticide was determined through the mortality rate. The mortality rate of the green leafhoppers was determined after applying the extract sprayed at intervals of 6 minutes for a total of 36 minutes. After the application, the insecticidal property was observed by identifying the mortality rate on the first day of application, after three days of application, and after the seventh day.

Figure 5 Application of leaf extract to *N. virescens.*

2.5. Statistical Analysis

Before analysis, the gathered data from laboratory experiments were corrected for the mortality rate of the control using Abbott's correction. An analysis of variance (ANOVA) was carried out to analyze the data, and the significant difference between the mean results of the several treatments was determined according to Tukey's honestly significance difference (HSD) with $\alpha = 0.05$.

3. Results and Discussion

3.1. Phytochemical Analysis of B. vulgaris and B. malingensis shoot and leaves extract

Table 1 data shows the qualitative results of phytochemical analysis of *B. vulgaris and B. malingensis* leaf and shoot extract used to treat *N. virescens* in this study. Five substances were present, namely alkaloids, saponins, flavonoids, tannins, and phenols. These combinations were considered responsible for the insecticidal property of the extract. Ayoola et al. (2008) also conducted a similar study that established the presence of alkaloids, saponins, and flavonoids.

Table 1 Results of the Phytochemical Analysis of *Bambusa Leaves and Shoots Extract*.

Phytochemical Components	A	B	C	D	E	F	G	H
Alkaloids	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Tannins	+	+	-	-	+	+	-	-
Glycoside	+	+	+	+	+	+	+	+
Reducing Sugar	+	+	+	+	+	+	+	+

3.2 Insecticidal activity

Table 2 shows the *Bambusa vulgaris* leaves extract with 50% and 100% volume concentration, and the corrected mortality was 79.49%-84.21%. One way analysis of variance showed that the corrected mortality of 84.21 was significantly different from the control. Furthermore, the effect of every treatment was reduced after 3 and 7 days of spraying the solution. It is seen that the corrected mortality on the first day is significantly higher than after 3 to 7 days.

Table 2 Activity of *Bambusa vulgaris* Leaves Extract (BVLE) against *Nephotettix virescens*.

Concentration (%v/v)	Corrected mortality (%) mean ± SD 95% confidence level		
	1 day after spraying	3 days after spraying	7 days after spraying
BVLE1 (100%)	79.49 ± 1.25	62.50 ± 1.63	0.00
BVLE2 (50%)	84.21 ± 0.82	71.67 ± 0.94	69.70 ± 0.94

Table 3 shows the *Bambusa vulgaris* shoots extract with 50% and 100% volume concentration, and the corrected mortality rate was 78.95% to 84.62%. One way analysis of variance showed that the corrected mortality rate of 84.62% was significantly different from the control. Corollary to Table 2, it is also observed that the mortality rate exhibits a reduction from the first day of exposure to the extract until the seventh day of observation.

Table 3 Activity of *Bambusa vulgaris* Shoots Extract (BVSE) against *Nephotettix virescens*.

Concentration (%v/v)	Corrected mortality (%) mean ± SD 95% confidence level		
	1 day after spraying	3 days after spraying	7 days after spraying
BVSE1 (100%)	84.62 ± 1.63	76.39 ± 1.25	0.00
BVSE2 (50%)	78.95 ± 0.82	60.00 ± 2.83	42.42 ± 0.47

Table 4 shows the *Bambusa malingensis* leaves extract with 50% and 100% volume concentration, and the corrected mortality rate was 75.64% to 77.19%.

Table 4 Activity of *Bambusa malingensis* Leaves Extract (BMLE) against *Nephotettix virescen*.

Concentration (%v/v)	Corrected mortality (%) mean ± SD 95% confidence level		
	1 day after spraying	3 days after spraying	7 days after spraying
BMLE1 (100%)	75.64 ± 1.25	70.83 ± 1.63	0.00
BMLE2 (50%)	77.19 ± 1.25	66.67 ± 1.70	39.39 ± 2.36

One-way analysis of variance showed that the corrected mortality rate of 77.19% exhibited a significant difference from the control. It also follows the same principle of the previous claims that the mortality rate decreases from the first day up to the seventh

day of observation. Comparing the data from table 2, both species exhibited an excellent insecticidal property at 50% volume concentration of leaves extract on the first day of application.

Table 5 shows the *Bambua malingensis* shoots extract with 50% and 100% volume concentration, and the corrected mortality rate was 80.77% to 84.21%. One way analysis of variance showed that the corrected mortality rate of 84.21% exhibited a significant difference from the control. The same trend also follows with the declining mortality rate from the first day of application to the seventh day of observation. Comparing it to the data obtained from table 3, *Bambusa vulgaris* showed a better result than the *Bambusa malingensis* at a 100% volume concentration of shoot extract.

Table 5 Activity of *Bambusa malingensis* Leaves Extract (BMLE) against *Nephotettix virescen*.

Concentration (%v/v)	Corrected mortality (%) mean ± SD 95% confidence level		
	1 day after spraying	3 days after spraying	7 days after spraying
BMSE1 (100%)	80.77 ± 0.82	56.94 ± 1.70	0.00
BMSE2 (50%)	84.21 ± 0.82	66.67 ± 1.25	39.39 ± 1.70

One factor analysis of variance was also used to compare the effectiveness of the extracts from both species according to 50% and 100% volume concentration. It showed a significant difference between the mean of the samples taken from the first day of application and observation until the seventh day of observation for both concentrations. Post-hoc Tukey HSD at a 95% level of confidence showed that there is a significant difference between the first day of observation and the seventh day of observation for 100% volume concentration extract, while the first day and the third day of observation showed a significant difference for 50% volume concentration extract.

Several studies have been carried out for the observation of bamboo's beneficial properties. One of which is the study by Hwang et al., 2006. They have observed that bamboo charcoal factories' bamboo vinegar produced as waste had been traditionally used as a sterilizing agent, deodorizer, fertilizer, growth-promoting agent, and antimicrobial agent. It has also been accounted for its sterilizing, antimicrobial, and antiviral activity against tobacco virus, porcine reproductive and respiratory syndrome virus, enveloped RNA virus from the Arteriviridae family, and the most famous air-borne foot-and-mouth disease virus. Studies on bamboo vinegar have been quite known for the past years, but very few about the bamboo tar. In the study of Nakajima et al., 2007 they have investigated the composition and uses of the bamboo wood tar. They have found out that the wood and vinegar from bamboo have identical tar pyrolysates, composed of phenolic compounds and aromatic hydrocarbons. These tars are said to be composed of various compounds, namely acetic acid, benzofuran, pyrroles, and naphthalene. It has also been studied to have an excellent wood coating performance with polyurethane coating by blending it with bamboo tar and castor oil. A polymeric toluene diisocyanate was used as a hardener which added an excellent coating to the wood (Lu and Hong, 2010).

The medicinal property of bamboo has also been carried out, one of which is the study on triterpenoid-rich extracts prepared using carbon dioxide supercritical fluid extraction from the shavings of various bamboo species. Succeedingly, an extract from *Phyllostachys nigra* var. *henonis* bamboo shavings showed decreased serum total cholesterol and triglyceride levels in hyperlipidaemic rats showing antihypertensive activity. The same study with the extract has been carried out and has exhibited a reduced systolic pressure in rates with spontaneous hypertension by vasodilatory effect. *In vitro* results have shown friedelin in the extract, the main triterpenoid compound that probably provided the vasodilatory effect (Jiao et al., 2007).

Cyanogenic glucoside performs an essential role as a protective defense against herbivores in plants (Zagrobelny et al., 2004). This type of glycoside is said to be present in bamboo. This chemical possesses an insecticidal activity which is an effective fumigant against stored-product insects and is sometimes used as soil fumigants (Park & Coats, 2002). Glycosides possessed a larvicidal activity against chikungunya and malarial vectors, making them a potential insect pest controller (Wimmer et al., 2007). Although many studies may have stated the broad scope of application of bamboo, people still cling to some unscientific myths about it, which often result in the loss of attention to its outstanding contribution to our lives. Therefore, further research is recommended on the production and processing of bamboo products to increase its potential against the emerging problems in our environment.

4. Conclusion

Based on the findings, it was concluded that the *Bambus Vulagaris* and *Bambusa Malingensis* shoot and leaf extracts contained alkaloids, flavonoids, tannins, saponins and phenols based on the phytochemical analysis results; There were significant differences in the insecticidal property of *Bambus vulagaris* and *Bambusa malingensis* shoot and leaf extracts of various concentrations compared with treated control (Azudran 5EC). Based on results, the highest concentration of 100% v/v *Bambusa vulgaris* shoot and 50% v/v leaves extract with 84.62%, and 84.21% corrected mortality rate respectively was comparably effective against the *Nephotettix virescens*. On the other hand, *Bambusa malingensis* 50% v/v shoot extract with a corrected mortality rate of 84.21% was comparably effective with the treated control. Both results were observed immediately on the first day of application.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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