



Evaluation of Concentration and Time Duration of Parthenium Hysterophorus and Melia Azedarach In Field Conditions for Mosquitoes Control

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Received: 25 February 2025 | Revised: 12 March 2025 | Accepted: 29 March 2025

ABSTRACT

Mosquitoes, mainly from the Culicidae family, are the feared vectors for the dangerous communicable diseases such as dengue, malaria, and several infectious diseases. This study determined the larvicidal activity of Parthenium hysterophorus and Melia azedarach extracts against mosquitoes with a particular focus on their combinatorial effect. The field experiment was conducted at the Nuclear Institute of Food & Agriculture (NIFA), Peshawar, Pakistan, 2021 to assess the mortality of larva from mosquitoes that were treated with varying concentrations of plant extracts. The formulations were conducted at two ranges of concentrations (0.1% and 0.3%) and sodium bicarbonate. The study established a significant correlation between concentrations and levels of time exposures with the larval mortality at its peak when a 0.3% concentration was used for 3 hours, achieving the highest mortality rate (95%). This research study provides valuable insights into the efficacy of biopesticides that are environmentally friendly, suggesting that these feel like a viable and sustainable alternative to chemical insecticides used to control mosquitoes. In conclusion, the data indicates that the highest mortality on the mosquito population was found with a concentration of 0.3% for 3 hours. Future studies are needed to determine whether these organic, non-harmful biopesticides remain effective in a longer timeframe and whether their improvement can be generalized for use in an integrated program for mosquito management.

Keywords: Mosquito control, Parthenium hysterophorus, Melia azedarach, larvicidal activity, biopesticides, plant extracts

INTRODUCTION

Mosquitoes (Culicidae: Diptera) are important arthropods that are vectors for some number of diseases: dengue, malaria, infectious diseases, and sometimes only dermatitis that comprises allergic symptoms to skin or body. (Utrio 1979) Mosquitoes have two flying wings attached to their thorax and are part of Culicidae, a family that is a part of one of the original Orders of Diptera. Currently there are almost 112 genera and 3555 species of mosquitoes in the world (Harbach, 2017). The first three of the stages, egg, larva, and pupa, are predominantly aquatic life stages that can last for periods of time between 5 and

14 days depending on temperature and species. Some species of Aedes will have eggs that will remain uninjured in diapause if they are dried, and will then hatch when subsequently soaked. For the adult stage of their life cycle, females will deposit between 100-200 eggs. There can be substantial mortality of eggs and intergenerational mortality, but if a female successfully breeds just one time she can support a colony of thousands overtime (Kosova al. 2003). Mosquitoes belong to a family called Culicidae that can be characterized as nearly 3,600 species of small flies.

Mosquitoes can be a vector for many microbes and viruses. Infected mosquitoes can also transfer viruses and microbes between people while remaining asymptomatic. Mosquito-borne viruses cause illness including viral infections like: dengue fever, yellow fever, and Chikungunya. Most of these are transmitted via *Aedes aegypti* mosquitoes. Dengue fever warrants attention because it can be serious; however, with appropriate treatment, less than 1% of dengue fever patients die (WHO, 2011). Malaria is a parasitic disease produced by one of the many species of plasmodium that can be carried by mosquitoes of the genus *Anopheles*. Lymphatic filariasis (the main cause of elephantiasis) is transmitted by many different mosquito species (WHO, 2011). The name mosquito is formed from *Musca* (which means fly) with the diminutive *Harbach*, Ralph (November 2, 2008). Mosquitoes have long, slender bodies which are covered by three segments (head, thorax and abdomen). Mosquitoes head is specialized to house sensory input organs (e.g., antennae) and mouthparts which pierce and suck for feeding on things like as blood and sugar.

All adult female mosquitoes must have blood in order to lay eggs, while the adult males have no need for blood! Mosquitoes have simple and compound eyes, as well as segmental antenna that are required to detect host's odors and breeding or laying places (Harzsch et al., 2006). The thorax is the third body section of the mosquito body plan. The thorax is modified specifically for locomotion. Mosquitoes have three pairs of legs and two pairs of wings. Insects have varying flying distances, some can fly centimeters; others can fly kilometers. Some species like *A. gambiae* (*anopheles* and several others) can engage in continuous flight for over five hours at 1-2 km/h (0.6 mph); and over 12 km away at night (see Anderson and Williams, 1996; Spielman et al., 2001!). Mosquito males can have wing beats of 450-600 times per second (Spielman et al., 2001). Yellow fever is now estimated to be prevalent in 47 countries with associated death rates of 29,000 to 60,000 people annually (WHO 2016). The global estimate of cases of apparent dengue in 2013 was 58.4 million (Stanaway, 2016). *A. aegypti* originated on the African subcontinents. *A. aegypti* expanded over the tropical, subtropical, and temperature regions. Just like other vector borne diseases, *A. aegypti* is now

common place in South America, Africa, and Central America, along with the Mediterranean, Southeast Asia, and some parts of Oceania, all the way to Northern Queensland, Australia (Powell 2013). Although *A. aegypti* has a relatively high vectorial capacity, it is responsible for many diseases including dengue fever, yellow fever, chikungunya etc.

Adult female mosquitoes lay their eggs on moist, inner walls of containers or above the water line. Mosquitoes lay 100 to 150 eggs per day. When the larvae hatch from the eggs, they originally feed on microorganisms found in the water or in their larval food. Larvae usually molt three times before entering the pupa stage. Once in the pupae, larvae grow until the newly formed adult flying mosquito comes out of the pupae. In an adult mosquito, once it emerges from the pupa, the male mosquito feeds on nectar from flowers and the female mosquito feeds of the blood of humans and animals to produce eggs. Dengue fever is one of the deadliest reemerging viral diseases that are transmitted by arthropods. The Global Burden of Disease estimates that 390 million dengue infections occur around the world each year. Today, more than 2.5 billion people also live in areas where they are at risk of infection worldwide, estimating that of those 390 million infections, there are ~96 million instances with clinical manifestations of dengue fever of which fever, rash, ocular pain, arthralgia, myalgias, and hemorrhage that were expressed (Bhatt and Gething, 2013). Mosquitoes have four distinct life stages: egg, larva, pupa, and adult. *P. hysterophorus* is a flowering plant that is in the Asteraceae family and native to the tropics of North America. Some common names include farmine weed, white top weeds, and Santa Maria feverfew.

A genus of East Indian and Australian deciduous trees (the type of the family Meliaceae) with pinnate or the ashes, white or lilac flowers usually fragrant in axillary panicles, and minuscule drupaceous fruit with hard bone seed. In human terminology, a weed is described as "an undesirable plant," which usually suggests that a weed in one location may or may not be a weed in another location (Wunch, 2019). To distinguish weeds, we do not often call them 'pests' in the same way as we call the other insect pest or other invertebrate or vertebrate pests. Most weeds do not impact humans directly and their threat to humans is

negligible. Although some weeds like *Parthenium hysterophorus*, have a magnitude of a health burden on humans (Naidu, 2012). They can irritate the human respiratory system and can in some cases aggravate allergies. A pest is simply any biological entity that causes harm to people. A weed is also an important pest because it resides in environments of the open-air community environment such as gardens, parks, yards, grass, scene, and landscapes and must be managed (Matusova et al., 2004).

According to Javed et al. (2005), allelopathy is a biological process in which an organism produces bio-chemicals that can inhibit the germination, growth, survival or reproduction of plants and animals. Some of the allelo-chemicals with herbicide, fungicide and growth regulator potential are flavonoids, terpenoids and strigolactones. Allelopathy has been demonstrated to be a widespread aspect of many plants (Hussain et al., 2011). The flowering plant *P. hysterophorus* is in the Asteraceae family. *P. hysterophorus* is native to America's tropics. Common names include Santa-Maria feverfew, white-top weed and hunger weed. In India, it is also known as *gajarghas*, congress grass and carrot grass. This is a generalized invasive species that is present in parts of Africa, Australia, and in India. Based on our knowledge and a preliminary literature review, there are no reports investigating the mosquito-larvicide activity of *P. hysterophorus* leaves against *Culex quinquefasciatus*. The weed *P. hysterophorus* is not native to India and has only prospered since the 1970s.

Parthenium is capable of rapidly acquiring land areas in abandoned lands, road margins, farmed lands, and overgrazed pasture lands. *Parthenium* is a very productive plant which can cause substantial economic loss, health concerns, and habitat degradation. Furthermore, it releases allelochemicals to stop pasture grasses and other plants from germinating and growing (Ranga Rao et al., 2007). *M. azedarach* commonly known as "Persian Lilac" also *Baka* in or 'thin' is a medicinal plant that is grown stating from the Himalayan region. In fact in Pakistan and India its growth is for medicinal and decorative purposes (Watt and Brand work, 1962). It is a perennial plant from the Meliaceae family. I call the China berry plant *Torashandi* in Khyber Pakhtunkhwa. Some *Melia azedarach* preparations

were used to treat a number of ailments (Bauer, 1989).

Numerous papers have been published across the plant's antimalarial, analgesic, anticancer antiviral, antibacterial, antifungal and antifertility actions (Vishnu anta, 2008). *M. azedarach* has been recorded as treating abdominal pains, intestinal disorders, and uterine problems (Perry, 1980). This plant powder is used in effective diabetic management (Ahmad et al., 2009). *M. azedarach* is part of the Meliaceae family, consisting of around 45 genera with about 750 species (Curtis, 1994). Vector control is important because some vectors, such as mosquitoes, are highly efficient vectors that promote development of malaria, filariasis, dengue, and other viral infections, and elicit pain after a bite (Curtis, 1994). Mosquito larvae have a broad head and long antennae. The front of the larval head has an oval gill inserted at the base of the antennae on the ventral side (Virk and Connelly, 2012).

MATERIAL AND METHODS

The study was carried out at the nuclear Institute of Food & Agriculture (NIFA), Peshawar, Pakistan 2021. In the current study, the laboratory protocols for mosquito rearing, and the synergistic effect of *Parthenium hysterophorus* and *Melia azedarach* against mosquito larval stages were tested. *Aedes aegypti* Mosquitoes do complete metamorphosis (four stages eggs, larvae, pupa, and adult). Eggs: The adult female mosquitoes lay their eggs above the water line on the inner wall of containers or vessels containing water. Mosquitoes only need a small quantity of water to lay eggs, bowls, cups, fountains, tires, barrels, vases or any container that holds water can be a great "nursery". Larva: Larvae are aquatic life. They hatch from mosquito eggs. This occurs when mosquito eggs are submerged in water (rain, sprinkle). Larvae can be discerned in water. They are called "wigglers", because they're always moving. Pupa: Pupae are aquatic organisms. Mosquito adult emerges from the pupa and flies away. Adult: After biting humans or animals, female mosquitoes need blood in order to produce eggs. *Aedes aegypti* don't travel long distances in their life span.

Aedes aegypti mosquitoes want to live with and bite people. Mosquitoes can live inside or outside a home. The keeping, feeding, breeding and providing the medical care of an insect (mosquitoes) or useful animal is called the rearing of insects or animal. Usually you rearing and care from when the eggs are raised to adult mosquito. *P. hysterophorus* is a flowering plant from the Asteraceae family. It is native to the tropics of North America. It is commonly referred to as Santamaria, Santamaria feverfew, white top weeds, and farmine weed. In India it is also called carrot grass, congress grass or Gajar Ghas.

Melia

A genus of the East Indian and Australian deciduous trees having pinnate or the ashes, fragrant white or lilac flowers in axillary panicles, and small drupaceous fruits with hard-boned seeds- see chinaberry sense.

Material Used/Equipment

The materials included consists in various pieces of equipment and materials used to conduct surveys of entomological diversity as well as conduct bioassays of insecticides. The pieces of equipment are all necessary for a variety of tasks, such as the collection and handling of mosquito larvae and they also fulfill other needs, such as safety (for the user's safety) and measuring. The aspirator and larval rearing trays are used to collect and rear larvae. The test tubes and ovicups are used to store samples and collect eggs. The adult cages, mass rearing cage, and general cage are used to house mosquitoes in different life stages. Steel trays and muslin cloth are used to collect insects and to act as a filter when collecting from the field. Gloves and masks, along with scissors are used for appropriate handling and safety. Spoons and droppers are used to measure and transfer. The scale is used to weigh materials such as *Parthenium* and *Melia*. The inch tape and deeper are used for determining size of,

positioning of and collecting of mosquito larvae from the field. Together, these tools facilitate the process of research and testing in terms of insecticide resistance and monitoring of mosquito populations.

Formulation of 100 g of Solution: 100 g of solution was formulated using the following ingredients: 40ml+5g Parth+5g Melia+50g NaHCO₃.

Formulation of 200 g of Solution: 200 g of solution was formulated with the following ingredients: 50ml+5g parth+5g Melia+120g NaHCO₃.

Formulation of 300 g of Solution: 200 g of solution was formulated by adding: 85g+25g parth+25gMelia+165g NaHCO₃.

Formulation of 400 g of Solution: 200 g of solution was formulated by adding: 160g+20g parth+20g melia+200gNaHCO₃.

Solutions were prepared in the lab before being applied in field conditions.

RESULTS

Mortality Rate at 100 g of Solution

The conducted analysis of variance for mortality rate at 100 g of solution demonstrated significance ($P < 0.05$) for factor time, concentration and the interaction of time and concentration. The means for mortality rate across different levels of concentration (20.33 to 95.00%) were shown in Figure 1. Moreover, a strong interaction between time and levels of concentration was apparent. The highest mortality rate (95.00%) was observed for 3 hours at the level of concentration of 0.3 and the lowest mortality rate, (20.33%) was observed for 3 hours at the level of concentration 0. Ding et al. (2012) conducted their study using an eco-friendly green synthesis approach to produce Mg O nanoparticles (NPs) by using the stabilization of MgO NPs with aqueous extract of *M. azedarach* seed and observed similar results.

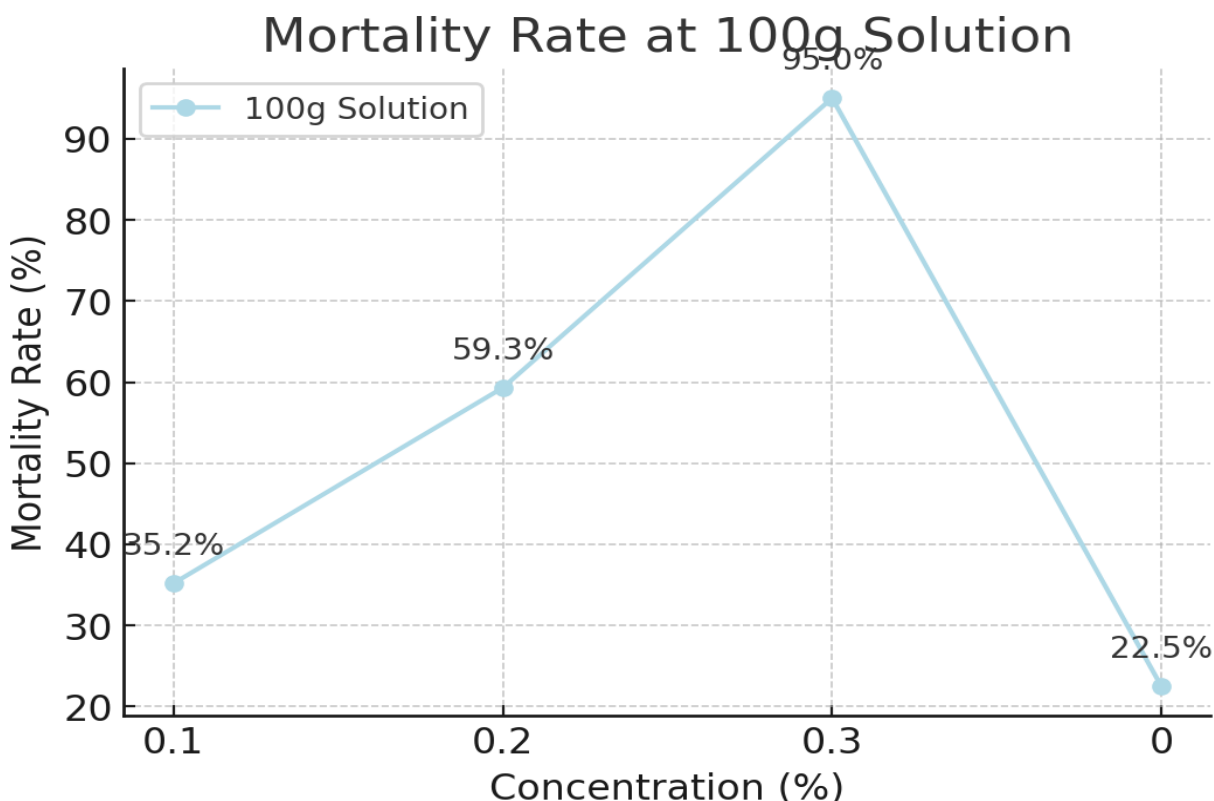


Figure 1. Mortality Rate at 100 g of Solution

Note. Treat; 1, 2, 3, 4= Concentration; 0.1, 0.2, 0.3, 0

Mortality Rate at 200 g of Solution

The analysis of variance based on mortality rate at 200 g of solution at the parameter level ($P < 0.05$), showed significant results for time, concentration and the interaction of time x concentration. The mean mortality rate values (in percentage) were described and represented graphically across levels of concentration (3.00 - 97.00%) (Figure 2). There was also a strong time and concentration interaction. The

highest mortality rate (97.00%) was observed at time (3 hours) and concentration (0.3), while the lowest mortality rate (3.0%) was observe at time (2 hours) and the (0) concentration of poison. Ali and Aljanabi (2020) looked at the efficacy of leaf extracts (hot, cold) and organic solvent extracts (ethyl alcohol, ethyl acetate, hexane) from *Melia azadirach* L. (Spain dales: Meliaceae) as a larvicide for *Culx pipiens* L. (Diptera: Culicidae) and had similar results.

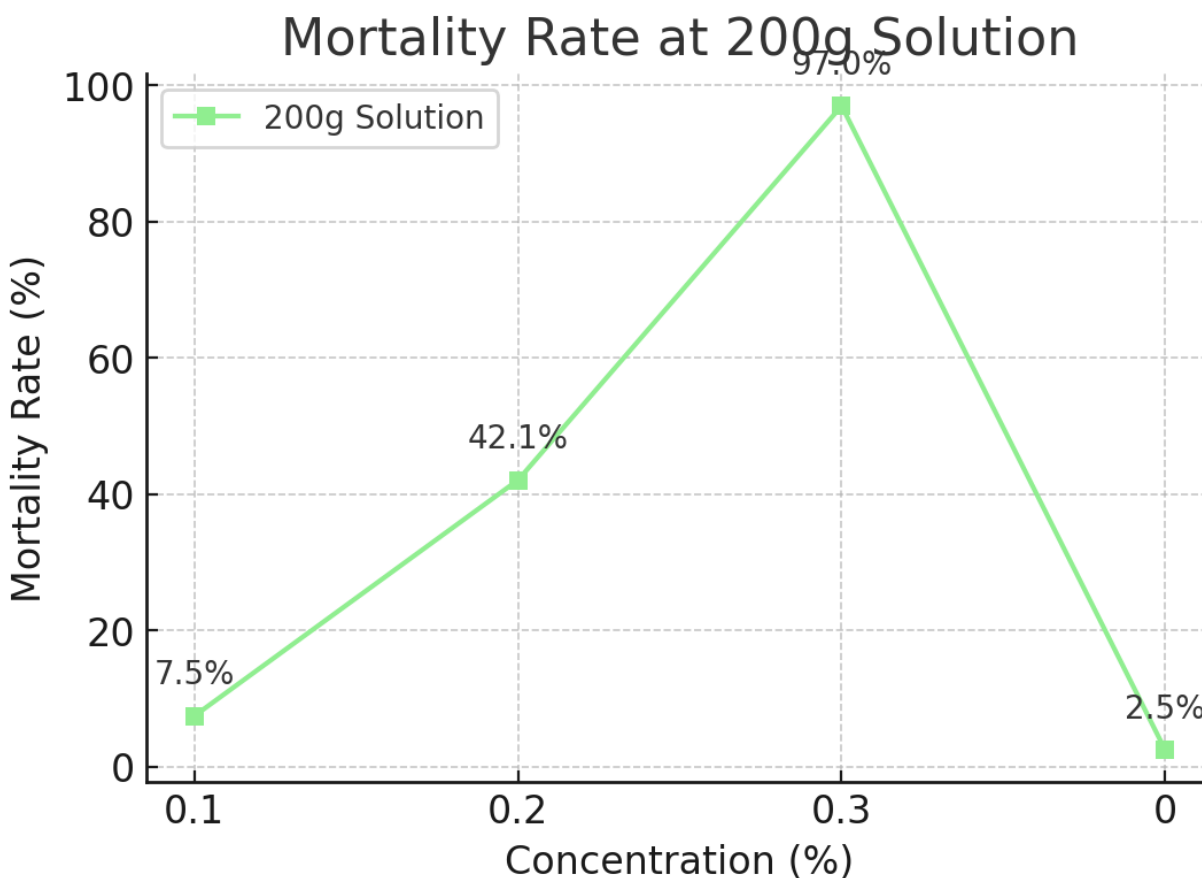


Figure 2. Mortality Rate at 200 g of Solution

Note. Treat 1, 2, 3, 4= Concentration, 0.1, 0.2, 0.3, 0

Mortality Rate at 300 g of Solution

The analysis of variance for mortality rate at 300 g of solution yielded significant results ($P < 0.05$) for time, concentration and the interaction of time and concentration. The mean values for mortality rate at various concentration levels ranged from 14.00 to 46.67% (Figure 3). However, no interactions occurred between time and levels of concentration, meaning that the effects of time and treatments were separate. The highest recorded mortality rate (46.67%) was found at level 0 concentration for 2 hours, while the lowest mortality rate (14.00%) was found at level 4.8 concentration for 1 hour. Jamal (2018) examined the larvicide activity of the synthetic pesticide cypermethrin compared to a leaf extract of the naturally occurring *P. hysterophorus* against 4th instar larvae of *Anopheles stephensi* and reported the same results.

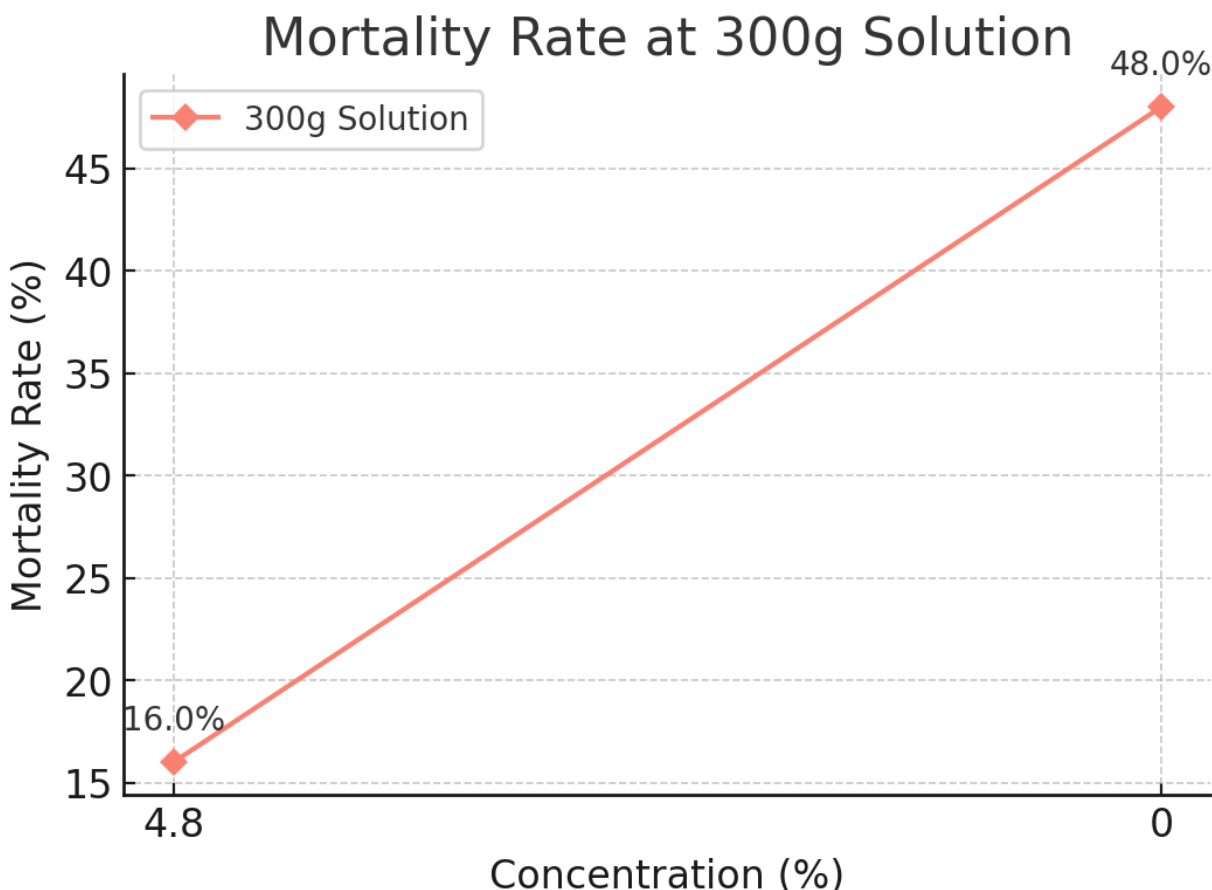


Figure 3. Mortality Rate at 300 g of Solution

Note. Treat; 1, 2= Concentration; 4.8, 0

Mortality Rate at 400 g of Solution

The one-way analysis of variance for mortality rates at 300 g of solution found all signs of significance ($P < 0.05$) for time, level of concentration, and interaction of time and level of concentration. The mean mortality rates for levels of concentration ranged between 22.67% and 80.00% (Figure 4). However, there was no interaction of time and levels of concentration suggesting that all other aspects of time and treatments were independent of the other aspects of mortality rates. The greatest mortality rate (80.00%) occurred at 5.2 level of concentration for 1

hour of exposure to treatment, the lowest mortality (22.67%) occurred 0 level of concentration for 2 hours of exposure. Kumar et al., (2011) synthesized extracts of *P. hysterophorus* leaves in 1,000 ppm solutions in acetone, benzene, petroleum ether, diethyl ether and hexane as solvents to test its effectiveness for the dengue disease vector, *Aedes aegypti*, and found similar results, as did Maggi et al., (2005) who studied the anti-insect properties of an ethanolic extract of aerial portions of *Artemisia annua* L. and artemisinin.

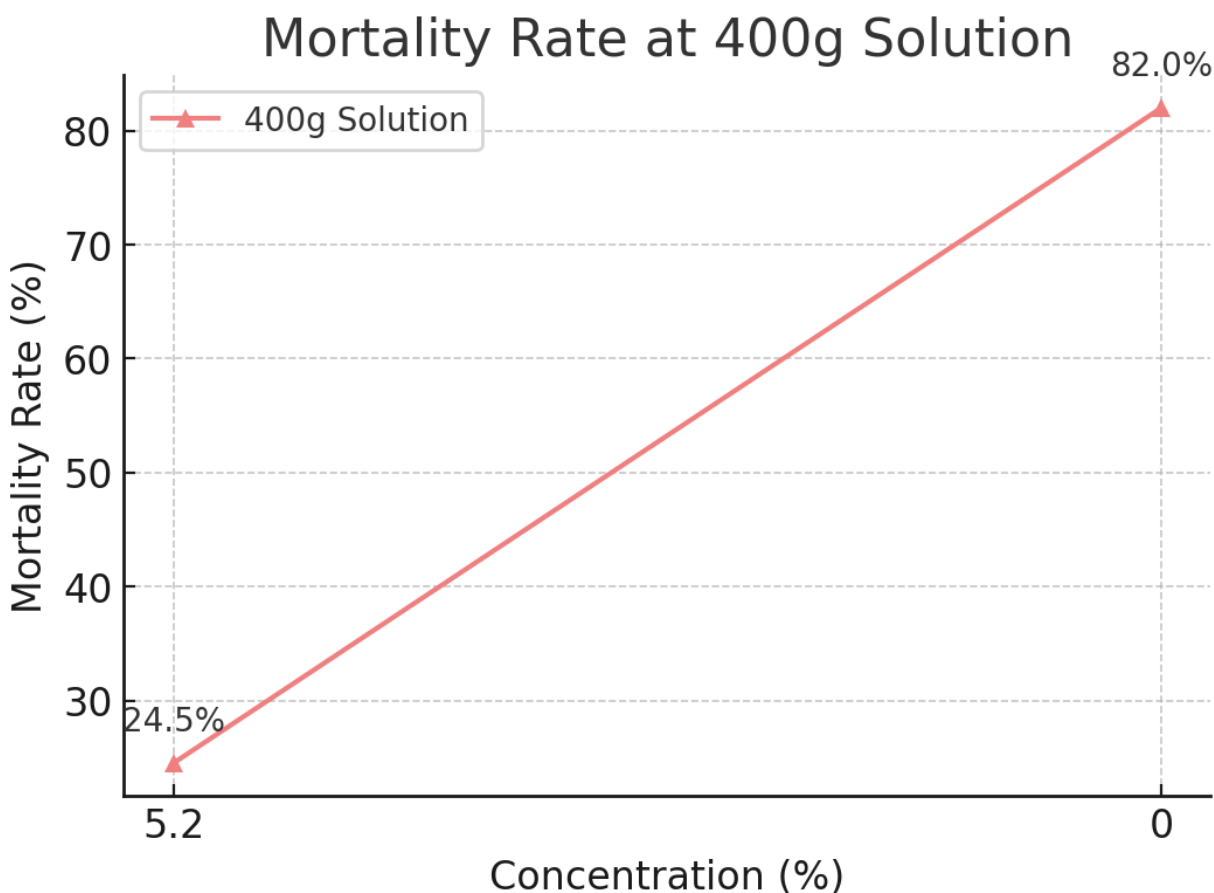


Figure 4. Mortality Rate at 400 g of Solution

Note. Treat; 1, 2= Concentration; 5.2, 0

DISCUSSION

Mosquitoes are a major source of vector-borne diseases on an international scale and the *Aedes aegypti* mosquito has been implicated in the transmission of dengue, Zika, and chikungunya viruses (Bhatt et al., 2013; Stanaway et al., 2016). As researchers become increasingly concerned about the negative effects that chemical insecticides can have on other living beings, many have started to look for plant-based options for sustainable mosquito control. The current study evaluated the larvicidal ability of *Parthenium hysterophorus* and *Melia azedarach* extracts by assessing mortality of *Cx. quinquefasciatus* larvae in field settings while also considering the effects of the concentration of the extract and exposure time. The results of this study confirm the considerable potential of both of these plants to be used as eco-friendly options for mosquito control. Our study revealed a strong relationship between concentration of the plant extract and the mortality of mosquito larvae. A concentration of

0.3% of the plant-based formulation yielded a mortality of 95% at the 3 hour exposure mark. These findings support and are similar to other studies, including those conducted by Ali and Aljanabi (2020), who reported similar larvicidal effects from *Melia azedarach* extracts against *Culex pipiens* larvae. The effects of *Parthenium hysterophorus* as a mosquito larvicide are also supported by other studies, like Jamal (2018) who monitored the effects of plant-leaf extract on *Anopheles stephensi* larvae. The interaction between *Parthenium hysterophorus* and *Melia azedarach* was clear in the mortality rates, with a combination of both plants showing stronger larvicidal potential. Dings et al. (2021) used *Melia azedarach* seed extract to synthesise MgO nanoparticles which also exhibited strong larvicidal properties. The chemistry of the natural plant extracts with sodium bicarbonate to have some facilitating role or contribution to enhancing bioactivity of the nanomaterials supports the case of the eco-friendly

nature of these natural products in integrated mosquito management (Kumar et al., 2011; Wubneh, 2019). The 0.3% treatments at 3 hours had mortality rates that were greater, but concentration, and retention are important in optimising plant-based larvicidal materials. The degree of mortality at 3 hours provides preliminary observations and is consistent with the findings of previous studies such as Iringa and Mange (1995) who evaluated the efficacy of *Melia azedarach* under laboratory controlled experiments against mosquito larvae.

The results are also consistent with the work of Aarathi and Morgan (2012), who asserted that plant-based systems provide viable alternatives for malaria vector control. Yet, while the initial results are promising, additional studies are required to determine the general applicability of *Parthenium hysterophorus* and *Melia azedarach* in a larger mosquito control framework. Studies assessing the long-term environmental impact of these biopesticides, especially on non-target organisms and overall ecosystem health, are essential (Ragnar et al., 2007). It would be prudent to also involve research examining the potential incorporation of these plant-based systems into current mosquito control operations- potentially decreasing the reliance of chemical insecticides.

CONCLUSION

The current study outcomes highlight that *Parthenium hysterophorus* (family: Asteraceae) and *Melia azedarach* (family: Meliaceae) can be evaluated as eco-friendly candidates in mosquito control. The aforementioned plant species indicate that both a combination of synergistic effects and great larvicidal effect make the two species viable alternatives to chemistry-based pesticides, which are commonly associated with adverse human and environmental effects. The study results support similar works on the effectiveness of the use of natural plant extracts and demonstrate a clear basis for future research aiming to apply sustainable methodology in pest control adaptations.

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