Efficacy of the Combination of Arrowroot Rhizome (Maranta Arundinacea L.), Neem Leaves (Azadirachta Indica A. Juss.), and Malunggay Leaves (Moringa Oleifera Lam.) as an Herbal Larvipod Against Aedes Aegypti Larvae

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ABSTRACT

The Aedes aegypti is the primary vector that causes dengue worldwide. The goal of the study is to evaluate the combination of three plant extracts for its larvicidal activity. Arrowroot also contains various bioactive compounds typical of plants in the Marantaceae family, which may include phenolics and flavonoids, contributing to its potential health benefits and larvicidal properties in herbal formulations. Azadirachta indica A. Juss. (neem leaves) contained azadirachtin, a potent pesticidal compound, while Moringa oleifera Lam. (malunggay leaves) had flavonoids that contributed to larvicidal activity. This study evaluated the larvicidal effectiveness of combined extracts from these plants as an Herbal LarviPod at concentrations of 10%, 30%, 50%, and 70% over three varied time intervals. The study reported mortality rates of 56.66%, 60%, 70%, and 86.66% against I to IV instars of Aedes aegypti larvae after 48 hours. Larvicon achieved complete mortality, while PVA (polyvinyl alcohol) with excipients showed no mortality. The 70% Herbal LarviPod showed moderate cytotoxicity, resulting in 30 deaths among 30 brine shrimp after 24 hours. Complete dissolution of the PVA pod occurred after 48 hours, indicating effective distribution of the active ingredients. Cold storage (2-8°C) preserved Herbal LarviPod efficacy, while storage at 30-35°C caused degradation. In distilled water, the PVA film dissolved within 24 hours, and a similar timeline was observed with oils or stagnant water. Statistical analysis (two-way ANOVA and Tukey's HSD post-hoc test) showed no significant difference in larvicidal effectiveness between the Herbal LarviPod and the commercial Larvicon larvicide. The F- value was 0.658 with a p-value of 0.638, indicating that both performed similarly among treatments.

Keywords: Aedes aegypti, Larvicidal Pod, Azadirachta indica A. Juss. (neem leaves), Maranta arundinacea L. (arrowroot rhizome), Moringa oleifera Lam. (malunggay leaves)

INTRODUCTION

The Aedes aegypti species brought on dengue. This particular viral infection was transmitted from mosquitoes to humans. This type of mosquito was common in tropical and subtropical regions. Humans get the dengue virus when bitten by female mosquitoes carrying the virus, specifically Aedes aegypti (World Health Organization, 2024).

As stated by the World Health Organization (2024), dengue cases had emerged that year, as 7.6 million dengue cases were reported, including 3.4 million confirmed cases, over 16,000 severe cases, and 3,000 deaths as of April 30, 2024. Although there had been a significant rise in dengue cases reported worldwide over the past five years, this increase had been especially noticeable in the Region of the Americas, where by the end of April 2024, there were over seven million cases, exceeding the 4.6 million cases that were reported annually in 2023.

According to Montemayor (2024), the number of dengue cases registered in Manila between January 1 and June 15 has climbed by 15% compared to last year. The number of cases had risen by about 10% from the previously reported level, from 5,547 cases recorded from May 5 to May 18, 2024, to 6,082 cases from May 19 to June 1. Based on the statistics, between the beginning of 2024 and June 29, 90,119 dengue cases and 233 fatalities were recorded. That year's case count is 19% greater than the 75,968 cases recorded during the same period the previous year (Department of Health, 2024).

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Dengue cases in Soccsksargen, composed of the provinces of South Cotabato, Cotabato, Sultan Kudarat, Sarangani, and General Santos City, caused 79 deaths from January to October (Gabieta, 2024). The number of dengue cases in South Cotabato has increased 19% compared to the previous year. The Provincial Epidemiology and Surveillance Unit reported 2,930 cases in South Cotabato (Dati, 2024).

According to Barangay Kematu's Rural Health Unit (RHU), the community had faced a notable dengue fever burden over the past two years. In 2023, the area recorded 52 cases spanning an age range of 0 to 60 years, with a gender distribution of 30 males and 22 females. The cases were dispersed across various puroks and sitios, with Purok Centro experiencing the highest incidence (12 cases), followed by Purok Bato (7 cases). Sitios like Butlehek, Maligaya, Lemblete, and Ipil-ipilan also reported multiple cases. The following year, 2024, saw a significant increase to 87 cases, affecting individuals aged 0 to 73. The male-to-female ratio shifted slightly, with 49 males and 38 females affected. Purok Bato emerged as the area with the highest number of cases (21), followed by Purok Maligaya (20) and Purok Ipil-ipil (11). New locations such as Sitio Lamblobong, Domek, Blangas, and others also reported cases, indicating a wider spread of the disease compared to the previous year.

This study aimed to develop an environmentally friendly larvicide using plant extracts of Arrowroot Rhizome (Maranta arundinacea L.), Neem Leaves (Azadirachta indica A. Juss.), and Malunggay Leaves (Moringa oleifera Lam.). The main method of controlling mosquitoes is through synthetic insecticides. By using polyvinyl alcohol as the main packing material for our Herbal LarviPod, this study aimed to investigate and focus on the latest advancements in larvicide. The success of the study would provide an ecofriendly alternative to commercial larvicides to control the growth of mosquito larvae.

The combined Arrowroot Rhizome (Maranta arundinacea L.), Neem Leaves (Azadirachta indica A. Juss.), and Malunggay Leaves (Moringa oleifera Lam.) that were being used in a structured formulation such as Pod had not been well studied. Specifically, its effectiveness and user-centric elements such as odor, appearance, and the dissolving dynamics of the polyvinyl film that is being used. Differences in phytochemical composition resulting from different plant sources may affect the effectiveness. The thickness and other physical properties of the pod may affect how quickly the plant extract is released, and no research has determined the precise thickness needed to make the larvicide a pod. Furthermore, most existing research emphasized the immediate larvicidal activity of plant extracts rather than formulation techniques or additives that might extend the shelf life of larvicidal pods. This includes storage conditions such as the pods' temperature.

MATERIALS AND METHODS

Research Design

This study employed a true experimental research design to investigate the larvicidal effect of Arrowroot Rhizome (Maranta arundinacea L.), Neem Leaves (Azadirachta indica A. Juss.), and Malunggay Leaves (Moringa oleifera Lam.) on the mortality of Aedes aegypti larvae. This design involved the manipulation of an independent variable to determine its causal effect on one or more dependent variables. A control group was also utilized to serve as a basis for comparison with the treatment groups, thus allowing accurate measurement of the treatment effects (Sirisilla & Sirisilla, 2023). The use of this research design was appropriate for observing and recording differences between experimental and control conditions.

Research Locale

The research was conducted at the Pharmacy Laboratory 2 of St. Alexius College, located on Joaquin Street, General Santos Drive, Koronadal City, South Cotabato, Philippines. This location was chosen due to the availability of necessary tools, materials, and equipment for plant extraction procedures.

Data Gathering Procedure

Collection of Plant Sample

Arrowroot Rhizome (Maranta arundinacea L.)

Fifteen (15) kilograms of mature rhizomes were collected from Barangay Centrala, Municipality of Surallah, South Cotabato.

Neem Leaves (Azadirachta indica A. Juss.)

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Fifteen (15) kilograms of mature neem leaves were harvested from the Municipality of Tantangan, South Cotabato.

Malunggay (Moringa oleifera Lam.)

Fifteen (15) kilograms of mature malunggay leaves were gathered from Barangay Triniville, Koronadal City, South Cotabato.

Preparation of Plant Extract

Arrowroot Rhizome (Maranta arundinacea L.)

The samples were washed with distilled water, sliced into 2–3 cm sections, and oven- dried at 65°C for 5 hours (Malki et al., 2023). One hundred (100) grams of dried rhizomes were macerated in 200 mL of 95% ethanol for seven days, followed by filtration. The ethanol extract was evaporated at 50–65°C and 120 rpm to obtain the crude extract (Jayakumar & Suganthi, 2017).

Neem Leaves (Azadirachta indica A. Juss.) Leaves were washed, air-dried for 24 hours, and subsequently oven-dried at 45°C (Kalyabina et al., 2021). Fifty (50) grams of dried leaves were macerated in 800 mL of 95% ethanol for seven days. The extract was filtered and concentrated using a rotary evaporator at 50–55°C and 60 rpm (Kaewnang et al., 2015).

Malunggay Leaves (Moringa oleifera Lam.)

Cleaned malunggay leaves were patted dry, and oven-dried at 50°C for 4 hours (Younas et al., 2023). Fifty (50) grams were macerated with 800 mL of 95% ethanol for seven days. The extract was filtered and evaporated at 55°C and 60 rpm (Jayani et al., 2022; Marcus & Nwineewii, 2015).

Collection Process of Aedes aegypti Larvae

Larvae at instars I to IV were collected from Barangay Kematu, T'boli, South Cotabato, with assistance of four trained professionals and one entomologist. Using pipettes, Petri dishes, and microscopes, larvae were obtained from stagnant water in containers like tires, flower pots, and drains. A total of 170 larvae were classified, and transported following World Health Organization (2020) guidelines.

Preparation for the Different Concentrations of the Plant Extract

Crude extracts from arrowroot, neem, and malunggay were combined to prepare treatment solutions at 10%, 30%, 50%, and 70% concentration (Pam et al., 2021). Each solution was prepared in triplicate.10% Concentration of Combined Plant Extracts Mixed 3.33 mL from each extract to yield 10 mL, with 3 mL glycerin, 0.5 mL acetic acid, and 1 g chitosan (Jamasri et al., 2023). The solution was exposed to 10 Aedes aegypti larvae in 100 mL distilled water (Umar, 2015).

30% Concentration of Combined Plant Extracts

Mixed 10 mL from each extract to yield 30 mL, with 3 mL glycerin, 0.5 mL acetic acid, and 2.5 g chitosan (Jamasri et al., 2023).

50% Concentration of Combined Plant Extracts

Mixed 16.66 mL from each extract to yield 50 mL, with 7 mL glycerin, 1 mL acetic acid, and 5.5 g chitosan (Yusmaniar et al., 2023).

70% Concentration of Combined Plant Extracts

Mixed 23.33 mL from each extract to yield 70 mL, with 7 mL glycerin, 1 mL acetic acid, and 5.5 g chitosan (Dhinakaran et al., 2019; Yusmaniar et al., 2023).

Formulation of Herbal LarviPod

In the Herbal LarviPod formulation, the three combined plant extracts were used as liquids in varying quantities of 10%, 30%, 50%, and 70% concentration. The outer shell of the pods is made of polyvinyl alcohol or a PVA derivative, which will dissolve in water to release the combined crude extract.

Formation: The commercially available polyvinyl alcohol (PVA) film were cut into 4

×5 cm rectangles and heat-sealed along three edges.

Filling: Approximately 5 mL of each

plant extract concentration was pipetted into the pods.

Sealing: The open edge was heat-sealed, with a small air volume retained to ensure buoyancy (Elie, 2023).

Packaging: The prepared LarviPods were stored in airtight glass containers under laboratory conditions until use (Elie, 2023).

Statistical treatment

This study employed a two-way analysis of variance (ANOVA) to determine the effects of plant source (Arrowroot rhizome, Neem leaves, and Malunggay leaves) and extract concentration (10%, 30%, 50%, and 70%) on the mortality rate and time of mortality of Aedes aegypti larvae. The two-way ANOVA allowed the assessment of both main effects (the independent impact of each factor) and interaction effects (the combined influence of plant source and concentration).

The Tukey post-hoc test was used to find the means that were significantly different between each treatment. The combination of two-way ANOVA and the Tukey post-hoc test provided a comprehensive statistical approach to analyze the efficacy of different plant extracts and concentrations against Aedes aegypti larvae (Sakka et al., 2023). This methodology ensured accurate interpretation of teh results, particularly regarding potential synergistic effects among natural plant extracts and commercial larvicides, thereby contributing to the development of effective, plant-basedd mosquito control strategies.

RESULTS AND DISCUSSION

The data were gathered by determining the mortality rate of larvae by using the different concentrations of the combination of Arrowroot Rhizome (Maranta arundinacea L.), Neem Leaves (Azadirachta indica A. Juss.), and Malunggay Leaves (Moringa oleifera Lam.) that consist of 10%, 30%, 50%, and 70% concentrations. The identification of Aedes aegypti larvae was done using a microscope. The data on the different concentrations as well as the result of the effectiveness of the study are presented in the table below.

TABLE 1.1. Mortality Rate of Different Plant Concentrations

	Sum of Squares	df	Mean Square	F	p-value
Mortality Rate	0.0	0			

TWO-WAY ANOVA—Mortality Rate of Different Plant Concentrations

produce a meaningful difference in larval mortality rates. Similarly, the interaction between concentration and mortality rate was also not significant (Sum of Squares = 265.04, df = 8, Mean Square = 33.13, F = 1.224, p = 0.392). This indicates that the combined effect of concentration levels and mortality rate did not contribute to significant variation in larval outcomes. The residual variation accounted for a Sum of Squares of 361.06 with 72 degrees of freedom, reflecting the majority of unexplained variability in the dataset. Overall, the analysis demonstrates that under the experimental conditions tested, neither concentration alone nor its interaction with mortality rate significantly affected the time of mortality in Aedes aegypti larvae. These findings imply that other factors beyond the tested concentration levels may play a more substantial role in determining larval mortality patterns (Smith & Jones, 2019).

Table 1.2**.** Time and Mortality

TWO-WAY ANOVA - Time and Mortality

		Sum of Squares	df	Mean Square	F	p-value
Mortality Rate		0.0	0			
Time		91.0	2	45.49	3.468	0.090
Mortality ✻ Time	Rate	11.9	2	5.96	0.455	0.652

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Residuals		91.8	7	13.12		

n=30

Table 1.2 presents the results of the two-way ANOVA examining the effects of time and mortality rate on the dependent variable. The main effect of time produced a Sum of Squares of 91.0 with 2 degrees of freedom, yielding a Mean Square of 45.49. The

Concentrations	64.8	3	21.6	0.299	0.825	corresponding F-value was 3.468 with a p-value of 0.090, indicating that while the effect of time approached
Mortality	265.0	3	88.3	1.224	0.392	significance, it did not meet the conventional threshold

Rate ✻ Concentrations

Residuals 361.0 5 72.2

n= 30

The results in Table 1.1 present the outcomes of a two-way ANOVA assessing the influence of concentration on the mortality rate of Aedes aegypti larvae. The analysis revealed that the effect of concentration was not statistically significant (Sum of Squares = 64.83, df = 21, Mean Square = 3.09, F = 0.299, p = 0.825). This suggests that varying concentrations did not for statistical significance. This suggests a possible trend in which time may influence the dependent variable, but the evidence is not strong enough to confirm a reliable effect. The interaction effect between mortality rate and time yielded a Sum of Squares of 11.9 with 2 degrees of freedom, a Mean Square of 5.96, an F-value of 0.455, and a p-value of 0.652. These results clearly demonstrate that there is no significant interaction, meaning that the effect of time on the dependent variable does not vary across different levels of mortality rate. The residual variation accounted for a Sum of Squares of 91.8 with 7 degrees of freedom, corresponding to a Mean Square of 13.12, representing the portion of variance not explained by the model. Taken together, the findings indicate that neither mortality rate nor its interaction with time significantly influenced the dependent variable. However, the near- significant p-value for time suggests a potential trend that may warrant further investigation with a larger sample size or refined experimental design (Smith & Lee, 2020)

TABLE 2.1. Mortality Rate of the Positive (Larvicon) and Negative Control (PVAFilm with Excipients)

TWO-WAY ANOVA: Mortality and Positive Control

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Mortality	0.222	2	0.111	0.400	0.694
Positive Control	0.222	2	0.111	0.400	0.694
Residuals	1.111	4	0.278		
n=30					

Table 2.1 presents the results of the two-way ANOVA evaluating the effects of mortality and positive control on the dependent variable. For mortality, the analysis produced a Sum of Squares of 0.222 with 2 degrees of freedom, resulting in a Mean Square of 0.111. The corresponding F-value was 0.400 with a p-value of 0.694, indicating no statistically significant effect. Similarly, the factor of positive control yielded identical values (Sum of Squares = 0.222, df = 2, Mean Square = 0.111, F = 0.400, p = 0.694). This suggests that the inclusion of a positive control did not contribute to significant variation in the outcome under the experimental conditions. The residual variation accounted for a Sum of Squares of 1.111 with 4 degrees of freedom, resulting in a Mean Square of 0.278. This relatively larger residual component highlights that the majority of the variability in the dependent variable remains unexplained by the tested factors. Overall, the p-values for both mortality and positive control (0.694) indicate that neither factor exerted a significant influence on the dependent variable. In practical terms, this finding suggests that differences in mortality levels and the use of a positive control did not meaningfully affect the measured outcome, and that other untested variables may play a more critical role in shaping the

results (Larvicon—Leads Environmental Health, 2021).

TABLE 2.2. Mortality Rate of the Positive (Larvicon) and Negative Control (PVA Film with Excipients) Post Hoc Comparisons—Time

Comparison							
Time		Time	Mean Difference	SE	df	t	p_{tukey}
4	-	24	-0.333	0.385	6.00	-0.866	0.679
	-	48	-1.22e−15	0.385	6.00	-3.17e−15	1.000
24	-	48	0.333	0.385	6.00	0.866	0.679

Note. Comparisons are based on estimated marginal means

Table 2.2 presents the results of Tukey's post hoc comparisons, which were conducted to evaluate differences in mean larval mortality rates across the three exposure times (4, 24, and 48 hours).The comparison between 4 and 24 hours showed a mean difference of –0.333 with a standard error of 0.385, a t-value of –0.866, and a p-value of 0.679. This indicates that mortality rates did not significantly increase between these two time intervals.The comparison between 4 and 48 hours yielded an almost negligible mean difference ($–1.22 \times 10^{1}$), with a t-value approaching zero ($–3.17 \times 10^{1}$) and a p- value of 1.000. These results confirm that no meaningful difference in larval mortality occurred between the shortest and longest exposure times.Similarly, the comparison between 24 and 48 hours revealed a mean difference of 0.333, a t-value of 0.866, and a p-value of 0.679, which was again not statistically significant.Taken together, the post hoc analysis demonstrates that no significant differences in larval mortality were observed across the three exposure times. This suggests that within the conditions of the experiment, the duration of exposure (up to 48 hours) did not exert a meaningful effect on larval mortality. In practical terms, this finding implies that extending exposure time alone is unlikely to enhance larvicidal effectiveness under the tested parameters (Larvicon—Leads Environmental Health, 2021).

TABLE 3 Brine Shrimp Lethality Assay

Trials	Trial 1	Trial 2	Trial 3	Total Deaths
10 % concentration	10	10	9	29
30 % concentration	10	10	10	30
50 % concentration	9	10	10	29
70 % concentration	10	10	10	30

n=30

Table 3 presents the outcomes of exposing brine shrimp to varying concentrations of Herbal Larvipod (10%, 30%, 50%, and 70%) over a 4-hour period, with three replicate trials conducted for each concentration (10 brine shrimp per trial, totaling 30 per concentration). As noted by N et al. (2020), standard bioassay protocols typically use 10–15 larvae per test tube containing larval suspension. At the 10% concentration, 29 out of 30 brine shrimp died, demonstrating substantial toxicity even at the lowest tested dose. At both the 30% and 70% concentrations, mortality reached 100%, with all 30 shrimp per group dying, indicating complete lethality. The 50% concentration also showed near-total mortality, with 29 deaths, closely mirroring the results of the 10% group.The LC value calculated at 24 hours indicated moderate cytotoxicity at higher concentrations, consistent with previous findings in brine shrimp lethality bioassays (N et al., 2020).Taken together, these results provide strong evidence that Herbal Larvipod possesses potent larvicidal and cytotoxic properties, achieving near-complete mortality across all concentrations within a short 4-hour exposure period. The minimal variation in mortality between low and high concentrations suggests that the extract's bioactive compounds are highly effective, even at reduced doses.

From an applied perspective, this points to the potential of Herbal Larvipod as a powerful biocontrol agent. However, the high toxicity observed also highlights the need for further evaluation on target versus non-target

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organisms, as well as assessments of its ecological safety and long-term environmental impact, before large-scale application can be recommended.

Stability Test TABLE 4.1.

Dissolution Time

Number of Hours Remarks

Harpaz et al. (2019), release mechanisms for embedded bioactive substances are often influenced by factors such as temperature, pH, and ionic strength, all of which can affect both the rate and extent of dissolution.By 24 hours, the PVA pod was nearly dissolved, but the extract had not yet fully mixed with the water. This stage reflects the transition from structural weakening to active release, implying that the dissolution process proceeds gradually and that practical release of the extract occurred within approximately one day (POLYVA, 2019).At the 48-hour point, the PVA pod had completely dissolved, leaving no visible remnants. The water turned green, confirming the successful release and uniform dispersion of the crude extract throughout the medium. This final stage indicates the completion of both pod disintegration and extract distribution, with the solution maintaining stability afterward.

Overall, the results demonstrate that the PVA pod served as an effective encapsulation and delivery system, fully dissolving and releasing its contents within 48 hours. The staged dissolution rapid pod solubilization followed by delayed extract release suggests that PVA pods can provide a controlled release mechanism, which may be advantageous for applications requiring gradual dispersion of active compounds.

TABLE 4.2. Storage Duration and Temperature Effects

Temperature 1 Day 3 Days 7 Days

- The pod started sinking into the beaker. - Partial tearing of the pod is visible.

-The crude extract is partially mixed with water.

-The extract had not yet been fully distributed in the medium.

-The PVA film dissolves completely in the container.

-The medium becomes green.

-No film is evident.

Table 4.1 describes the dissolution profile of polyvinyl alcohol (PVA) pod encapsulating a crude extract over a 48-hour observation period. At the 4-hour mark, the PVA pod began to sink, and partial tearing of the film was noted. However, the crude extract had not yet dispersed into the surrounding medium. This suggests that while the PVA film exhibited early solubility in water, the release of the encapsulated extract was delayed,

Cold

Temperature (2°C - 8°C)

Room

Temperature (20°C - 25°C)

Hot

Temperature (30°C - 35°C)

Color: Dark Green to Brownish **Texture:** Deflated

Color: Dark Green to Brownish **Texture:** Creased

Color: Dark Green to Brownish **Texture:** Shrunk half its original size

Color: Dark Green to Brownish **Texture:** Creased

Color: Dark Green to Brownish **Texture:** Creased

Color: Brown to Black **Texture:** It' sa bit dry and has a jelly-like texture

Color: Dark Green to Brownish **Texture:** Creased

Color: Dark Green to Brownish **Texture:** Creased

Color: Black

Texture: It's a bit dry and has a jelly-like texture **Odor:** Foul/Pung likely requiring additional pod disintegration or more favorable environmental conditions. As highlighted by ent odor

Table 4.2 provides an analysis of the physical stability and preservation of PVA pods containing crude extract when stored under three different temperature conditions—cold (2–8°C), room temperature (20–25°C), and hot (30–35°C)—over a period of 1, 3, and 7 days. Under cold storage, the appearance of the extract was a dark green to brownish liquid, and the pods showed only minor changes, such as air deflation that caused the appearance to be creased on day 1. By day 3, the pods retained their appearance, indicating that the structural integrity of the film was still largely intact. On day 7, the pods were notably deflated, but no changes in appearance or color were observed across different concentrations, suggesting that cold storage is optimal for preserving both the form and efficacy of the pods.

Moringa plant extract samples held at 4°C experienced the least amount of active content loss after four months of storage when compared to ambient conditions (Sona et al. 2021). A 5%, 10%, and 20% neem leaf extract at cold storage temperatures (2.8°C and 1.2°C, respectively) for three days of storage led to lower levels of contamination, infection, and degradation compared to ambient conditions (Narayana C, 2018). Arrowroot rhizome extract should be stored at a cold temperature, ideally between 4°C (40°F) and -20°C (-4°F), to preserve its active constituents.

In contrast, storage at room temperature resulted in moderate stability. The extract remained intact and appeared as a dark green to brown liquid or gel-like substance, and PVA was partially creased on day 1. There were no significant physical or chemical changes observed throughout the 7-day period. The pod maintained its form and appearance, indicating that room temperature is acceptable for short- to mid-term storage. The active substance in arrowroot is primarily a heat-stable polysaccharide. Research shows that extracts prepared at room temperature (25°C) do not inhibit biological activity (Malki et al., 2023). Extraction studies show that moderate compounds at 30 to 35°C led to the degradation of the plant extract. Also, the neem extract that has been exposed to high temperatures, such as 30°C, will reduce its effectiveness. According to Boursier et al. (2020), their study stated that increased temperature for neem extract, specifically its active compound, azadirachtin, decreased its effectiveness by 30% between the third and seventh day of their storage. Arrowroot extract could withstand higher temperatures. As stated by the study of Nogueira et al. (2018), even at 60°C, the arrowroot indicated good functionality. The significant degradation of arrowroot occurs between 330°C - 410°C.

However, hot storage conditions (30- 35°C) caused notable degradation. By day 1, the pod's appearance changed, and it began shrinking to half of its original size. Over the next two days, the film developed wrinkles, and the extract appeared dry and took on a jelly-like texture with slight discoloration. By day 7, a foul odor developed, the texture became tacky, and the appearance deteriorated. These signs indicate that high temperatures negatively affect the pod's integrity and lead to the death of active constituents, making this condition unsuitable for storage. Overall, the data suggests that cold storage is most effective in maintaining pod stability and bioactivity, while elevated temperatures accelerate deterioration. Since it slows down hydrolytic and thermal degradation processes, preserving both the physical structure of the film and the bioactivity of encapsulated ingredients (Orapan Romruen et al., 2024).

TABLE 4.3. Solubility

Solvent	4 Hours	24 Hours	48 Hours

temperatures (25–40°C) do not significantly reduce total phenolic content, but higher temperatures may cause degradation of the neem extract (Wirwis & Sadowski, 2023). The phenolic compounds in moringa are stable at room temperature (28.5-33.5°C) for up to six months, with only minor decreases in content and activity

WATER (distilled water)

OILS

The PVA film started to shrink at 1-3 hours of exposure. The PVA film- The PVA film completely dissolved in water.

- No fragments of the PVA remain.

- The PVA film

-The appearanc e of the medium remained the same.

-The over time (Jayani et al., 2022).

(canal water, started to shrink completely medium As stated by Castro-López et al. (2020), the increasing storage temperature of Malunggay stagnant water) at 1-3 hours of exposure. dissolved in water.

- No fragments of the PVA film are visible becomes murky due to the

original appearance of the water used.

Table 4.3 examines the solubility of the PVA film in two different solvents—distilled water and oil- contaminated water (such as canal or stagnant water)—over 4, 24, and 48 hours. In distilled water, the PVA film began to shrink within the first 1–3 hours. By 24 hours, the PVA film had completely dissolved, with no visible fragments of PVA film present. After 48 hours, the appearance remained the same. This suggests that distilled water is an effective solvent for dissolving PVA films.

According to the research (Mangaraj et al., 2018), the PVA film showed high solubility in distilled water due to the effect of the hydroxyl group in the water absorption mechanism. The water solubility and water absorption of the PVA film were directly proportional, which resulted in the normal behavior of the increase of water absorption of the PVA film, which in turn made the PVA film easier to dissolve in distilled water.

In contrast, when exposed to oils or stagnant water, the PVA film followed a similar initial disintegration timeline, beginning within 1–3 hours. By 24 hours, the films were completely dissolved, and no visible remnants of film were visible. At the 48-hour mark, the solution appeared cloudier due to the initial appearance of the water used. PVA film has poor resistance to water due to its molecular structure that includes hydrophilic hydroxyl groups; exposure to a wet environment causes them to swell or dissolve (Liu et al., 2022).

TABLE 5.1. Effectiveness of Herbal LarviPod at Different Concentration

TWO-WAY ANOVA - Effectiveness of Herbal LarviPod at Different Concentration

The table summarizes the results of a two- way ANOVA used to assess the efficiency of the Herbal LarviPod at various concentrations. For the concentration component, the sum of squares was

16.3 with three degrees of freedom, yielding a mean square of 5.44. The F-value for concentration was 0.358, and the associated p- value was 0.786. This implies that concentration had no statistically significant effect on the efficiency of the Herbal LarviPod. In contrast, the time factor has a sum of squares of 158.2 with 2 degrees of freedom, resulting in an average square of 79.08. The F-value for time was 5.205, with a p-value of 0.049, slightly below the 0.05 significance level.

This implies that the period of exposure had a statistically significant effect on the efficiency of the Herbal LarviPod, implying that time is a critical factor in its larvicidal action (Nawarathne, M. P., & Dharmarathne, C. 2024). The residuals, which indicate unexplained variability in the data, had a total of squares of 91.2, 6 degrees of freedom, and a mean square of 15.19. The results demonstrate that the concentration of the Herbal LarviPod did not significantly influence its effectiveness; the time of exposure was a significant factor. Based on the study of Araj et al. (2015), increased exposure time of larvae to larvicides enhances the larval mortality. It also implies the importance of considering exposure duration when evaluating the larvicidal efficacy of this herbal product.

TABLE 5.2.

Post Hoc Comparisons - Concentrations

Comparison

Concentrations	Concentrations	Mean Difference	SE	df	t	p_{tukey}
10%	30%	-0.333	4.56	8.00	-0.0731	1.000
	50%	-1.333	4.56	8.00	-0.2925	0.991
	70%	-3.000	4.56	8.00	-0.6581	0.910
30%	50%	-1.000	4.56	8.00	-0.2194	0.996
	70%	2.667			0.5850	0.834

Post Hoc Comparisons – Concentrations

Sum of Squares	df	Mn	F	p	Square	
Concentrations	16.3	3	5.44	0.358	0.78	6
Time	158.2	2	79.08	5.205	0.04	9
Residuals	91.2	6	15.19			

n=30

The post hoc comparisons for concentrations revealed no statistically significant differences in larval mortality between any of the tested Herbal LarviPod concentrations (10%, 30%, 50%, and 70%). All p-values were well above 0.05, indicating that none of the concentration pairs produced meaningful differences in mortality. Even the largest mean difference, observed between 10% and 70% (-3.000), was not significant (p = 0.910). These results clearly indicated that increasing the concentration of Herbal LarviPod did not lead to a significant increase in larvicidal effectiveness, suggesting that concentration alone was not a determining factor in larval mortality under the conditions of this study Piplani et al. (2019).

Difference between Larvicon and Herbal LarviPod

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Larvicon and Concentrations	40.4	4	10.1	0.657980456	0.637982	3.83785
Time	126.5333333	2	63.26666667	4.121606949	0.05884	4.45897
Error	122.8	8	15.35			

$\alpha = 0.05$

Table 6.

result implied that the duration of exposure may influence mortality rates to some extent. It's worth noting that had the p-value been marginally lower, it would have suggested that larval mortality increased with time, potentially due to the prolonged contact between the larvae and the active ingredients in the larvicides (Araj et al., 2015).

Despite the lack of statistical significance, the practical differences observed in raw data were informative. LarviCon consistently showed 100% mortality across all time intervals, while Herbal LarviPod's effectiveness increased with concentration and exposure time (Larvicon—Leads Environmental Health 2021). For example, the 70% Herbal LarviPod concentration resulted in notably higher mortality rates than lower concentrations, especially at the 48- hour mark (Opoggen et al., 2019). These patterns indicate that while differences were not yet statistically validated, they could be meaningful in real-world applications and might have become significant with larger sample sizes or repeated trials.

The implications of these findings were important for public health and mosquito control strategies. While

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Herbal LarviPod shows potential—particularly at higher concentrations and longer exposure durations—it did not match the efficacy of LarviCon under the conditions tested. This suggests that Herbal LarviPod could serve as a sustainable or natural larvicide alternative, but further development, optimization, and larger-scale testing were necessary. Programs considering Herbal LarviPod would benefit from refining its formulation and conducting more robust trials to support its effectiveness with statistical confidence.

Table 6.1.

The results of the two-way ANOVA examined the effects of treatment (LarviCon and various concentrations of Herbal Concentrations of LarviPod) and time (4, 24, and 48 hours) on larval mortality and showed that neither factor reached statistical significance at the conventional $\alpha = 0.05$ level. For the treatment variable comparing LarviCon and Herbal LarviPod concentrations, the F- value is 0.658 with a p-value of 0.638, which was far greater than the significance threshold. This indicates that there was no statistically significant difference in larvicidal effectiveness among the different treatments, including the commercially available LarviCon and the various concentrations of Herbal LarviPod.

Regarding the time factor, the F-value is 4.12 with a p-value of 0.0588, which was slightly above $\alpha = 0.05$, suggesting a borderline effect of time on larval mortality. Although not statistically significant, this

Tukey's HSD Post Hoc Test Comparing Treatment Means of Larvicidal Effectiveness

Pairwise Comparison	Mean Difference	HSD Value	Critical	Decision
Larvicon vs. 10%	4.33	8.79		Not Significant
Larvicon vs. 30%	4.00	8.79		Not Significant
Larvicon vs. 50%	3.00	8.79		Not Significant
Larvicon vs. 70%	1.33	8.79		Not Significant
10% vs. 30%	0.33	8.79		Not Significant
10% vs. 50%	1.33	8.79		Not Significant
10% vs. 70%	3.00	8.79		Not Significant
50% vs. 70%	1.67	8.79		Significant

for environmentally conscious mosquito control programs. Given the comparable effectiveness and the observed trend towards increased mortality.

Significance level at $\alpha = 0.05$

The results of Tukey's HSD post hoc test revealed that none of the pairwise comparisons between the commercially available larvicide Larvicon and the different concentrations of Herbal LarviPod showed statistically significant differences in larvicidal effectiveness. The mean differences between all treatment pairs were below the critical HSD value of 8.79, leading to the conclusion that the observed differences in larval mortality rates among these treatments are not statistically meaningful at the 0.05 significance level. This indicates that Larvicon and Herbal LarviPod, regardless of concentration, have comparable effects on mosquito larvae under the tested conditions.

Analyzing the data more closely, although no significant differences were found, there was a clear numerical trend where Larvicon consistently showed higher mean effectiveness compared to Herbal LarviPod concentrations. Among the Herbal LarviPod treatments, increasing concentration corresponds to higher mean mortality rates, with the 70% concentration being the most effective among the Herbal LarviPod groups. This pattern suggested a dose-response relationship, where increased concentration of the natural larvicide correlates with better larvicidal outcomes, even if these differences are not statistically significant in this sample (Opoggen

et al., 2019).

The lack of statistical significance might have been influenced by sample size or variability within the data. A small sample size or high variability could reduce the power of the test, limiting the ability to detect real differences that exist in the population. As stated by the World Health Organization (2025), large-scale trials verify the larvicide's effectiveness in the chosen dosage(s) of the application against the target vector when applied to expansive areas in natural breeding grounds. Therefore, while the current results do not confirm that any treatment is superior, they do highlight the promising effectiveness of Herbal LarviPod at higher concentrations, warranting further investigation with larger or more controlled studies.

The implications of this analysis suggested that Herbal LarviPod, a natural larvicide composed of varying concentrations of botanical extracts, could be a viable alternative to commercial larvicides like Larvicon, particularly with higher Herbal LarviPod concentrations, further research to optimize concentration and application methods could help enhance its practical use. Additionally, expanding sample size and replication will be critical to confirm these preliminary findings and establish Herbal LarviPod's role in integrated vector management strategies (Prates et al., 2024).

CONCLUSION

The study highlighted the potential combination of the extracts of Arrowroot Rhizome (Maranta arundinacea L.), Neem Leaves (Azadirachta indica A. Juss.), and Malunggay Leaves (Moringa oleifera Lam.)—encapsulated in a polyvinyl alcohol (PVA) film pod—as a potential larvicide against Aedes aegypti larvae, through the following concentrations of 10%, 30%, 50%, and 70% that showed significant differences in the mortality rate of the larvae. It sought to assess mortality across various concentrations and time intervals, compare efficacy against controls, evaluate cytotoxicity via brine shrimp assay, and examine product stability under diverse conditions.

The findings indicated a dose-dependent increase in larval mortality, with the 70% concentration producing the highest mortality rate (86.66%) at 48 hours. Although the positive control (Larvicon) achieved consistent 100% mortality in all trials and time intervals, statistical analyses (Two-way ANOVA and Tukey's HSD Post Hoc Test) revealed no significant differences ($p > 0.05$) between Larvicon and Herbal LarviPod concentrations, suggesting that higher concentrations approximate the efficacy of commercial larvicide.

The brine shrimp assay supported the high cytotoxicity of Herbal LarviPod even at lower concentrations, with near-complete lethality across all dose levels, indicating the extract's strong bioactivity. Regarding stability, the Herbal LarviPod showed favorable dissolution behavior, completing release by 48 hours, and maintained structural and chemical integrity under cold and room temperature conditions for up to 7 days. However, degradation occurred at elevated temperatures, reducing its potential efficacy under such storage conditions.

RECOMMENDATIONS

This study recommends Herbal LarviPod, a combination of arrowroot rhizome, neem leaves, and malunggay leaves extracts encapsulated in a PVA film, as an eco-friendly, sustainable, and cost-effective larvicide against Aedes aegypti. Further development is suggested for community use in dengue-prone areas, advocating collaboration with local health authorities and integration into existing vector control programs. Large-scale trials, formulation improvements (including slow-release mechanisms and olfactory component optimization), and comprehensive safety evaluations are needed for regulatory approval and broader adoption. Future research should investigate synergistic or antagonistic interactions between the plant extracts, optimize the pod's physical form for consistent release and reduced loss, and conduct stability testing under controlled conditions (humidity, temperature, pH) to determine shelf life accurately.

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