Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society
ANALIS Persatuan Sains Analisis Malaysia

# SCREENING OF PLANTS FOR INSECTICIDAL ACTIVITIES AGAINST ORIENTAL FRUIT FLY *Bactrocera dorsalis* (HENDEL) FOR PEST MANAGEMENT OF MANGO *Mangifera indica*

(Saringan Tumbuhan bagi Aktiviti Serangga Perosak Terhadap Lalat Buah Oriental *Bactrocera dorsalis* (Hendel) untuk Pengurusan Serangga Perosak Mangga *Mangifera indica*)

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Received: 20 November 2019; Accepted: 13 June 2020; Published: 11 August 2020

**Abstract**

The mango, *Mangifera indica*, is one of the many fruits exported by the Philippines. However, the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is becoming a threat to its production; thus, its control and management are now imperative. This research conducted a preliminary screening of different plants with insecticidal properties that could be used for the development of potential biocides. Twenty plants were screened for their insecticidal activities against *B. dorsalis*. The plant that showed the highest mortality was further investigated using various extraction methods such as hot infusion, maceration, hot continuous reflux (Soxhlet), and fermentation. Several solvents were also considered in the extraction, including rice wine, rice wash, vinegar, and distilled water. The extracts were tested in terms of mortality against *B. dorsalis* after 6, 12, and 24 hours of exposure using an improvised olfactometer. *Azadirachta indica, Andrographis paniculata, Indigofera zollingeriana*, and *Lantana camara* gave 100% mortality at 24 hours exposure, but *A. indica* was the most effective, having 46.67%, 91.67%, and 100% mortality at 6, 12, and 24 hours, respectively. Further investigations employing different extraction–solvent combinations revealed that fermentation of *A. indica* leaves using rice wash is the most economical method for extracting the active components against *B. dorsalis* and can be utilized in the development of biocide for mango pest management.

**Keywords:** neem, biocide, fermentation, mangoes, pest management

**Abstrak**

Mangga, *Mangifera indica* adalah satu dari buah-buahan yang dieksport oleh Filipina. Namun, lalat buah oriental, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) menjadi ancaman bagi penghasilannya. Oleh itu, pengawalan dan pengurusan mestilah dimulakan. Kajian ini dijalankan melalui saringan awal bagi tumbuhan yang mempunyai sifat aktiviti serangga perosak yang boleh digunakan bagi pembangunan potensi biosida. Dua puluh tumbuhan telah disaring bagi mengkaji aktiviti serangga perosak terhadap *B. dorsalis.* Tumbuhan yang menunjukan kematian tertinggi telah dikaji lebih terperinci mengunakan pelbagai kaedah pengekstrakan seperti seduhan panas, maserasi, refluks berterusan panas (Soxhlet), dan fementasi. Pelarut berbeza juga dipertimbang semasa pengekstrakan termasuklah wain beras, basuhan beras, cuka dan air suling. Ekstrak yang diuji bagi kematian terhadap B. dorsalis selepas 6, 12 dan 24 jam pendedahan mengunakan olfaktometer yang telah ditambahbaik. *Azadirachta indica, Andrographis paniculata, Indigofera zollingeriana*, dan *Lantana camara* memberikan 100% kematian pada masa 24 jam pendedahan, tetapi *A. Indica* adalah paling efektif mempunyai 46.67%, 91.67%, dan 100% kematian masing-masing pada 6, 12 dan 24 jam. Kajian selanjutnya dijalankan dengan kombinasi pelarut pengekstrakan berbeza mendedahkan bahawa fementasi daun *A. Indica* mengunakan basuhan beras adalah kaedah paling ekonomikal bagi pengekstrakan komponen aktif melawan *B. dorsalis* dan boleh di manfaat bagi pembangunan biosida terhadap pengurusan serangga manga.

**Kata kunci:** margosa, biosida, fementasi, manga, pengurusan serangga

## Introduction

The fruit fly species *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), also known as the oriental fruit fly, has been one of the most serious economic pests affecting agricultural fruits in India, East Asia, and the Pacific [1]. These fruit flies deposit their eggs into fruits and vegetables, whose flesh is subsequently consumed by developing larvae [2]. The fruit flies' invasive action has caused major economic losses in horticultural crop production [3]. Oriental fruit flies attack more than 150 kinds of fruits and vegetables, including avocadoes, bananas, citrus, coffee, figs, guavas, mangoes, papayas, passion fruit, pineapples, and tomatoes. *B. dorsalis* has been identified as the principal pest of mangoes in the Philippines [4], having caused a decrease in production of 34.36 and 33.13 thousand metric tons in 2014 and 2015, respectively [5]. As the mango is of valuable importance in both domestic and international markets, control and management of *B. dorsalis* is necessary to prevent significant losses in fruit production. This research conducted a preliminary screening of different plants reported to possess insecticidal properties, followed by screening of extraction procedures and solvents that could be employed for the development of potential biocides against *B. dorsalis*.

Three criteria were used in the selection of plants for the screening. The first was that the plants were reported to possess insecticidal properties. The second was their availability in the locality, and the last was that the plants were indigenous or endemic. Twenty plants were screened for their activities against *B. dorsalis.* Most of these plants were reported to possess insecticidal properties. These are *Andrographis paniculata* (Burm f.) Wall. ex Nees (whole plant ethanolic extract has larvicidal, pupicidal, adulticidal, and ovicidal properties against the malarial vector *Anopheles stephensi* Liston [6], and adulticidal against cowpea [7]; contains andrographolide, a diterpene against *Aegis egypti* [8]); *Annona muricata* L. (antiparasitic [9] and has mosquitocidal activities [10]); *Annona squamosa* Linn. (seeds extract contains antifeedant activity against lepidopteran pests [11,12]).

*Azadirachta indica* (effective against malarial vector *Anopheles stephensi* [13]); *Blumea balsamifera* (Linn.) DC. (used in livestock healthcare as poultice for abscesses in swine and ruminants [14]); *Cassia alata* L. (Roxb.) and *Chromolaena odorata* Linn. (weeds with a strong aromatic odor sometimes used to drive away insects in stored products [15]; *Chrysophyllum cainito* and *Tabernaemontana pandacaqui* (repellents against hematophagous insects [16]); *Cymbopogon citratus* (insecticidal properties against the house fly, *Musca domestica* [17]); *Cyperus rotondus* (tuber with toxicity to the diamondback moth [18]); *Euphorbia tithymaloides* (with high toxicity to the oriental fruit fly [19]), *Garcinia vidalii* (no reported properties but represents an endemic species); *Gliricidia sepium* (rodenticide [20]); *Indigofera zolligeriana* (used as feed for goats and sheep; contains flavanols, glycosides, and megastigmane glucosides [21]); *Lantana camara* (contains pentacyclic triterpenes called lantadene [22]), *Moringa oleifera* (bioinsecticide with mechanism of action involving carbohydrate–lectin interactions on the surface of the digestive tract, with glycoproteins and other glycosylated structures in the midgut and resistance to enzymatic digestion [23]); *Ocimum sanctum* (with larvicidal effect on *Aedes aegypti* and *Culex quinquefasciatus* [24]); *Tridax procumbens* (exhibits activity against scale insects (*Phenacoccus solenopsis*) of *Psidium guajava* [25]); and *Zamioculcas zamiifolia* (in combination with *C. odorata* exhibits insecticidal properties against the harlequin cockroach

[26]). *Euphorbia tithymaloides* was the only plant in this study reported to have insecticidal effects on the oriental fruit fly [19].

The extraction methods and the solvents used are crucial in the evaluation of plants' secondary metabolites. Numerous studies have been conducted in search of the best technique and solvent to extract plants' secondary metabolites [27]. Among these techniques are the use of liquid, steam, and supercritical extractions [28]. The conventional methods like hot infusion, maceration, decoction, Soxhlet extraction, etc. are being modified, and pretreatments of samples like microwave and ultrasound processes are adopted [29]. Soxhlet is a conventional extraction method still considered as one of the reference methods in comparing the success of a newly developed methodology [30]. Rice wine, rice wash, and vinegar have been employed in the extraction of phytochemicals as substitutes for methanol, ethanol, hexane, ether, etc., which are conventionally used in the extraction process. Rice wine contains 15-20% ethanol and 120-440 mg/mL glucose levels [31, 32]. Rice wash contains para-aminobenzoic acid (PABA), ferulic acid and allantoin [33], and volatile fatty acids [34]. Vinegar, on the other hand, contains not less than 3% but no more than 5% acetic acid, 0.5% ethanol and other polyphenols [35]. This study aimed at employing a "green approach" using household chemicals in the preparation of biocide for *B. dorsalis*.

## Materials and Methods

### Plant materials collection, preparation, and extraction

The leaves of the different plants were detached from the stems and washed thoroughly with tap water. The clean leaves were air dried for 3-5 days in the shade during the summer season. Drying for more than 5 days is not advisable, since molds will grow during this period. The sample was considered to be dry when it was easily crushed by the hand. The dried leaves were ground in a blender and stored in a tightly covered container until further use. Hot infusion was done by boiling 150 mL of distilled water, then adding 15 grams of the previously ground leaves. The mixture was set aside for 15 minutes. The extract was filtered using Whatman No. 42 filter paper, and the filtrate was placed in an amber bottle and stored in the refrigerator until it was used for analysis. This was to prevent reaction with light or degradation at room temperature.

### Trapping of male fruit flies

Male fruit flies were trapped inside a canister with cotton wet with 10 drops of methyl eugenol. The trap was placed under a mango or guava tree from 6:00 to 8:00 in the morning, as this is the time when the most fruit flies are trapped. The canister was covered when there were enough fruit flies inside. Cotton dipped in sugar solution placed on the net covering of the canister served as food for the fruit flies.

### Mortality test

The mortality test was done using an improvised olfactometer adopting the method of Chang and co-workers [36] with some modifications. The olfactometer was a transparent plastic food container measuring 4.5 in. in top diameter, 3.5 in. in bottom diameter, and 4 in. high. The cover was cut, forming a 2-in.-diameter hole, which was covered with silk fabric to prevent the fruit flies from suffocating during the test.

Male fruit flies were introduced into the improvised olfactometer after 30-60 minutes using a transparent tube made of acetate. A small cotton ball was wet with 10 drops of the crude extract and placed at the top center of the container 5-10 minutes after the male fruit flies were introduced. The container was covered, and the flies were observed after 6, 12, and 24 hours for the screening study and 3, 6, 12, 18, 22, and 24 hours for further investigations of the most effective plant. The fruit flies' reaction to the extract was noted. A fruit fly was considered dead when no more movement was observed. Percent mortality was computed by dividing the number of dead fruit flies by the total number of flies inside the plastic container.

### Data analysis part A

Adult fruit flies' percent mortality was compared per plant extract using analysis of variance and post hoc using the Duncan multiple range test. The plant extract that showed the highest mortality rates at 6, 12, and 24 hours and the plant extract that showed the highest mortality at 6 hours exposure time were further subjected to efficacy tests applying the following procedure. For the first cycle, male fruit flies were introduced into the improvised olfactometer after 30-60 minutes using a transparent tube made of acetate. A small cotton ball was wet with 10 drops of the crude extract and placed at the top center of the container 5-10 minutes after the male fruit flies were introduced. The container was covered, and the flies were observed after 6, 12, and 24 hours.

Mortality rates were recorded, after which the dead fruit flies were transferred into vials and stored in the refrigerator. For the second cycle, the same olfactometer and cotton ball were used. The olfactometer was cleaned by wiping it with tissue paper wet with 70% ethanol to remove the remaining particles from the dead flies. The same cotton ball with extract (no addition of fresh extract) was moistened with 10 drops of distilled water, a new set of fruit flies were introduced into the olfactometer, and the mortality rates were read after 6, 12, and 24 hours. Mortality rates were recorded, the dead fruit flies were placed into vials and stored in the refrigerator, the olfactometer was cleaned with ethanol, and the cotton ball was wet with distilled water. The third cycle used the same procedure as the second cycle. The plant extract that gave higher mortality rates was subjected to further investigations employing the four solvents and four extraction methods.

**Effects of solvent and extraction method on insecticidal properties**

Four different extraction methods were tested: Soxhlet or hot continuous extraction, hot infusion, fermentation, and maceration. The Soxhlet method was done as follows: The ground plant material (15 g) was loaded into the cellulose thimble, which was then placed inside the Soxhlet extractor. The solvent (150 mL) was heated using the heating mantle, and the solvent began to evaporate, moving through the apparatus into the condenser. The condensate then dripped into the reservoir containing the thimble. When the level of solvent reached the siphon, it poured back into the flask, and the cycle began again. The process ran for a total of 8 h to ensure that the sample was in contact with the solvent long enough; thus, extraction of the active components was more efficient. Hot infusion was done by adding 15 g of previously weighed ground leaves into 150 mL of boiling solvent. The mixture was set aside for 15 minutes. The extract was then filtered using Whatman No. 42 filter paper, and the filtrate was placed in an amber bottle and stored in the refrigerator until it was used for analysis. The fermentation method was carried out by soaking 15 g of the plant material in 150 mL of the solvent. The container was wrapped with aluminum foil and stored inside a locker. The mixture was set aside for 5 days, after which the mixture was filtered, and the filtrate was placed in an amber bottle and stored in the refrigerator until it was used for analysis. Maceration was done by placing 15 g of the plant material in 150 mL of the solvent. The mixture was set aside for 1 hour, then filtered, and the filtrate was placed in an amber bottle and stored in the refrigerator until it was used for analysis.

Four green solvents were used in this experiment primarily to test the effect of household chemicals and waste in rice cooking. These are readily available in a Filipino household in the provinces where mango production is usually located. These are rice wine, rice wash, vinegar, and distilled water. These materials contain polar substances that can extract the insecticidal components of the plant under study. The rice wine was produced in Panupdupan, Lamut, Ifugao, Philippines. The rice wash was prepared by washing 100 g of bungkitan glutinous rice with 100 mL of distilled water. The rice-washing water was then drained into a beaker. Another 100 mL of distilled water was added to the glutinous rice, which was then washed, and the second washing was added to the first washing. The combined rice wash was filtered to remove the suspended solids. The vinegar, called '*sukang basi*' is a byproduct of sugar production.

**Data analysis part B**

The adult fruit flies' percent mortalities were compared per solvent–extraction method combination using analysis of variance and post hoc using the Duncan multiple range test. The solvent–extraction combinations that showed high mortality rates were evaluated for their economic value by subjecting them to probit analysis to determine the lethal dose ($LD_{50}$).

This is the concentration of the extract that is lethal to 50% of the insect population tested.

**Results and Discussion**

The percent mortalities of the different aqueous extracts against *Bactrocera dorsalis* (Hendel) were observed after 6, 12, and 24 hours (Table 1). There were two plant extracts that showed mortality of >20% after 6 hours exposure; 11 plants after 12 hours, the highest of which was *A. indica* (91.67%); and 16 plants after 24 hours, of which *A. paniculata*, *A. indica*, *I. zollingeriana*, and *L. camara* showed mortality of 100%.

Analysis of variance and post-hoc analysis (Duncan multiple range test at the 5% level) of the activity of the extracts at 6-hour exposure indicate that *A. indica* leaf extract is the most effective, with 46.67% mortality, followed by *O. sanctum* (40.00%) and *C. alata* (33.33%). However, the mortality rates of *L. camara* (20.00%), *B.* balsamifera, *G. sepium*, and *I. zollingeriana* (13.33%) cannot be ignored when compared to those plants with 0% mortality. Effectivity may increase at a longer time of exposure, as seen in their activities at 12 and 24 hours. These extracts may contain smaller amounts of the active components; thus, diffusion into the olfactometer is slower. But at longer periods, the concentration of extract inhaled or ingested by the fruit flies increases, leading to mortality.

At 12-hour exposure, *A. indica* still gave the highest mortality (91.67%), but this was not significantly different from the activities of *L. camara* (73.33%), *O. sanctum, G. sepium*, and *A. paniculata* (66.67%). This could mean that these five plants' components namely, azadirachtin, lantadane, eugenol, oxytroside and andrographolide, respectively, possess insecticidal effect against *B. dorsalis*.

At 24-hour exposure, four extracts gave 100% mortality: *A. paniculata*, *A. indica*, *I. zollingeriana*, and *L. camara*. Those extracts with mortality ranging from 53.33% to 93.33% are not significantly different from the four extracts with 100% mortality at 5% level of significance using the Duncan multiple range test (Table 1). The plants with mortality rates of 53.33–93.33% after 24 hours are *G. sepium*, *T. pandacaqui, O. sanctum*, *M. oleifera*, *A. squamosa*, *A. muricata*, *B. balsamifera*, *C. alata*, *E. tithymaloides*, *C. rotondus*, and *Z. zamiifolia*. All these plants were reported to possess insecticidal properties against different pests. The mode of action of the different plant extracts against the oriental fruit fly cannot be explained further at this point. Neurotoxicity may be one of the reasons [33]. Of the 20 plants screened in this study, only *C. odorata*, *C. cainito*, *C. citratus*, *G. vidalii*, and *T. procumbens* did not show promising results using water as the extracting solvent. This may be because the insecticidal properties reported in the literatures cited above were extracted using organic solvents [15–17, 25].

Two extracts were chosen for the efficacy test: *A. indica*, which had the highest mortality at 12 hours, and *A. paniculata*, which did not show activity at 6 hours but gave 100% mortality at 24 hours. The dried cotton balls with extract used in the test in Table 1 were wetted with 10 drops of distilled water, the improvised olfactometers were cleaned and loaded again with fruit flies for the second and third cycles, and mortality rates were again recorded (Table 2).

Post-hoc analysis denotes that the *A. indica* and *A. paniculata* aqueous extracts are still effective up to the third cycle. However, a decrease in activity was observed at 6 and 12 hours for *A. indica* and at 12 hours for *A. paniculata*. Moreover, at 24 hours, there was a slight decrease in the activities in the third cycle for *A. indica* and in the second and third cycles for *A. paniculata*, but still no significant differences from the first cycle. This result implies that the extracts can be used against *B. dorsalis* not only once but up to three times or maybe more. The significant decrease in the activity of both plant extracts in the second and third cycles at 12 hours may be due to the decrease in concentration of the active components as a result of inhalation or consumption by the first batch of fruit flies. This part of the study further implies that *A. indica* (also known as neem) is the most effective plant in the management of *B. dorsalis.* This result conforms with the report that the azadirachtin present in the neem has antifeedant and growth inhibition effects against insects [31].

Table 1. Percent mortality of the different aqueous plant extracts against fruit fly *Bactrocera dorsalis* (Hendel)

| Scientific Name | Percent Mortality* | | |
|---|---|---|---|
| | 6 hours | 12 hours | 24 hours |
| *A. paniculata* | $0.00^{d}$ | $66.67^{abc}$ | $100.00^{a}$ |
| *A. muricata* | $6.67^{cd}$ | $20.00^{efg}$ | $66.67^{abc}$ |
| *A. squamosa* | $0.00^{d}$ | $33.33^{def}$ | $73.33^{ab}$ |
| *A. indica* | $46.67^{a}$ | $91.67^{a}$ | $100.00^{a}$ |
| *B. balsamifera* | $13.33^{bcd}$ | $33.33^{def}$ | $66.67^{abc}$ |
| *C. alata* | $33.33^{abc}$ | $60.00^{bcd}$ | $66.67^{abc}$ |
| *C. odorata* | $0.00^{d}$ | $0.00^{g}$ | $6.67^{ef}$ |
| *C. cainito* | $0.00^{d}$ | $0.00^{g}$ | $0.00^{f}$ |
| *C. citratus* | $0.00^{d}$ | $6.67^{fg}$ | $26.67^{bcdef}$ |
| *C. rotondus* | $0.00^{d}$ | $6.67^{fg}$ | $53.33^{abcde}$ |
| *E. tithymaloides* | $6.67^{cd}$ | $26.67^{efg}$ | $60.00^{abcd}$ |
| *G. vidalii* | $0.00^{d}$ | $0.00^{g}$ | $20.00^{cdef}$ |
| *G. sepium* | $13.33^{bcd}$ | $66.67^{abc}$ | $93.33^{a}$ |
| *I. zollingeriana* | $13.33^{bcd}$ | $40.00^{cde}$ | $100.00^{a}$ |
| *L. camara* | $20.00^{bcd}$ | $73.33^{ab}$ | $100.00^{a}$ |
| *M. oleifera* | $0.00^{d}$ | $0.00^{g}$ | $75.00^{ab}$ |
| *O. sanctum* | $40.00^{ab}$ | $66.67^{abc}$ | $80.00^{a}$ |
| *T. procumbens* | $0.00^{d}$ | $6.67^{fg}$ | $13.33^{def}$ |
| *T. pandacaqui* | $6.67^{cd}$ | $26.67^{efg}$ | $86.67^{a}$ |
| *Z. zamiifolia* | $6.67^{cd}$ | $20.00^{efg}$ | $60.00^{abcd}$ |

*: In a column, values with the same letter superscript are not significantly different at a 5% level using the Duncan multiple range test

Table 2. Mean percent mortality of fruit flies using the cotton balls with extracts from first to third cycle

| Plant Extract | Mean Percent Mortality | | |
|---|---|---|---|
| | 6 hours | 12 hours | $24 \text{ hours}^{ns}$ |
| *A. indica* | | | |
| 1st cycle | $46.67^{a}$ | $91.67^{a}$ | $100.00^{a}$ |
| 2nd cycle | $16.67^{b}$ | $58.33^{b}$ | $100.00^{a}$ |
| 3rd cycle | $18.92^{b}$ | $65.19^{b}$ | $93.64^{a}$ |
| *A. paniculata* | | | |
| 1st cycle | 0.00 | $66.67^{a}$ | $100.00^{a}$ |
| 2nd cycle | 0.00 | $33.33^{b}$ | $91.67^{a}$ |
| 3rd cycle | 0.00 | $22.29^{b}$ | $91.17^{a}$ |

*: In a column, values with the same letter superscript are not significantly different at a 5% level using the Duncan multiple range test

**Effect of method of extraction**

Mortality of fruit flies treated with the leaf extracts prepared by four extraction methods and four extracting solvents was recorded and is presented in Figure 1. The insecticidal experiments were replicated thrice. Observations were done at 3, 6, 12, 18, 22, and 24 hours to determine which of the extraction methods and solvents would be the best in extracting the insecticidal properties of *A. indica* against the fruit fly, *B. dorsalis*.

It was observed that Soxhlet extraction yielded the highest mortality at 3- and 6-hour exposure times irrespective of what solvent was used (Figure 1). This effect may be due to the higher amount of bioactive components extracted in this method. The total phenolic content and antioxidant activity of *Quercus infectoria* galls was reported to be higher with the Soxhlet technique than with supercritical carbon dioxide extraction [38]. A similar study on the total phenolic and anti-radical capacity of extracts from *Pinus radiata* bark showed that total phenols and tannin content were highest with the Soxhlet technique compared to microwave-assisted and ultrasound-assisted extractions [29]. Soxhlet extraction causes a more rapid rupture of cells, facilitating the release of their contents [28]. This may explain why the neem leaf extract via Soxhlet extraction showed higher fruit fly mortality at 6 hours than those from hot infusion, maceration, and fermentation. Based on the study of Subramanian et al. [39], a binary extraction approach using a Soxhlet extraction device with a 50:50 solvent mixture (hexane: ethanol) resulted in a yield of 720 $mg_{azadirachtin}/kg_{leaves}$ at 6 hours extraction.

At 12, 18, and 22 hours exposure, on the other hand, hot infusion gave the lowest mortality, while Soxhlet, fermentation, and maceration had no significant differences. Hot infusion was done by adding 15 g of ground leaves into 150 mL of boiling solvent. The mixture was set aside for 15 minutes and filtered. The duration of solvent–sample interactions in the hot infusion process may not be long enough to extract a significant amount of azadirachtin, resulting in low fruit fly mortality at 12–22 hours. According to the study of Ramirez-Rodrigues et al. [40], hot infusion resulted in higher amounts of aldehydes, while cold infusion or maceration yielded higher amounts of alcohols. Azadirachtin has more affinity to polar solvents. Alcohols are more polar than aldehydes, and this may explain why hot infusion extracts yielded lower fruit fly mortality.

At 24 hours, the extraction method has no significant effect on mortality. This result suggests that a small amount of active component in the extract (toxicant) can also lead to death in fruit flies when they are exposed to it for 24 hours or longer.

**Effect of solvent on percent mortality**

The effect of the green solvents used in the extraction of the insecticidal properties of neem leaves is revealed in Figure 2. Mortality rate was observed to be comparable at 3- and 6-hour exposure of the fruit flies to the different extracts. At 12, 18, 22, and 24 hours, rice wash, vinegar, and distilled water had no significant differences in terms of mortality, though it can be observed in the graph that vinegar gave the highest percent mortality from 12 to 24 hours exposure. Rice wine gave the lowest mortality from 3 to 24 hours. This can be attributed to the volatiles in rice wine, such as ethanol and acetic acid. Fruit flies are attracted to some volatile wine and vinegar compounds. The fruit flies that did not die immediately thrived on the wine components. In contrast to rice wine, vinegar, which also has undergone fermentation and might contain some volatile compounds that attract fruit flies, was able to register the highest mortality. Vinegar contains polyphenols that have antioxidant properties [41]. The polyphenols in vinegar (amounting to approximately 0.30-32.67 GAE) [42], in addition to the bioactive components extracted from the neem leaves, might have a synergistic effect on the anti-fruit fly activities, causing the highest mortality after 22 and 24 hours. The following effects of the extracts on the oriental fruit fly (Figure 4) are more to do with primary antifeedancy, which targets mouthparts and other chemoreceptors, and whose mode of action is deterrent cell stimulation and sugar cell inhibition [43]. An observable effect was darkened and damaged proboscises. The other effect was cellular processes targeting the muscles, whose mode of action was loss of muscle tone. Torn wings and damaged legs were the documented effects on the fruit flies.

The effect of rice wash as an extracting solvent cannot be disregarded in this study. It was not significantly different from vinegar and distilled water from 12 to 24 hours exposure time. In fact, it yielded higher mortality than the distilled water extract. The use of rice wash in the extraction of phytochemicals has not been given attention by natural product chemists who practice green chemistry. This rice byproduct (also called rice broth or rice water) is used by Filipinos in the preparation of '*burong mustasa*' [44] or pickled flat mustard green. Rice wash provides a small amount of lactic acid bacteria (LAB) that produce lactic acid as they act on the sugars from the mustard. Acetic acid may also be produced in the pickled product due to the presence of acetobacter [45]. The results of this experiment reveal the potential of rice wash in extraction of neem's insecticidal components. The polar substances present in rice wash may have extracted the insecticidal components against fruit flies, which is why neem leaf rice wash extract was as effective as vinegar. Higher concentration of azadirachtin is extracted by polar solvents than nonpolar solvents [39].

**Solvent and extraction method combinations**

Analysis of variance of the solvent and extraction method combinations showed significant differences at the 5% level (Table 3). There were 16 extraction × solvent combinations in this study. Of these, there were seven effective combinations with insecticidal effects on *B. dorsalis*, namely, Soxhlet–vinegar (S-V), Soxhlet–rice wash (S-RWa), Soxhlet–distilled water (S-D), fermentation–rice wash (F-RWa), fermentation–vinegar (F-V), fermentation–distilled water (F-D), and maceration–vinegar (M-V). The use of hot infusion combined with all four solvents and the use of rice wine in the four extraction methods were not effective.

The above statistics were calculated to determine which method is most economical in terms of utilization of materials. Fermentation using rice wash was found to be the most economical way of extracting the insecticidal components of neem leaves. Rice wash is a household waste material generated every day among Filipino families. Meanwhile, fermentation is done without the use of electricity. The rice wash and the organisms in the neem leaves will ferment even without the addition of commercial yeast; thus, no additional cost will be incurred in the production of the biocide from neem leaves.

**Lethal concentration of the extracts (with high mortality rates)**

The lethal concentrations of the fermentation–rice wash extract (F-RWa) (Figure 3a) and Soxhlet–vinegar (S-V) extract (Figure 3b) were calculated using probit analysis. This was done to compare the lethality of the two extracts, S-V being the one with the highest interaction effect and F-RWa being the most economical among the 16 extraction × solvent methods defined in this study. The lethal dose for F-RWa extract was found to be 2.7% v/v (mL extract per 100 mL solution), versus 1.9% for the S-V extract. These lethal doses for F-RWa and S-V extracts mean that 50% of the fruit flies will die if the toxicant is introduced into the olfactometer. This result shows that neem extracts prepared by fermentation using rice wash and Soxhlet extraction using vinegar are effective biocides against *B. dorsalis* when the extracts are introduced via inhalation, as was done in this experiment. Rice wash when fermented contains small amounts of alcohol and acetic acid, while vinegar contains acetic acid and small amounts of alcohol. These are polar substances and may contribute to the extraction of azadirachtin from neem leaves.

The above results conform to the reports that neem leaf extracts have insecticidal properties. In the first few hours after the fruit flies were exposed to the neem extracts, hyperactivity was observed, and after about 4–6 hours, the fruit flies' mobility was observed to be depleted. This may be attributed most probably to the plant's azadirachtin, a triterpenoid [46]. Triterpenoids cause loss of tyrosine hydroxylase which can lead to a depletion of dopamine and norepinephrine in the brain due to the lack of precursor L-3,4-dihydroxy-phenylalanine (L-DOPA), which then results in inhibition of locomotor activity and death [47]. Saponins have the same effect as triterpenoids on tyrosine hydroxylase [48]. Hyperactivity may have been due to neuroexcitation, and the slow mobility could have been due to energy depletion, neuromuscular fatigue, and neuroinhibition by possible oxygen deprivation and/or reduced respiratory capacity, which may lead to mortality.

The manifestations of the observed effects on the fruit flies exposed to the neem extracts were damaged wings, legs, proboscises, and antennae and empty abdomens (Figure 4). Damaged wings and legs might be due to the flies' hyperactivity after a few hours of exposure to the neem extracts. Ceaseless movement of the legs, rubbing against each other to remove the substances attached to the legs and other parts, was observed. This may have been the effect of cellular processes targeting the muscles, resulting in loss of muscle tone. The flies' death may also have been due to the inhibition of protein synthesis in various tissues. Since the extracts were introduced into the cap of the improvised olfactometer, the flies either inhaled or tasted the extract. The taste organs of fruit flies are widely distributed in the adult body, legs, and wings and the proboscis's labellum, the external gustatory centers. All these taste organs detect tastants through the gustatory receptor neurons (GRNs) [49]. The biocide is the tastant to which the fly's initial reaction was movement of the legs and spreading of the wings without body movement, as if it sensed something. But after a few hours, this behavior manifested as movement of legs and suppressed proboscis extension [50], as shown in Figure 4b. Disappearance of the sensory hairs, called sensilla, was observed. These sensilla are usually made of odor-binding proteins (OBP) and can react to the active components, causing the proboscis to darken.

One behavior that was documented in this study when the fruit flies were exposed to the plant extracts is the manner of courtship. The flies were all males, as they were trapped using methyl eugenol, a male sex pheromone. There were flies that showed courting behaviors, such as movement around one fly as if guarding it from other flies. Attempting copulation with another male fly was also observed. In the study conducted by Liu's research group [51], dopamine level reduction in *Drosophila melanogaster* enhanced the attractiveness of male *Drosophila* to other males. Dopamine is an important neuromodulator in animals, and it plays an important role in mammalian behavior associated with sexual encounters. When the dopamine level decreases due to inhibition of tyrosine hydroxylase, male–male courtship behavior is enhanced.

The empty abdomens among the dead flies can be associated with the behavior of the living fruit flies recorded by the researcher. The living flies were attracted to the dead flies and fed on the pheromone reservoir near the anus. Methyl eugenol is a pheromone of *B. dorsalis* which is ingested and metabolized into *E*-coniferyl alcohol (ECF) and 2-allyl-4,5-dimethoxy phenol (DMP). ECF and DMP are stored in the rectal gland and eventually released as sex pheromone during courtship at dusk [52, 53].

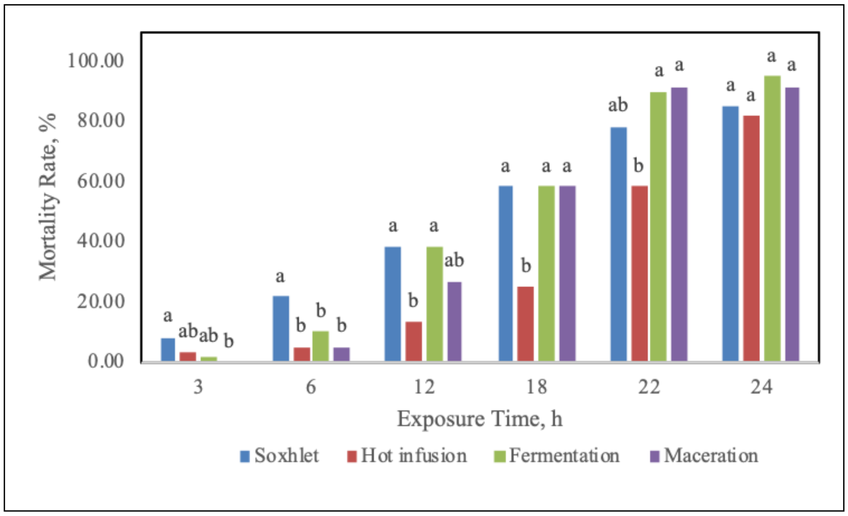


Figure 1. Effect of methods of extraction on the anti-fruit fly properties of neem leaf extract

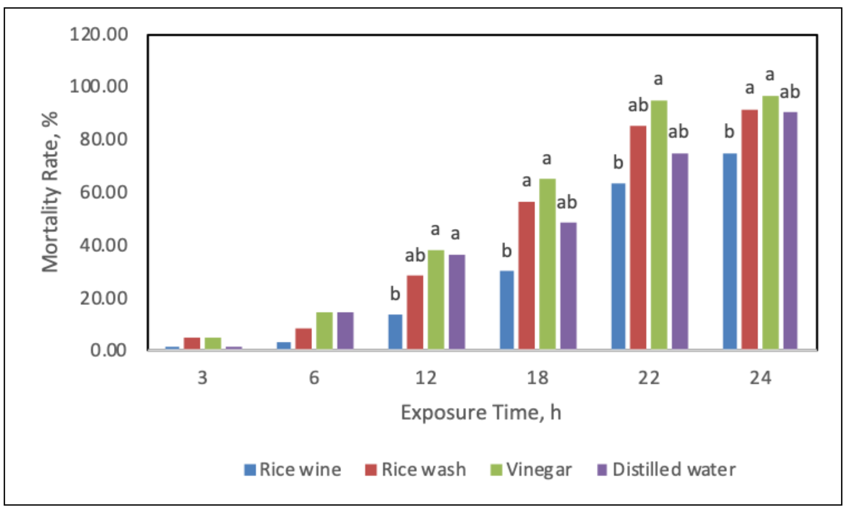


Figure 2. Effect of green solvents on the anti-fruit fly properties of neem leaf extract

Table 3. Effect of extraction method–solvent combinations on the percent mortality of *B. dorsalis*

| Extraction Method | Mean % Mortality (Extraction × Solvent Combinations)* | | | |
|---|---|---|---|---|
| | Rice Wine (RW) | Rice Wash (RWa) | Vinegar (V) | Distilled Water (D) |
| Soxhlet (S) | $27.78^{g}$ | $54.44^{ab}$ | $61.11^{a}$ | $50.00^{abcd}$ |
| Hot infusion (HI) | $15.56^{h}$ | $37.78^{defg}$ | $40.00^{cdefg}$ | $31.11^{fg}$ |
| Fermentation (F) | $35.56^{efg}$ | $51.11^{abcd}$ | $56.67^{ab}$ | $52.22^{abc}$ |
| Maceration (M) | $45.56^{bcde}$ | $40.00^{cdefg}$ | $52.22^{abc}$ | $44.44^{bcdef}$ |

*: Means with the same letter superscript are not significantly different at the 5% level using Duncan's multiple range test

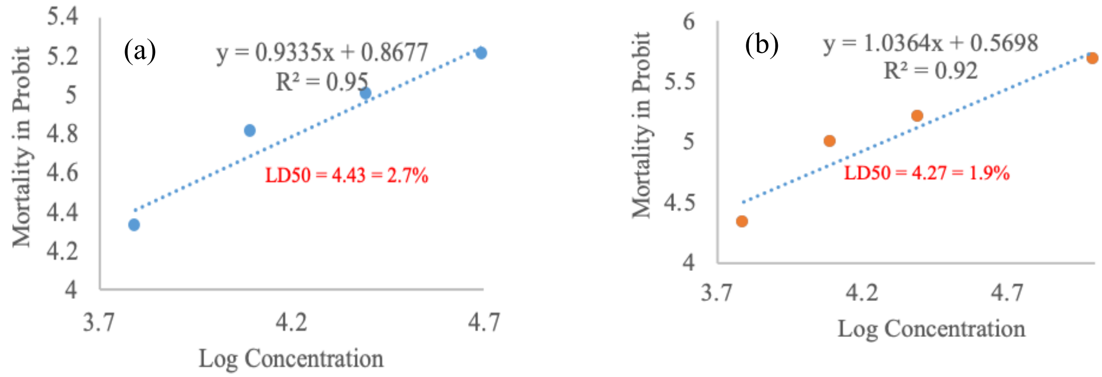


Figure 3. Graphical presentation of the lethal dose for (a) F-RWa and (b) S-V extracts using probit analysis

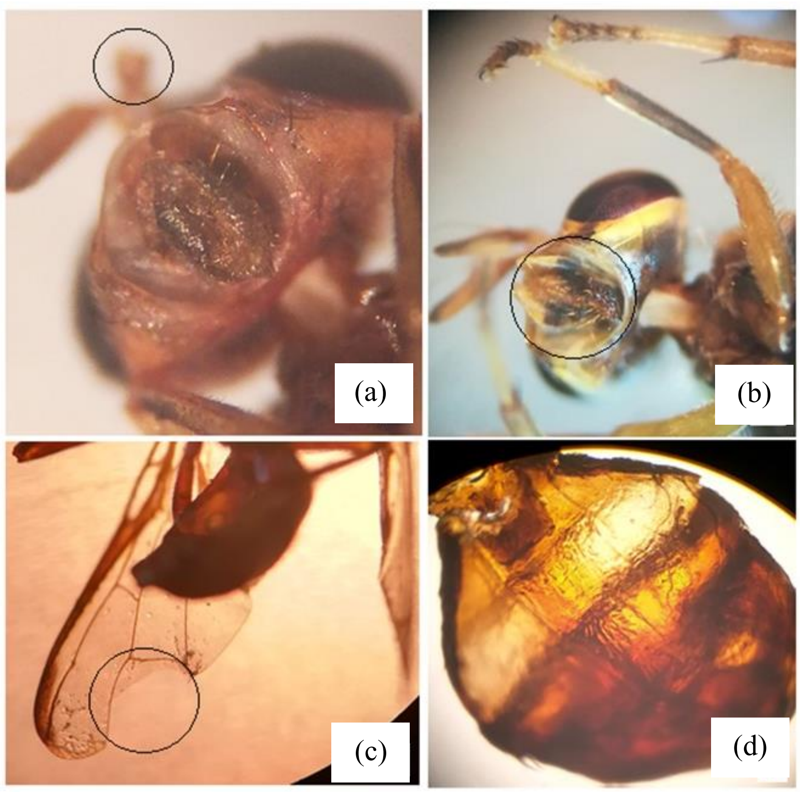


Figure 4. Observed effects of the plant extracts on oriental fruit flies (a–damaged antennae; b–darkened and retracted proboscis; c–torn wings; d–empty abdomen).

## Conclusion

The findings cited above suggest that there are plants that showed activities against *Bactrocera dorsalis* and can be used in the formulation of biocide for the management of oriental fruit flies. These are the following: *Azadirachta. indica, Andrographis paniculata*, *Indigofera zollingeriana*, and *Lantana camara* with 100% mortality; *Glericidia sepium*, *Tabernaemontana. pandacaqui*, *Ocimum sanctum*, *Mangifera oleifera*, *Annona squamosa*, *Annona muricata*, *Blumea balsamifera*, *Casia alata*, *Euphorbia tithymaloides*, *Cyperus rotondus*, and *Zamioculcas zamiifolia* with 60–93% mortality after 24 hours. *A. indica* (neem) leaf extract was the most effective against *B. dorsalis* and was used for the screening of extraction methods and solvent. Fermentation using rice wash was the most economical method but was found to be effective in the preparation of biocide from neem leaves against *B. dorsalis.*

**Acknowledgement**

The authors wish to acknowledge Mr. and Mrs. Juanito Palaganas, Mary Ann Gabriel, John Paul Maximo, Orly Neuda, and Luzviminda Quitos for their assistance in the collection of plant samples. Acknowledgment is also due to Ms. Ella Joyce S. Paragas for the statistical analyses.

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