Bima Journal of Science and Technology, Vol. 9(2A) Jun, 2025 ISSN: 2536-6041

DOI: 10.64290/bimagombe.v9i2A.1068

Impact of Fresh and Powdered Extracts of *Azolla pinnata* on Larval Mortality, Oviposition and Ovicidal Activity on *Aedes aegypti* Mosquitoes

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ABSTRACT

This study was carried out at Zaki and Katagum Local Government Areas of Bauchi State, Nigeria. The study aimed to assess the impacts of fresh and powdered extracts of *Azolla pinnata* on larval mortality, Oviposition and Ovicidal activity of *Aedes aegypti.* Eight (8) concentrations of five replicates were used for the experiment. The doses were; 500, 600, 700, 800, 1000, 1500, and 1700 mg/L respectively. Samples of *A. pinnata* were taken, laboratory-dried, mixed, and sieved into a fine powder. Larvicides bioassays were conducted in compliance with the World Health Organization recommendation protocol. To test for larval susceptibility, five replicates of *A. aegypti* larvae were exposed to powdered and fresh *A. pinnata* at varying doses for 24hours. *A egypti* gravid females, consisting of five replicates, were used to perform the Oviposition deterrent. However, five replicates of each concentration was used to test the ovicidal action. Microscope was used to count the egg in each treatment the eggs were moved into a cup filled with distilled water to monitor hatching. 48 hours after treatment, hatch rates and the proportion of eggs that died were evaluated. Mortality was subjected to log-probit analysis in order to calculate LC_{50} and LC_{90} with a 95% confidence limit. Utilizing formulas, ovicidal activity and oviposition deterrent were determined. With increasing concentrations in both treatments, there was a discernible rise in mortality. For fresh and powdered *A. pinnata* extracts, the oviposition deterrence assay demonstrates effective repellency at 100% for concentrations of 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1700 mg/L. Ovicidal activity revealed that 81%, 85%, 91%, and 97% of *Aedes aegypti* eggs died when submerged in trays containing both extracts. *Aedes* egg hatchability ranged from 96% to 100% in the control treatments. The result of this study shows that *A. pinnata* crude extracts, both fresh and powdered, were efficient and might be used as an insecticide for the control.

Keywords: *Azolla pinnata, Aedes aegypti,* larval mortality, Oviposition, Ovicidal activity.

INTRODUCTION

According to Reuda (2004), one of the most significant insect groups responsible for spreading diseases including dengue fever, yellow fever, chikungunya, malaria, encephalitis, and filariasis to humans is the mosquito. Many regions of the world now face mosquito problems, particularly those that are tropical or subtropical (Fradin, 2001). In addition to spreading illness, their bites can result in allergic reactions, dermatitis, and secondary infections, not to mention a great deal of discomfort (Keiser *et al*., 2005) Numerous Aedes species are known to transmit arboviral diseases, including Zika virus infections, dengue, yellow fever, chikungunya, and Rift Valley fever, which

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have a significant impact on public health (Nelson, 2010). Currently, sylvatic, rural, and epidemic cycles perpetuated by wild and urban vectors are causing these illnesses to spread throughout West Africa (Ghosh, 2012).

Dengue is one of the most significant tropical infectious illnesses that were growing worldwide (Ghosh, 2012).Over 40% of the world's population are currently at risk of dengue, according to the World Health Organization (WHO). According to current WHO estimates, 50–100 million cases of dengue fever may occur annually (WHO, 2018). Currently, over 100 nations in Africa, the Americas, the Eastern Mediterranean, South-east Asia, and the Western Pacific have an endemic case of the illness. According to official data provided by Member States, the number of cases across the Americas, South-East Asia, and Western Pacific topped 1.2 million in 2008 and over 2.3 million in 2010. According to WHO (2017), 2.5% of people with severe dengue die. High fever, headache, pain behind the eyes, joint and muscle aches, nausea, vomiting, and rash are some of the symptoms of diabetic foot syndrome.

In addition to these symptoms, patients with Dengue Shock Syndrome (DSS) also experience restlessness and chilly, clammy skin (WHO, 2017). Until vaccines are developed, the main strategy for managing these infectious diseases is to decrease the numbers of *Aedes* mosquitoes, which transmit the dengue virus, and *Culex pipiens* which transmit the West Nile virus (Delette, 2011). Although the use of insecticides to manage vectors has been shown to be successful in killing *Aedes* mosquitoes, there is still a risk because of the emergence of chemical pesticide resistance and subsequent rebound in vector capacity.

MATERIALS AND METHODS

Study Area

This study was carried out at Zaki and Katagum Local Government Areas of Bauchi State, Nigeria. The area located on the coordinates 12° 8'0" N, 10^0 17' 0" E and 12° 17'N 10^0 21'E (BASG, 2018). The majority of the residents are farmers, which include raising cowpeas, cotton, sorghum, millet, rice etc. The soil in the area is loamy soil that is rich in nutrients for plant fertility, leading to the majority of the population being peasant farmers.

Collection and Preparation of *A. pinnata*

Fresh *A. pinnata* was obtained from the Gololo pond in the Zaki local government of Bauchi State. The tubs had a diameter of 21 cm and were filled with a layer of rice field soil (4-5 cm) that had been saturated with tap water up to 4-5 cm above the soil surface. The tubs were stored outside in areas with some shade. For a month, superphosphate and carbofuran were sprayed once every five days at a dosage of 2g Based on physical traits, the *A. pinnata* species was identified at Department of Plant Biology, Bauchi State University Gadau.

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A. pinnata Samples in Powdered Form

Fresh *A. pinnata* samples weighing 60g were taken out of plastic tubs and allowed to dry in the sun for two days. The dried samples were then mixed using an electric blender and sieved into a fine powder**.**

Maceration Extraction of Powdered Sample

One liter of methanol solvent was then added to a beaker containing 60g of the measured powdered dry plant material of *A. pinnata.* Additionally, the samples were left for seven days in order for the solvents to enter and soften the plant cellular structure. Periodically, the setup was shaken to aid in the extraction process.

Fresh *A. pinnata* Samples

Before being used, 30g of fresh *A. pinnata* leaves were sampled from the plastic tubs and cleaned with chlorine-free water to get rid of any contaminants from other sources. The plastic tubes holding the distilled water were filled with the Pinnata sample.

Collection and Rearing of *Aedes* larvae

Aedes mosquitoes in their immature stages were collected from various freshwater breeding sites in Katagum (Azare), primarily from makeshift puddles and little ponds. Using a scooping spoon, water was collected and transferred into little, clear plastic pails. After carefully inspecting the buckets for the presence of predators, the predators were removed with a pipette. The collected mosquito larvae were taken in clearly labeled plastic buckets to the Postgraduate Laboratory, Department of Biological Sciences, Bauchi State University, Gadau. The collected larvae were identified like jet black scales, silvery-white scales on the side of the thorax and abdomen and ringed with white bands on each leg, using Kraemer's (2015) taxonomic keys, and they were fed yeast capsules (Singh, 2016). A portion of the larvae were utilized in a test for larvicidal susceptibility. When the adults emerged from the water after two to five days, the nets kept them from fleeing and imprisoned them at the top of the rearing bowls. Using an aspirator, emerging adults were removed from the rearing bowls, carefully moved to adult cages, and recognized as *Aedes aegypti* pending the duration of their exposure to *A. pinnata*. While females were fed grass cutter blood, adult males were administered a 10% sugar solution. Every stage was kept at 28± 2°C and 65± 5% relative humidity in the lab.

Larvicidal Bioassay

The larval susceptibility test protocols recommended by the World Health Organization (WHO, 2005) were followed while conducting the larvicidal bioassays. Five replicate of *A. aegypti* late larvae were used in the bioassay tests for powdered *A. pinnata* in concentrations of 500 mg/L, 600 mg/L, 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1700 mg/L; fresh *A. pinnata* was chosen as the test concentrations, with concentrations of 500 mg/L, 6000 mg/L, 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1700 mg/L after 24 hours of exposure. For every experimental replicate, the control solutions consisted of 10% of the corresponding methanol solvent mixed with 1 milliliter of distilled water. This test was conducted using plastic containers that each had 500 mL of distilled water, 25 mosquito larvae, and *A. pinnata* powder and extract. The mortality rates of *A. Aegypti* larvae were ascertained after a 24-hour period. When a larva completely stopped moving even when touched it was deemed dead.

Oviposition Prevention

The oviposition deterrent test was carried out in a laboratory. Five clones of a 25-gravid female *A. aegypti* were placed under room-temperature in the insectary cages measuring

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30 cm by 30 cm by 30 cm each. A 10% glucose solution was given to the adults and was always available. *A. pinnata* extracts were placed in two 50 mL plastic cups for oviposition, and one 50 mL plastic cup contained one milliliter of distilled water and ten percent of the methanol solvent. In various cages, *A. pinnata* powder and fresh were utilized in concentrations of 500 mg/L, 600 mg/L, 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1500 mg/L Oviposition was supported by inserting a piece of filter paper (Whatman No. 1) on the inside of each plastic cup so that the upper half of the paper was above the solution where the mosquitoes would lay their eggs and the lower half was submerged in the treated solution or untreated control for the entire paper to get moistened. To neutralize any impact of their placement on oviposition, the control and treatment cups were positioned at alternating diagonally opposite sites for every replicate. The number of eggs deposited in the treated and control cups were counted under a microscope after three days. The following formula was used to determine the percentage of effective repellency (ER %) for each concentration of oviposition repellent:

$$ER = (NC - NT) / NC) \times 100\%$$

Where ER = percent effective repellency; NC = number of eggs in control; and NT = number of eggs in treatment.

Ovicidal activity of *A. aegypti*

Six trays were filled with one hundred *A. aegypti* eggs. Five trays containing 500 mg/L, 600 mg/L, 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1500 mg/L of powdered and fresh *A. pinnata*, respectively. One milliliter of pure water and ten percent methanol solvent were placed in the control cup. For every concentration, there were five replicate of the test run. Following treatment, eggs were counted under a microscope and placed into a cup of distilled water to be monitored for hatching. Based on the inability of eggs with closed opercula to hatch, the proportion of eggs that died was computed. 48 hours after treatment, the hatch rates were evaluated using a formula:

$$\text{Egg mortality (\%)} = \frac{\text{number of unhatched larvae}}{\text{Total number of eggs}}$$

Data Analysis

Using SPSS (23.0 version), data on larval mortality were converted to percentage and then subjected to log-probit analysis to calculate LC_{50} and LC_{90} with a 95% confidence interval. Analysis of variance (ANOVA) was used to assess if differences in oviposition deterrent and ovicidal activity were statistically significant at the 95% confidence level or p= 0.05.

RESULTS

For both fresh and powdered *A. pinnata* extracts, the oviposition deterrence assay demonstrated excellent repellency at 100% for concentrations of 700 mg/L, 800 mg/L, 1000 mg/L, 1500 mg/L, and 1700 mg/.Therefore, fresh *A. pinnata*500 mg/L caused 83% and 600 mg/L caused 95% repellence against *A. aegypti*, whereas for powdered 500 mg/L caused 87% and 600 mg/L caused 98% repellence. Meanwhile all other values indicated 100% repellency as shown in **Table 1** below:

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Table 1: Showing Percentage of Adult *Aedes aegypti* Exposed to Varying amount of *Azolla pinnata* leaf extracts that do not oviposit

Graded Azolla Conc	Oviposition Deterrence		
(Mg/L)	**Aqueous Extract (%)**	**Methanoic Extract (%)**	**Control**
500	83	87	1/0
600	95	98	
700	100	100	
800	100	100	
1000	100	100	
1500	100	100	
1700	100	100	

However, the LC_{50} and LC_{90} result for fresh and powdered leaf extract indicated that the powdered extract was found to be more significant than the fresh extract, The powdered extract indicated that lethal concentrations of both fresh and powdered has effects on the population exposed. Whereas, the powdered extract was found to have greater effect on the population exposed than the fresh as indicated in Table 2 below.

Table 2: Mean LC_{50} and LC_{90} of *A. pinnata* powdered and fresh extracts after 24h of exposure on *A. aegypti* mosquitoes

Pinnata extract	**LC_{50}(mg/L)**	**LC_{90}(mg/L)**	**Regression Equation**	**95%confidence interval**	
				Lower bound	**Upper bound**
Powdered	937.59	2687.54	Y= -13.83 + 0.77X	3.08	4.11
Fresh	1011.61	2657.41	Y= - 14.83 + 0.80X	3.40	4.45

LC_{50}, lethal concentration required to kill 50% of the population exposed; LC_{90}, lethal concentration required to kill 95% of the population exposed and mg/L, milligram per litre

Table 3 shows the mortality of adult *Aedes aegypti* exposed to graded concentrations of *Azolla* leaf extracts; our findings revealed that fresh *A. pinnata*, at 500 mg/L concentration resulted in 81% mortality, whereas for powdered *A. pinnata*, the figure was 85%. Moreover, 600 mg/L produced 91% mortality, 700 mg/L produced 97% mortality for fresh extracts, and 600 mg/L produced 95% mortality for powdered extracts. Every other concentration had a 100% death rate. Conversely, under control treatments, Aedes eggs had a hatchability of 96% to 100%.

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Table 3: Percentage Egg Mortality of adult *Aedes aegypti* exposed to graded concentrations of *Azolla pinnata* leaf extracts.

Graded Azolla Conc	Oviposition Deterrence		
(Mg/L)	Aqueous Extract (%)	Methanoic Extract (%)	Control
500	81	85	1/0
600	91	95	
700	97	100	
800	100	100	
1000	100	100	
1500	100	100	
1700	100	100	

DISCUSSION

The results of this investigation shows that *A. pinnata*, fresh and powdered, have insecticidal action against *A. aegypti*. The findings demonstrate the possible use of *A. pinnata* powder and fresh as a mosquito larvicidal agent and, larval mortalities were observed at various concentrations. This is in line with the findings of Zulkrnin *et al.,* (2018), who found that powdered *A. pinnata* had a higher mortality rate than fresh *A. pinnata*. Meanwhile both powdered and fresh *A. pinnata* had recorded LC_{50} and LC_{90} values at different concentrations. Research by Ravi *et al*., (2018) showed the effectiveness of *A. pinnata* crude extract bioactive compounds and their potentials for development into biolarvicides for the control of *Aedes* mosquito vectors. With respect to *A. aegypti* early 4th instar larvae, their results showed the maximum larvicidal effect, with LC_{50} and LC_{90} values, respectively.

The larval bioassay test for *A. albopictus* revealed values in its Soxhlet extraction, with LC_{50} and LC_{90} values, compared with the maceration extraction LC_{50} and LC_{90} values were proven to have effect on the target organism. Meanwhile, the maceration extraction chemicals were recorded with the LC_{50} and LC_{90} values, respectively. In a similar vein, Ravi *et al*., (2020) showed how *Azolla pinnata* might be used as a substitute bio-insecticide. The results of their four distinct tests demonstrated the potency of plant extracts from *Azolla pinnata* against *Aedes Albopictus* and *Aedes aegypti* mosquitoes. The adulticidal test showed a statistically significant rise in mortality with increasing test concentration.

Furthermore, the effectiveness of *Azolla pinnata* extract generated in six different concentrations as an *Aedes aegypti* larvicides was examined by Ravi *et al.,* (2020b) using Nano-synthesized silver particles. After 24 hours of exposure, the insecticide resistance (IR) results showed that there was no death of larvae in the control groups, and the lowest mortality was at 10 ppm, where there was only 7.5% mortality, while the highest concentration showed 95% mortality. Both fresh and powdered *A. pinnata* extracts exhibit effective repellency at 96% - 100% varied concentrations according to the oviposition deterrence assay.

On the other hand, *Aedes aegypti* mosquitoes, prepared laying eggs in the untreated (control)

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cups. This study supported the findings of Ravi *et al.,* (2020a), which showed that during the oviposition deterrence test, the tested samples of *A. aegypti* and *A. Albopictus* chose to lay eggs in the plastic cups filled with water rather than in the plastic cups containing *A. pinnata* extract. Numerous laboratory experiments have demonstrated that, in several Asian nations, completely covering the water's surface with fresh and powdered *Azolla* extracts significantly lowers the death rate of larvae and the emergence of adult Anopheles and *Culex* mosquitoes (Mogi, 1988). According to field research conducted in India, *Azolla* growths are nearly entirely responsible for suppressing mosquito breeding in their natural environments (Ansari & Sharma, 1991).

Rajendraan and Reuben (1991) discovered that *Azolla microphylla*, which covers more than 80% of the water surface, greatly reduced the number of immature mosquitoes in an agro-ecosystem of rice fields in South India. Bracco (2007) noted a decrease in mosquito larval development in China in paddy areas where *Azolla pinnata* was grown alongside the paddy. In a similar vein, Sithira and Kamalaveni (2008) reported another conclusion from a laboratory-based investigation with *A. pinnata*, which influences *Culex quinquefasciatus* and *Cx*. *Culicifacies* mosquito oviposition. When 50% of *A. pinnata* is present in laboratory containers, *Cx*. *oxinquefasciatus* and *Cx*. *culicifacies* mosquitoes exhibit dramatic behavioral changes that restrict their ability to lay eggs.

Furthermore, it was discovered that *Anabaen aazollae* decreased the larval densities and productivity of *A. gambiae*, *anfunestus*, and Cx. *Quinquefasciatus* in Tanzanian paddy fields. According to Mwingira data, mosquito productivity is reduced in paddy fields with high (>80%) *Azolla* coverage.

CONCLUSION

Based on the findings of this study, it was concluded that the crude extract of both fresh and powdered *A. pinnata* are effective and have the potential to be developed as insecticidal activities against *Aedes* mosquito, but greater effect of insecticidal activity was found in powdered extract, and can be used as vector control strategy. Further research on bioactive compound and their effects on non-target organisms should be carried out.

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