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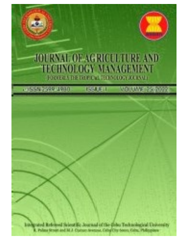

Original article

Larvicidal property of sugar apple (*annona squamosa*) and san francisco (*codiaeum variegatum*) against yellow fever mosquito (*aedes aegypti*)

Florieza M. Mangubat

College of Arts and Sciences, Cebu Technological University – Tuburan Campus

ABSTRACT

Two Philippine plants, sugar apple (*Annona squamosa*) and San Francisco (*Codiaeum variegatum*), reported having insecticidal properties were selected for investigation, Samples were screened and assayed for their larvicidal property against *Aedes aegypti* by exposing 3rd-4th instar larvae with a unified amount of concentrations with two solvents. Trials were performed to determine the average mortality rate of concentrations after the LD50 value to compare the larvicidal property of the two identified plants and to compare the susceptibility of *Aedes aegypti*. Findings of the study showed that San Francisco-chloroform obtained the highest larvicidal activity after 24 hours of exposure while atis- ethyl alcohol signified the highest larvicidal activity after 48 hours of exposure against larvae of *Aedes aegypti*.

KEYWORDS: *aedes aegypti, larvicidal property, san francisco, sugar apple*

1 INTRODUCTION

Annually, two billion humans are at risk of being infected with one of the four dengue viruses as the mosquito that carries the virus is even more spread now (Halstead, 2008). Mosquito is a severe insect threat to public health, transmitting several dangerous diseases like dengue (Madhumathy et al., 2007; Kumar et al., 2012). Dengue worldwide disease is spread by Aedis aegypti (Borah et al., 2010). Southeast Asian and Western Pacific countries bear the brunt of global illness due to dengue (WHO, 2009a). The recurring dengue cases all over tropical countries are very alarming because it impacts the population mortality rate. However, the most pressing challenges now are the complicated causative factors driving the spread of severe dengue disease (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS) rather than the remarkable expansion of *Ae. aegypti* (Halstead, 2008).

Aside from the diseases caused by mosquitoes such as dengue, malaria, yellow fever, and West Nile virus, mosquitoes are annoying in that they disrupt outdoor activities (Dickens & Bohbot, 2013). Personal protection from mosquito bites is currently the most important measure to reduce mosquito-related diseases (Das et al., 2003). Repellents based on chemical insecticides are commercially sold in the market to help reduce and prevent mosquito vectors. Insect repellents work by exerting their effect by interacting with the olfactory receptor neurons (ORNs) and gustatory receptor neurons (GRN) in mosquitoes. Diethyltoluamide (DEET) modifies responses in *Ae. aegypti* where DEET changes the responses of ORNs to their ligands. Additionally, DEET initiates a specific GRN that is sensitive to feeding deterrents in *Ae. aegypti* (Dickens and Bohbot, 2013).

However chemical insecticides containing DEET are not safe for humans, specifically children because of the probability of skin irritation, hot sensation, rashes, or allergy (Das et al., 2003, quoted: Mayoora Soonwera et al., 2014). In contemporary years, as dengue cases rise, there has been an increasing public concern about the safety of many chemical products, instigating a renewed interest in using natural products of plant origin for mosquito vector management (Pavela, 2008). The shift to the use of organic or herbal insect repellants arose.

Various plants' essential oils, in general, have been documented as significant natural sources of insecticides and insect repellents; numerous essential oils have also been documented to exhibit acute toxic effects compared to insects, including mosquitos (Pavela, 2008). Essential oils of sugar apple or Atis (*Annona squamosa*), cedrene and caryophyllene (Joy & Rao, 1997), and San Francisco (*Codiaem variegatum*) possess phytochemical contents that are highly insect repellent ingredients due to their relatively low toxicity, comparable efficacy, and customer approval (Katz et al., 2008). Natural extracts of these plants are widely used to control pests and insects in organic farming.

The key to mosquito control is larval by modification of habitat thru insecticides (Batabyal et al., 2007; Dua et al., 2009). Along with personal protection from mosquitoes, mosquito larvae control is important to control the spread of mosquitoes and the diseases they carry (Das et al., 2003). Natural pesticides, also called

*corresponding author: florieza.mangubat@ctu.edu.ph
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botanicals, have a high potential as an alternative to synthetic pesticides and their associated negative effects (Wezel et al., 2014). Plant essential oils and their constituents have also been suggested as potential alternatives to currently used insect control agents, according to Isman (2006), because they are a potential source of bioactive chemicals that are perceived as relatively safe by the general public, pose less risk to the environment, and have minimal human impacts.

Sugar apple *(Annona squamosa*) is a tiny evergreen tree that is cultivated for its fruits (Pandey & Barve, 2014). Sugar apple, locally known as Atis in the Philippines, is used in folklore medicine to cure a variety of ailments. The origin of A. squamosa is unclear. It is a semi-deciduous tree that can be found throughout tropical South America and the West Indies (Ma et al., 2017). Before 1590, Spaniards were thought to have brought seeds from the New World to the Philippines, while the Portuguese were thought to have introduced the sugar apple to southern India (Morton, 1987). Extensive phytochemical testing on various parts of the A. squamosa plant include diterpenes (DITs), alkaloids (ALKs), annonaceous acetogenins (ACGs), cyclopeptides (CPs), and essential oils (Ma et al., 2017). Along with many biological activities, A. squamosa has insecticidal activity (Wezel et al., 2014). Annona species, such as sugar apple, have been proven to be promising biological insecticides among tropical plants because of the presence of ACGs (Ma et al., 2017).

Likewise, San Francisco (Codiaeum variegatum) is a group of beautifully variegated leafy perennial, tropical ornamental shrubs or trees with glabrous branches and prominent leaf scars native to the Philippines, Thailand, Malaysia, Indonesia, and other Pacific islands (Saffoon et al., 2010). The presence of important phytochemicals, minerals, and proximate parameters in ovalifolium found in C. variegatum implies that these plants may possess a good potential for food and medicine (Babatunde et al., 2017). In a study by Saffoon et al.; (2010), saponins, reducing sugars, tannins, and gums were revealed from methanolic leaf extracts of C. variegatum, which indicated promising cytotoxic properties. This indicates that potential larvicidal activity is shown in plant tissue extraction that can minimize the occurrence of dengue cases.

Studies proved varied evidence of the insecticidal property of many plants available in the locality. However, there was no contributory factor of larvicidal plant extracts combining sugar apple and San Francisco that kill *Ae. aegypti* before they turned into adult biting mosquitoes causing dengue. Considering this scenario, the larvicidal property of the extract of the phytochemicals found in sugar apple and San Francisco leaves against *Ae. aegypti* larvae associated with a unified amount of solvents and concentrations are explored by the researcher. Therefore, the study is designed to determine the larvicidal effect of sugar apple and San Francisco extracts.

2 MATERIALS AND METHODS

Plant Material. The samples were collected within Cebu Technological University Tuburan Campus. San Francisco is grown as an ornamental plant inside the campus, while Atis is widely available around the vast space of the campus. Samples of fresh leaves from mature San Francisco and sugar apple (Atis) were obtained. For the Atis sample, leaves were collected regardless of whether the plant was bearing fruit or not. Samples used were matured leaves, and the extraction procedure was patterned from Tiwari et al. (2011).

Mosquito rearing. Aedes aegypti larvae were intentionally grown from artificial containers such as pails, and drums within the Chemistry Laboratory room. Larvae were sorted out and only strong and healthy 3rd-4th instar larvae were selected for assays. Clean water in various types of artificial containers is preferred by the larvae and pupae of *Ae. aegypti* (Halstead, 2008). The containers were left uncovered during the duration of larvae-rearing.

Repellency evaluation. For each of the plants assayed, a sufficient amount of fresh leaves were weighed, cut into smaller pieces, blended, and added with a unified amount of solvent concentrations that amounted to 100 g %(w/v). The mixture was filtered to separate fibrous material and other large particles. Filtered extracts were used during phytochemical screening and in the test proper with solvents utilizing ethyl alcohol and chloroform. Tests were achieved in triplicate for each plant species. All glass chambers were filled with corresponding concentrations; fifteen healthy and active 3rd-4th instar larvae previously sorted out were transferred into each glass. A total of 375 mosquito larvae were used during the assay. The experiments were performed at room temperature and exposed to normal daylight hours. After 24 hours and 48 hours, observations were done to record the number of dead larvae in each glass chamber. Probit analysis was employed to determine LD50, which constituted the lethal dose of the extracts (Randhawa, 2009).

3 RESULTS AND DISCUSSION

As shown in Figure 1, the comparison of LD50 after 24 hours and 48 hours shows that the larvicidal activity values after 48 hours are lower than those after 24-hour results. On LD50 at 24 hours, San Francisco-chloroform denotes the highest larvicidal activity (17.46 g%) and Atis-ethyl alcohol (30.14 g%) is the least effective. San Francisco-chloroform is the most potent larvicidal

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activity because it takes only 17.46 g% of the extract to kill 50% of the mosquito larvae. The combination of San Francisco-chloroform shows immediate release without delaying the larvicidal activity of the extract, causing a higher mortality rate of the mosquito larvae after 24 hours of exposure. While on LD50 at 48 hours, it discloses that atis-ethyl alcohol (2.49 g%) exhibits the highest larvicidal activity while San Francisco-chloroform (11.7 g%) shows the least larvicidal effect. This gives details that atis-ethyl alcohol showed the highest level of lethality when mosquito larvae were exposed for 48 hours. This further illustrates that the larvicidal effect of atis-alcohol is a slow-release combination wherein larvicidal activity was extended to a longer period of time. Furthermore, the results explained that the larvicidal efficacy of the different concentrations also differs. San Francisco-chloroform is most effective in 24 hours of exposure, while the larvicidal effectiveness of atis-ethyl alcohol is at 48 hours.

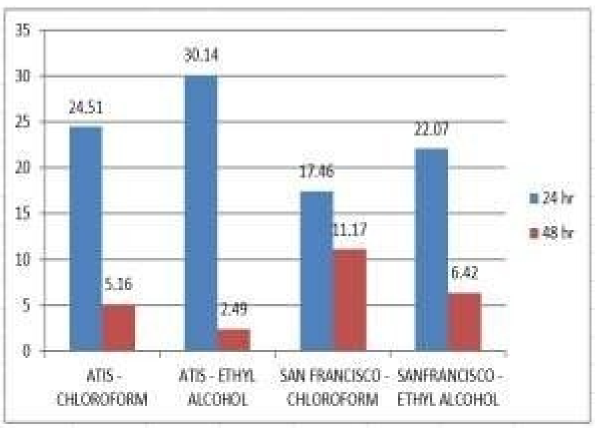

Figure 1. Comparison of Larvicidal Activity of the Leaf Extracts - LD50 after 24 Hours and 48 Hours of Exposure (100g/100ml)

To counter check and validate the efficacy of the concentration, LD50 after 48 hours was recorded. It is noted that the most effective concentration at LD50 after 48 hours is Atis-ethyl alcohol concentration (2.49 g%) and the least effective in San Francisco– chloroform concentration (11.17 g%). All parts of the Atis plant can be used as insecticides (Mondal et al., 2018). The phytochemical component works as a contact and stomach poison and is used as an insecticide, repellent, and antifeedant. It has been used to kill *Ae. aegypti* larvae (Novasari et al., 2017; Wahyuni et al., 2015;). Another study found that Atis extract was 20.62 ppm or 0.00206% (Wahyuni et al., 2015) showed that the toxicity of Atis leaf extract is still better when compared to Atis seed extract. This means that among the concentrations, Atis-ethyl alcohol concentration shows a promising larvicidal property that kills mosquito larvae before they turn into adult biting mosquitoes, causing dengue.

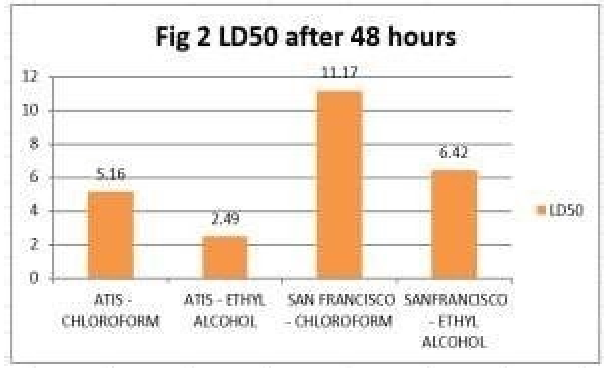

Figure 2. Larvicidal Activity of the Leaf Extracts- LD50 after 48 hours of exposure (100g/100ml)

4 CONCLUSIONS AND RECOMMENDATION

Results obtained from this study revealed that the degree of potency of the two selected plants was associated with the different solvent extracts against species of *Ae. aegypti* differs significantly. They were shown to contain certain components that can effectively kill mosquito larvae. At 24-hour exposure, San Francisco-chloroform extract showed the highest larvicidal activity, followed by San Francisco-ethyl alcohol, Atis -chloroform, and the least effective was Atis-ethyl alcohol. At 48-hour exposure, the effectivity rate in descending order is reflected as Atis-ethyl alcohol, Atis-chloroform, San Francisco-ethyl alcohol, and San Francisco-chloroform. All extracts are made up of 100g/ 100ml concentrations. To attain an immediate response to the larvicidal effect in killing mosquito larvae before they turn into adult biting mosquitoes causing dengue, San Francisco-chloroform extract is recommended.

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REFERENCES

Azhari, H. N., Jessinta, D. O., & Abdurahman, H. N. (2012). Larvicidal activity of extracts from different parts of Neem (*Azadirachta indica*) against Aedes aegypti mosquitoesâ€™ larvae. *Scientific Research and Essays*, *7*(31), 2810-2815.

Babatunde, E. E., Banji, A. F., Foluke, O., Ayandiran, A. D., & Fatima, K. (2017). Comparative study of leaf morphology, phytochemical, mineral and proximate analysis of *Codiaeum variegatum* (L.) A. Juss.(Malpighiales: Euphorbiaceae) and its stable mutant. *Brazilian Journal of Biological Sciences*, 4(7), 25-34.

p-ISSN: 2599-4875 e-ISSN: 2599-4980

Batabyal, L., Sharma, P., Mohan, L., Maurya, P., & Srivastava, C. N. (2007). Larvicidal efficiency of certain seed extracts against *Anopheles stephensi*, with reference to Azadirachta indica. *Journal of Asia-Pacific Entomology*, *10*(3), 251-255.

Borah, R., Kalita, M. C., Kar, A., & Talukdar, A. K. (2010). Larvicidal efficacy of Toddalia asiatica (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus. African Journal of Biotechnology*, *9*(17), 2527-2530.

Das, N. G., Baruah, I., Talukdar, P. K., & Das, S. C. (2003). Evaluation of botanicals as repellents against mosquitoes. *Journal of Vector Borne Diseases*, *40*(1/2), 49.

Dickens, J. C., & Bohbot, J. D. (2013). Mini review: Mode of action of mosquito repellents. *Pesticide Biochemistry and Physiology*, *106*(3), 149-155.

Dua, V. K., Pandey, A. C., Raghavendra, K., Gupta, A., Sharma, T., & Dash, A. P. (2009). Larvicidal activity of neem oil (*Azadirachta indica)* formulation against mosquitoes. *Malaria Journal*, *8*(1), 1-6.

Halstead, S. B. (2008). Dengue virus–mosquito interactions. *Annu. Rev. Entomol., 53*, 273-291.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*., *51*, 45-66.

Joy, B., & Rao, J. M. (1997). Essential oil of the leaves of Annona squamosa L. *Journal of Essential Oil Research, 9*(3), 349-350.

Katz, T. M., Miller, J. H., & Hebert, A. A. (2008). Insect repellents: Historical perspectives and new developments. *Journal of the American Academy of Dermatology*, *58*(5), 865-871.

Kumar, S., Wahab, N., & Warikoo, R. (2011). Bioefficacy of Mentha piperita essential oil against dengue fever mosquito Aedes aegypti L. *Asian Pacific journal of tropical biomedicine*, *1*(2), 85-88.

Ma, C., Chen, Y., Chen, J., Li, X., & Chen, Y. (2017). A review on *Annona squamosa* L.: phytochemicals and biological activities. *The American Journal of Chinese Medicine*, *45*(05), 933-964.

Madhumathy, A. P., Aivazi, A. A., & Vijayan, V. A. (2007). Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *Journal of Vector Borne Diseases*, *44*(3), 223.

Mayura, S., & Siriporn, P. (2014). Mosquito repellent from Thai essential oils against dengue fever mosquito (*Aedes aegypti* (L.)) and filarial mosquito vector (*Culex quinquefasciatus* (Say)). *African Journal of Microbiology Research*, *8*(17), 1819-1824.

Mondal, P., Biswas, S., Pal, K., & Ray, D. P. (2018). *Annona squamosa* as a potential botanical insecticide for agricultural domains: a review. *IJBS*, 5(1), 81-89.

Morton, J. F. (1987). Fruits of warm climates. JF Morton, pp. 69–72.

Novasari, A. M., & Sasongkowati, R. (2017). The effect of Sugar Apple *(Annona squamosa* L.) seeds solution as an insecticide against mortality of *Aedes aegypti* mosquito with liquid electric method. *Jurnal Kesehatan Lingkungan*, *9*(2), 200-208.

Mwine, J., Damme, V. P., Kamoga, G., Nasuuna, M., & Jumba, F. (2010). Ethnobotanical survey of pesticidal plants used in South Uganda: Case study of Masaka district; *Journal of Medicinal Plants Researc, 5*(7) 1155-1163, 2011).

Pandey, N., & Barve, D. (2011). Phytochemical and pharmacological review on *Annona squamosa Linn*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *2*(4), 1404-1412.

Pavela, R. (2008). Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *22*(2), 274-278.

Saffoon, N., Alam, M. A., & Uddin, G. M. (2010). Phytochemical and Cytotoxic Investigation of *Codiaeum variegatum Linn*. leaf. *Stamford Journal of Pharmaceutical Sciences*, *3*(2), 51-53.

Randhawa, M. A. (2009). Calculation of LD50 values from the method of Miller and Tainter, 1944. J *Ayub Med Coll Abbottabad*, *21*(3), 184-185.

Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K., & Harleen, K. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

Wahyuni, D., & Loren, I. (2015). Difference between Toxicity of Betel Leaf Extract (Piper betle L) and Sugar Apple (Annona squamosa L) Seed Extract against *Aedes aegypti* Mosquito Larvae. *Jurnal Saintifika, 17*(1), 38-48.

Wezel, A., Casagrande, M., Celette, F., Vian, J. F., Ferrer, A., & Peigné, J. (2014). Agroecological practices for sustainable agriculture. A review. *Agronomy for sustainable development*, 34(1), 1-20.

World Health Organization. (2009). Epidemic, & Pandemic Alert.. Dengue: guidelines for diagnosis, treatment, prevention and control. Special programme for research, training in tropical diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization.

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