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Phytotoxic Potentials of *Azadirachta Indica* Methanolic Extracts against Species of Mosquito Larval Found in Jalingo LGA of Taraba

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ABSTRACT

This study assessed the potency of methanolic extracts from several neem tree components against mosquito larvae, a common disease vector in the study area. Fresh leaves, seeds, roots, and bark of A. indica plants were obtained in their natural settings and then macerated in a methanol solvent to provide the crude extract used in this study. Ten larvae (2nd and 3rd instars) that were actively swimming were put into 25-mL beakers with 16 mL of each larvicide extract solution made from different plant sections. As a control, an untreated group of larvae in (tap) water was used. At the highest concentration tested, neem seed extracts had the highest larvicidal activity, with a mortality rate of about 80%. Neem Bark extracts caused mortality of around 65%, whilst Neem Leaf and Neem Root extracts had less potency and caused mortality of about 50% and 40%, respectively. These results highlight neem extracts' potential as environmentally friendly mosquito larval control agents, especially neem seed and bark. To maximise the use of these extracts in integrated mosquito management techniques while taking ecological impact and safety issues into consideration, more research and practical evaluations are required.

Key Words: Disease Vector, Larvicidal Efficacy, Mosquito Larvae, Public Health.

I. INTRODUCTION

Vector-borne infections are among the most difficult to avoid and manage of all infectious diseases. Infectious diseases caused by insects account for more than 17% of all infectious disorders (Karunamoorthi, 2015). Mosquitoes are responsible for the transmission of over ten serious human and/or animal diseases, including malaria, lymphatic filariasis, yellow fever, dengue fever, West Nile virus, rift valley fever, chikungunya, Japanese encephalitis, Venezuelan equine encephalitis, and Murray Valley Encephalitis (Mukandiwa *et al.,* 2016). Mosquito-borne diseases are frequent in over 100 countries, with 700 million people worldwide afflicted each year (Ghosh *et al.,* 2012), and over one million people dying from these infections. Human malaria, mostly caused by *Plasmodium falciparum* and *P. vivax,* is the most common, especially in the tropics and subtropics. Aside from malaria, Aedes-transmitted viral infections such as dengue, yellow fever, chikungunya, and Zika have been a major source of worry in recent years over much of the tropics and subtropics around the world (Campbell *et al.*, 2015).

Malaria, while being a preventable protozoan disease of humans, is extremely common and ubiquitous in many tropical nations, and the malaria situation in these areas is dire. Malaria infection threatens an estimated 3.3 billion people, with 1.2 billion living in high-risk areas. Malaria endangers the lives of 40% of the world's population, or more than 2,200 million people. Each year, an estimated 300-500 million clinical cases are reported. Malaria is expected to kill over one million people each year, the vast majority of whom are children. Sub-Saharan Africa accounts for almost 90% of all malaria deaths worldwide (Hemingway, 2014). Malaria is a primary source of morbidity and mortality worldwide, despite control efforts. Yellow fever (YF) is a viral infection caused by the yellow fever virus, which is spread to humans and other vertebrate hosts by the bite of infected female Aedes mosquitos. The virus is still present in many countries of Africa and South America. It is a severe public health hazard in 45 endemic nations, 32 of which are in Africa and 13 in Central and South America, putting about 900 million people at risk (WHO, 2010). In many parts of Africa, declining vaccination rates and the abandonment of vector control methods have resulted in the recurrence of yellow fever sickness and periodic outbreaks (Beasley *et al.,* 2015).

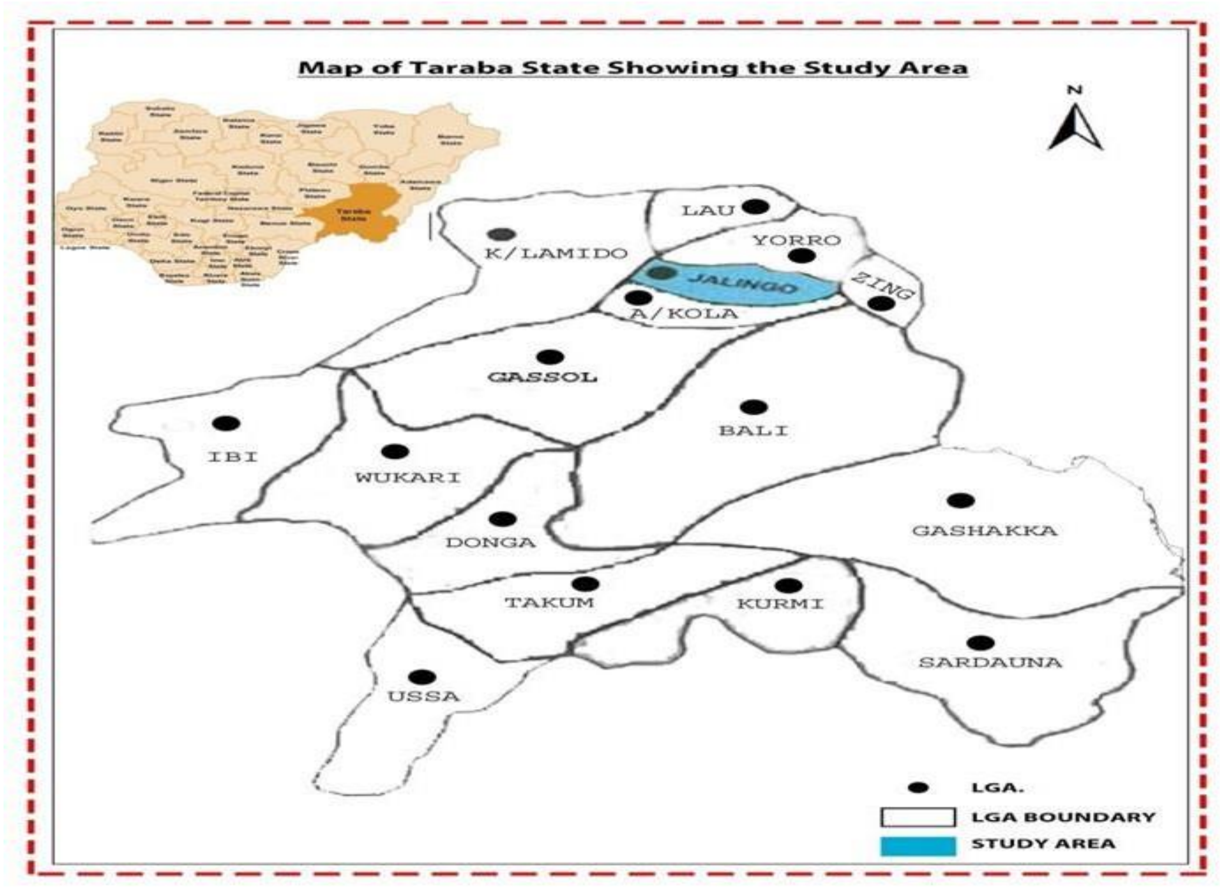
Dengue, chikungunya, and Zika outbreaks have been observed throughout the Americas, putting an estimated 3.9 billion people in 120 different countries at risk (Shragai *et al.,* 2017). Dengue fever is the most common mosquito-borne viral disease in the world, infecting an estimated 390 million people each year and putting 40% of the world's population at risk. Dengue fever causes an estimated 50-100 million occurrences every year, as well as tens of thousands of more severe and sometimes deadly dengue hemorrhagic fever/shock syndrome (DHF/DSS syndromes).

Mosquito vector control has long been a major component of the global plan to manage mosquito-borne diseases, and insecticides are the most essential component of this endeavour (Liu, 2015). However, pesticide resistance is becoming a major issue, and the development of novel insecticides is required to preserve present gains and minimise worldwide transmission loads (Hemingway, 2014). Mosquito control using synthetic chemical insecticides has become a serious concern, as has their indiscriminate use. The significant dependence on chemical pesticides, as well as repeated and ineffective insecticide applications, are major drivers of resistance. (Karunamoorthi & Sabesan, 2012). Synthetic chemical insecticides also have a negative impact on the environment, disrupting ecological equilibrium. The majority of chemical pesticides are dangerous to humans and animals, with some being less biodegradable and spreading damaging effects. Previously, severe concerns about the ecotoxicity of chemical insecticides have been expressed. For these reasons, naturally occurring chemicals and their derivatives are gaining popularity in the creation of new insecticidal agents against disease-causing pathogen vectors. (Anstrom *et al.*, 2012).

II. RESEARCH METHODOLOGY

Study Area

Jalingo is the capital of the state of Taraba in northeastern Nigeria (Figure 3.1). It lies between 8° 47'30 & 9°0'0 north latitude and 11° 18"30 & 11°32'15 east longitude. According to the 2006 census, its population is predicted to be 118,000 people. It has a tropical climate with dry and wet seasons. The wet season is hot and cloudy, whereas the dry season is partially cloudy and warm all year. Throughout the year, temperatures range between 16°C and 37°C, rarely falling below 13°C or rising above 41°C. Its diverse foliage supports a diverse range of animals, flying birds, and fish. For some, agriculture is their primary source of income. Jalingo has numerous primary, secondary, and tertiary medical facilities, as well as private and religious facilities.



Above is the Map of Taraba State showing the study area (Jalingo LGA)

Collection and Preparation of Plant Samples

Fresh *A. indica* plant leaves, seeds, roots, and bark were collected in their natural environment, Jalingo LGA, based on ethnobotanical descriptions and with the assistance of a taxonomist. And the plant samples were cleansed of extraneous elements by carefully washing them with clean water, then air-dried under a shade at room temperature before being chopped and trimmed to proper size. They were then manually pounded to powder using a mortar and pestle. For future usage, the powdered formulations were stored in a sterile plastic plate.

The crude extracts of powdered components, including bark (30 g), leaves (50 g), and roots (40 g), were extracted with acetone and macerated in ethanol. After 5 days, the macerated extracts were filtered and concentrated by evaporating the solvents using a rotary evaporator and drying under open air. The fixed oil (seed oil) was extracted from Neem seeds using official technique No. Aa 4-38 from AOCS (1993) (Angers *et al.*, 1996). The extraction was carried out for 6 hours in a soxhlet extractor with n-hexane as the solvent.

Phytochemical Analysis

Secondary metabolites were detected in the plant using ethanol extracts of plant parts. Thus, testing for alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, anthraquinones, and cardiac glycosides were carried out in the Chemistry Laboratory of Ahmadu Bello University Zaria, using the standard test techniques described by Gavamukulya *et al.* (2014). Following that, the qualitative analysis of each secondary metabolite will be performed and recorded.

Larvicidal bioassays

The larvicidal bioassay approach proposed by Dua *et al.* (2009) was carried out with minor modifications. Ten actively swimming larvae (2nd and 3rd instars) were placed in 25-mL beakers containing 16 mL of each larvicide extract solution from various plant parts. A control group of untreated larvae in (tap) water was employed as the control. The beakers were kept at room temperature at 29°C 2°C and 12L:12D. Moribund larvae were counted as dead (mortal) after 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, and 12 hours of exposure.

III. RESULTS AND DISCUSSION

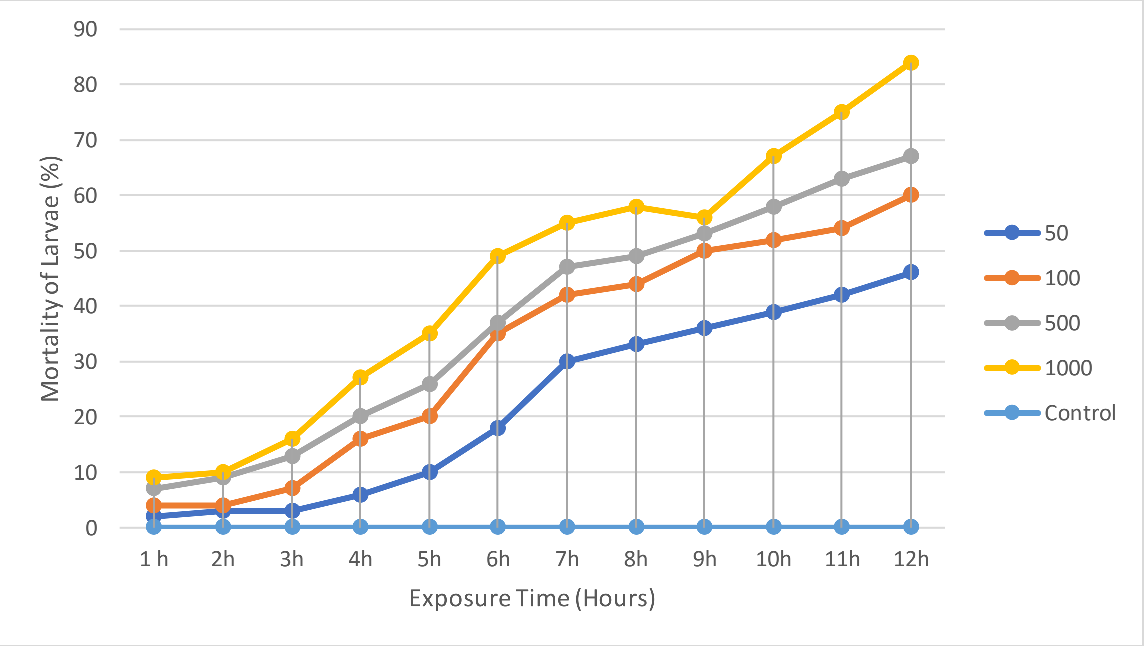


Figure 1: Larvicidal activity of Neem Seed methanolic extracts against Mosquito Larvae

Figure 1 depicts the results of the larvicidal activity of Neem Seed methanolic extracts against mosquito larvae at various doses and time intervals. The concentrations examined were 50 ppm, 100 ppm, 500 ppm, and 1000 ppm, with the absence of the Neem Seed extract (0 ppm) serving as a control group. The results reveal that the larvicidal activity of the methanolic extracts of Neem Seed increases with increasing concentrations and longer exposure durations. At 50 ppm, 2% of mosquito larvae were destroyed after 1 hour, and the number rapidly rose over time, reaching 46% larvae killed after 12 hours. Similarly, at 100 ppm, 60% of the larvae were dead after 12 hours. The larvicidal action grew more obvious as the concentration increased to 500 ppm and 1000 ppm, with 67% larvae destroyed at 500 ppm and 84% larvae killed at 1000 ppm after 12 hours. The control group did not show any larvicidal activity, showing that the observed effects were related to the Neem Seed methanolic extracts and not other factors. These findings indicate that methanolic preparations of Neem Seed have strong larvicidal efficacy against mosquito larvae.

The extracts appear to have a dose-dependent effect, which means that larger concentrations result in greater larval mortality; this study's findings are connected to those of Ayinde *et al.* (2020). The activity is also time-dependent, with the number of larvae killed rising over time. These findings are crucial in the context of mosquito control and disease prevention caused by mosquitos. Mosquito larvae are an important stage in the life cycle of the mosquito, and targeting them can help reduce mosquito populations and the risk of disease transmission. The larvicidal activity of methanolic extracts of Neem Seed indicates their promise as a natural and environmentally acceptable method of mosquito larvae control.

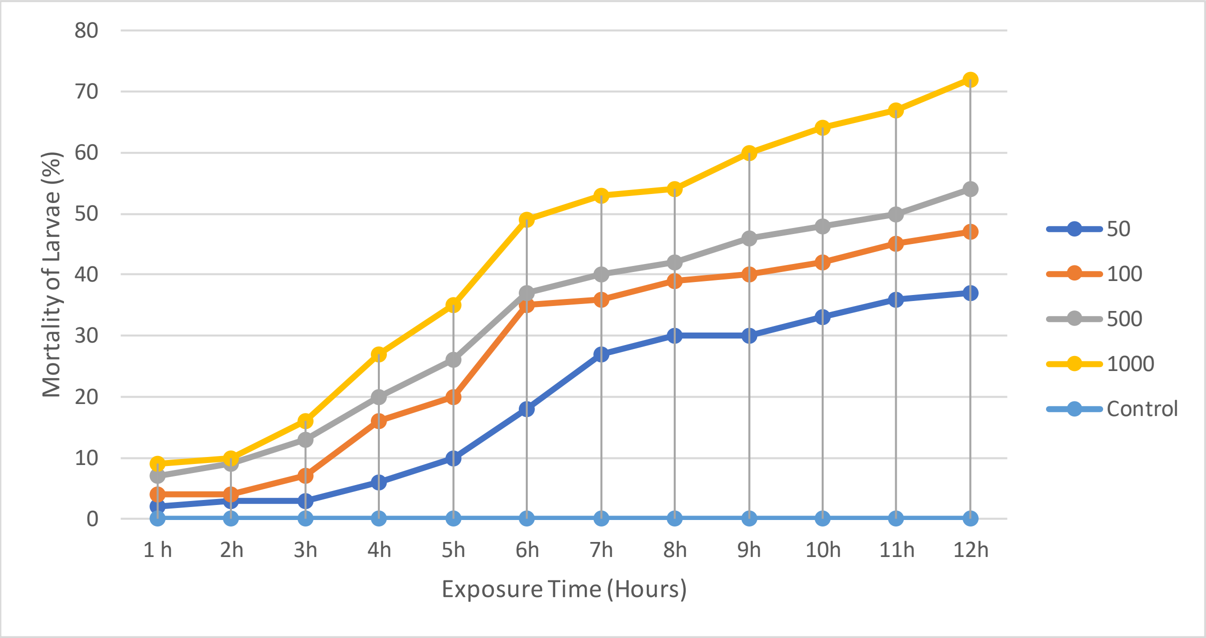


Figure 2: Larvicidal activity of Neem Bark methanolic extracts against Mosquito Larvae

Figure 2 depicts the larvicidal action of Neem Bark methanolic preparations against mosquito larvae. The results show how varied concentrations and exposure times affect mosquito larvae mortality. The larvicidal activity is relatively modest at the lowest dose of 50 ppm. The amount of larvae destroyed gradually increases over time, from 2% at 1 hour to 37% at 12 hours. The extract's potency appears to plateau after around 8 hours, since the number of larvae killed remains rather steady. The larvicidal activity is slightly stronger at 100 ppm than at 50 ppm. The number of larvae destroyed increases more rapidly over time, reaching 47% at 12 hours. Again, after around 8 hours, there is a plateau in effectiveness. The larvicidal activity gets more prominent as the concentration increases to 500 ppm and 1000 ppm. At each time period, higher amounts kill a greater number of larvae. After 12 hours, 54% of the larvae are killed at 500 ppm, whereas 72% are killed at 1000 ppm. The extract's efficiency appears to be more sustained over time at larger concentrations; this discovery is connected to that of Sayono *et al.* (2019).

The lack of larvicidal activity in the control group, which did not receive any Neem Bark extract, reaffirmed that the observed effects were due to the Neem Bark methanolic extracts. Overall, these findings suggest that methanolic preparations of Neem Bark have larvicidal efficacy against mosquito larvae. The extracts' effectiveness is concentration-dependent, with larger concentrations resulting in higher larval death. However, there appears to be a limit to the effectiveness, as larvicidal activity appears to plateau after a given amount of exposure time.

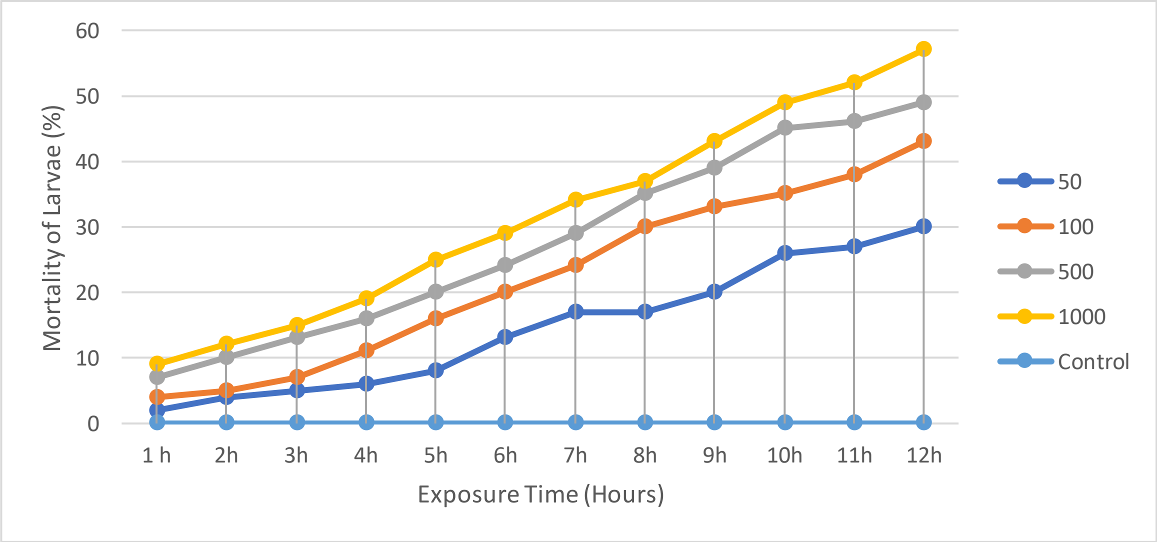


Figure 3: Larvicidal activity of Neem Leave methanolic extracts against Mosquito Larvae

The figure 3 above presents the larvicidal activity of Neem Leaf methanolic extracts against mosquito larvae at different concentrations (in parts per million, ppm) and time intervals (in hours). The concentrations examined were 50 ppm, 100 ppm, 500 ppm, and 1000 ppm, with the lack of Neem Leaf extract (0 ppm) serving as a control group. The results show that different concentrations of Neem Leaf methanolic extracts have larvicidal action against mosquito larvae. The larvicidal activity is rather modest at a concentration of 50 ppm. The amount of larvae killed gradually increases over time, reaching 30% after 12 hours. The extract's potency appears to plateau after around 8 hours, since the number of larvae killed remains rather steady. Larvicidal activity is slightly higher at 100 ppm concentration than at 50 ppm concentration. Over time, the number of larvae destroyed increases, reaching 43% larvae killed after 12 hours. The effectiveness, like the lesser concentration, peaks after about 8 hours. The larvicidal activity gets more prominent as the concentration climbs over 500 ppm. The amount of larvae destroyed grows significantly with time, reaching 49% after 12 hours. Throughout the testing period, the extract demonstrated consistent effectiveness with no plateauing. The highest concentration examined, 1000 ppm, reveals a considerable increase in larvicidal action. The number of larvae destroyed increases gradually over time, reaching 57% after 12 hours. Throughout the testing, the effectiveness remains steady.

The absence of larvicidal activity in the control group, which received no Neem Leaf extract, confirms that the observed effects were caused by the Neem Leaf methanolic extracts. Overall, the results show that methanolic extracts of Neem Leaf have larvicidal action against mosquito larvae. The extracts' efficacy is concentration-dependent, with higher concentrations resulting in higher larval death. The effectiveness is also determined by the exposure length, with longer exposure periods resulting in higher larval mortality, which is consistent with the findings of Iqbal et al. (2022). These findings imply that methanolic extracts of Neem Leaf could be a natural and environmentally friendly solution for reducing mosquito larvae.

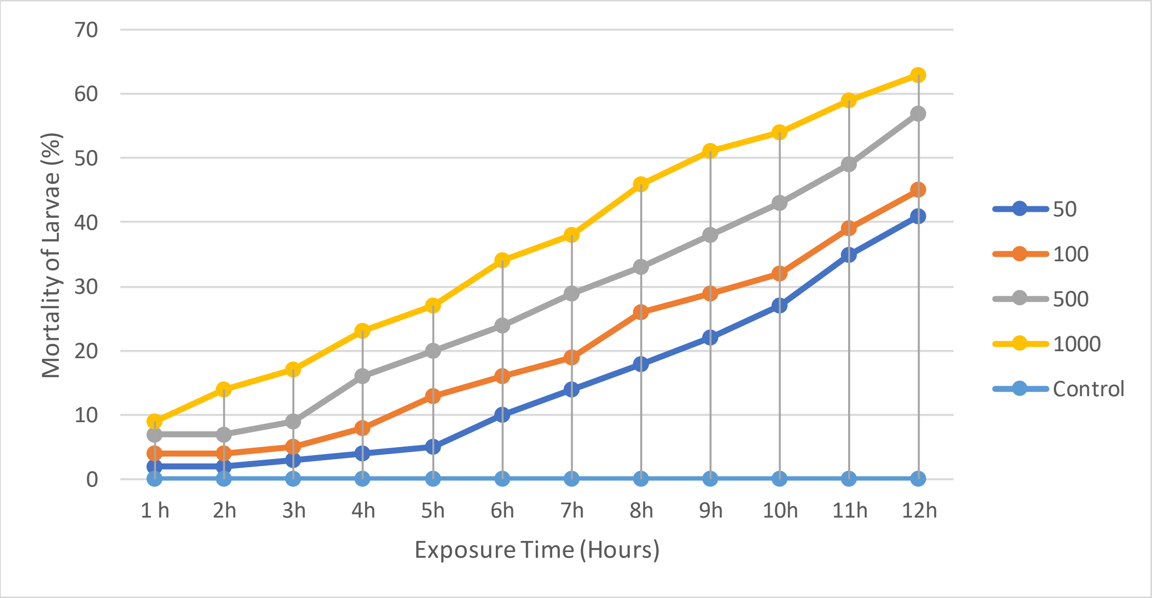


Figure 4: Larvicidal activity of Neem root methanolic extracts against Mosquito Larvae

The figure 4 above provided shows the larvicidal activity of Neem Root methanolic extracts against mosquito larvae at different concentrations (in parts per million, ppm) and time intervals (in hours). The concentrations examined were 50 ppm, 100 ppm, 500 ppm, and 1000 ppm, with the lack of Neem Root extract (0 ppm) serving as a control group. The findings emphasized the impact of varied concentrations and exposure times on mosquito larvae mortality at various concentrations. At 50 ppm, the larvicidal activity is relatively poor. The amount of larvae destroyed gradually increases with time, with 41% eliminated after 12 hours. The extract's efficiency gradually increases, indicating a possible impact on larval mortality; at 100 ppm, the larvicidal activity is consistent with the prior concentration. The number of larvae killed rises over time, reaching 45% after 12 hours. The extract is helpful in lowering larval populations over time. The larvicidal activity becomes more prominent at 500 ppm as the concentration increases. The amount of larvae killed rapidly increases over time, reaching 57% after 12 hours. The extract has a long-term and considerable effect on larval mortality. The highest concentration examined, 1000 ppm, shows a significant increase in larvicidal action. The number of larvae destroyed increases significantly with time, reaching 63% after 12 hours.

The absence of larvicidal activity in the control group, which received no Neem Root extract, indicates that the observed effec ts were caused by the Neem Root methanolic extracts. Overall, the findings indicate that methanolic preparations of Neem Root have larvicidal efficacy against mosquito larvae. The extracts' potency is concentration dependent, with larger concentrations resulting in increased larval death. The effectiveness appears to be impacted by exposure time as well, with longer exposure durations resulting in higher larval mortality; this observation is consistent with that of Amala *et al.* (2021). These findings suggest that methanolic extracts of Neem Root could be a natural and environmentally benign method of suppressing mosquito larvae.

The results suggest that Neem seed, stembark, leaf root methanolic extracts have larvicidal properties against mosquito larvae.

These findings have important public health ramifications, especially in areas where malaria and dengue fever are common mosquito-borne illnesses. Neem extracts could be used as a larvicidal chemical to control mosquito populations in these locations in an eco-friendly and long-lasting manner. Neem extracts are a safer option for the environment than synthetic chemical pesticides because they are biodegradable and have little effect on organisms that aren't their intended targets.

Neem-based larval control strategies can possibly reduce the population of disease-carrying mosquitoes, hence minimising the risk of diseases spread by mosquitoes among the local people. This is in line with the more general objectives of improving public health and preventing disease. Nevertheless, it's crucial to remember that even while neem extracts are generally regarded as harmless, the right dosage and application techniques should be established to guarantee successful mosquito control without endangering non-target creatures or the ecosystem.

IV. CONCLUSTION AND RECOMMENDATION

Overall, the results show that methanolic extracts of various sections of the Neem plant (Seed, Bark, Leaf, and Root) have larvicidal action against mosquito larvae. The extracts' efficacy is concentration-dependent, with higher concentrations resulting in higher larval death. The exposure time also has an effect on the effectiveness, with longer exposure times resulting in higher larval mortality. These findings show that methanolic extracts of the Neem plant could be a natural and environmentally friendly solution for reducing mosquito larvae. Further research is needed to determine the individual chemicals responsible for the larvicidal activity, as well as to optimise doses and exposure times for maximum efficacy. Furthermore, non-target organism investigations and field trials are required to evaluate the practical applicability of Neem Root extracts in mosquito control tactics.

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