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Infertilization of dengue vector (*Aedes aegypti*) with Bromelin Solution and Extract From Ananas comosus

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Abstract

Dengue fever remains a significant public health problem in the tropics, with *Aedes aegypti* as the primary vector. Insecticide resistance among mosquitoes has been increasing, so an environmentally friendly alternative approach with biolarvicides is needed. This study aimed to evaluate the effects of bromelain and pineapple (*Ananas comosus*) extracts on the reproductive parameters of *Aedes aegypti*. Experiments were conducted in a controlled laboratory, where mosquitoes were exposed to various concentrations (0% to 10%) of bromelain and pineapple extracts. The number of egg production, hatching rate, and lifespan of adult mosquitoes were measured. Our results showed Bromelain, especially at higher concentrations, significantly reduced egg production, hatching rate, and longevity compared to the control and pineapple extract. At 6% and 8% bromelain concentrations, egg production and hatchability were very low, and mosquito lifespan was reduced. Pineapple extract also showed an inhibitory effect on egg production, although less consistently than bromelain. The sugar solution used as a control produced more eggs and live longer. In conclusion, bromelain from Ananas comosus showed promising insecticidal potential by reducing mosquito fertility and longevity, indicating its utility as an alternative vector control strategy for managing dengue outbreaks.

Introduction

Dengue fever continues to be a dominant public health threat in tropical and subtropical regions, with *Aedes aegypti* identified as the primary vector responsible for its transmission. As of recent estimates, approximately 50 million dengue infections occur annually, leading to significant morbidity and mortality worldwide(Yang *et al.*, 2021). Efforts to control the spread of dengue have traditionally focused on reducing mosquito populations, primarily through chemical insecticides(Achee *et al.*, 2015). However, with the rapid rise of insecticide resistance in mosquito populations (Konan *et al.*, 2021) alternative approaches to vector control are becoming increasingly critical. There is a pressing need for new, environmentally friendly, and sustainable solutions to tackle the ever-growing challenge of vector control in dengue-endemic regions.

Botanical insecticides have emerged as a promising area of research in recent years due to their biodegradable nature and low toxicity to non-target organisms(Damalas and Koutroubas, 2020; Duarte *et al.*, 2020; Riyaz *et al.*, 2022). Among the natural compounds with

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potential insecticidal properties, bromelain—an enzyme extracted from the pineapple (Ananas comosus)—has garnered attention for its ability to influence reproductive processes in various insects, acari and nemathoda(Domingues *et al.*, 2013; Castro *et al.*, 2023). Bromelain is known for its proteolytic activity, which disrupts cellular processes(Hikisz and Bernasinska-Slomczewska, 2021; Varilla *et al.*, 2021), and recent studies have shown its potential to interfere with the reproductive mechanisms of insects(Castro *et al.*, 2023). Pineapple extracts also contain serotonin(Istiqomah *et al.*, 2021), which can influence reproductive cycles by stimulating uterine contractions in mammals, hinting at potential applications for mosquito infertility. Previous research on the effects of bromelain on the reproductive system of insects, especially the *Aedes aegypti* mosquito, is limited. Most studies related to mosquito control have focused on the use of chemical or other biological agents, such as larvicides and plant-based insecticides(Tominik and Haiti, 2018). But the potential of proteolytic enzymes, such as bromelain, in affecting mosquito egg and larval development has not been widely studied. However, since bromelain is known to have the ability to affect uterine contractions in mammals, researchers hypothesized that this enzyme may affect the development of the reproductive system in insects, including mosquitoes(Almeida *et al.*, 2018).

The objective of this study was to explore the effects of pineapple extract and bromelain on the fertility of *Aedes aegypti* mosquitoes. This research aims to evaluate the egg production, hatch rate, and longevity of mosquitoes exposed to varying concentrations of pineapple extract and bromelain enzyme. By understanding how these natural compounds influence mosquito reproduction, we hope to offer an alternative, eco-friendly vector control strategy that could complement existing measures in combating dengue fever outbreaks.

Method

The research was conducted at the Entomology Laboratory of Poltekkes Kemenkes Banjarmasin, Banjarbaru, Indonesia, under controlled conditions simulating a natural mosquito habitat. Laboratory temperatures were maintained between 25°C and 30°C with a relative humidity of 70–80%. Banjarbaru, situated in South Kalimantan, is a region with high dengue incidence, making it a suitable location for studying mosquito behavior and exploring potential control methods. The study was ethically approved by the Health Ethics Commission of Muhammadiyah University of Banjarmasin under certificate number 100/UMB/KE/VII/2019. Mature pineapples (*Ananas comosus*) were procured from local farms in Banjarbaru for preparing the experimental extracts. The preparation process involved washing, peeling, and chopping the fruit, followed by blending with distilled water in a 1:2 ratio to produce a clear solution. Bromelain was extracted from the pineapple cores using a 24-hour maceration process, followed by centrifugation at 4000 RPM for 15 minutes. The resulting bromelain was purified using gel filtration chromatography. Both pineapple and bromelain extracts were diluted to seven concentrations: 0% (control), 1%, 2%, 4%, 6%, 8%, and 10%. A sugar solution was also prepared as a control to evaluate mosquito responses to a carbohydrate-based diet.

The *Aedes aegypti* larvae were collected from natural breeding sites around Banjarbaru, such as stagnant water in flower pots and discarded tires. Only third and fourth-instar larvae were selected for the study. These larvae were transferred to breeding tanks containing distilled water under conditions conducive to their growth, including temperatures of 25°C–27°C and a pH range of 6.5–7.5. They were fed ground liver powder, facilitating their development into pupae within 7–10 days. The pupae were transferred to mosquito cages (30 cm x 30 cm x 30 cm) to allow emergence into adults. Upon emergence, male and female mosquitoes were separated. Female mosquitoes were fed a blood meal from a marmot to support egg production, while males were sustained with a sugar solution. This step was crucial for ensuring adequate protein for oogenesis in females. Experimental treatments involved exposing groups of 10 paired adult mosquitoes to pineapple extract, bromelain extract, or sugar solution for three consecutive days, following a 12-hour mating period. Each treatment was replicated three times. Mosquitoes were given

oviposition opportunities, and the eggs laid were counted daily. Observations included egg morphology and viability, and hatch rates were calculated by counting larval emergence after five days.

Longevity was assessed by monitoring the survival of mosquitoes daily until death. Any abnormalities in mosquito behavior were recorded, alongside environmental data such as temperature and humidity levels in the cages. The data were analyzed using a two-factor factorial design to evaluate the effects of treatment type and concentration on egg production, hatch rates, and mosquito longevity. When assumptions of normality and homogeneity were unmet, non-parametric tests like the Friedman test were applied. Post-hoc analyses were conducted using Wilcoxon Signed Rank tests where applicable. Statistical analyses were performed using IBM SPSS software version 26 (George and Mallery, 2019), with a significance level set at $p < 0.05$.

Results and Discussions

The findings of this study are presented in the tables and figures below, showing the effects of pineapple extract, bromelain, and sugar solution on the reproductive parameters of *Aedes aegypti*. The results of this study are summarized in the following tables and figures, showing the effects of pineapple extract, bromelain, and sugar solution on the reproductive and longevity parameters of *Aedes ae*gypti mosquitoes.

This table shows that *Aedes aegypti* exposed to higher concentrations of bromelain (6% and 8%) produced significantly fewer eggs compared to the control and other treatments. Pineapple extract also showed a reduction in egg production, but the effect was more variable (Table 1).

Bromelain demonstrated a marked reduction in the hatchability of eggs, particularly at higher concentrations, while the sugar solution maintained relatively high hatch rates, indicating its neutral effect on egg viability (Table 2).

This table shows that bromelain significantly shortened the life span of mosquitoes, particularly at lower concentrations (1%-4%). In contrast, mosquitoes exposed to sugar solution lived the longest, indicating the neutral effect of the sugar solution on longevity (Table 3). This study revealed significant differences in the reproductive performance of *Aedes aegypti* when exposed

TABLE 1. Total Egg Production by *Aedes aegypti* at Different Concentrations

Concentration (%)	Pineapple Extract	Bromelain Extract	Sugar Solution
0%	994	994	994
1%	702	1003	1031
2%	514	1198	1235
4%	643	1652	1038
6%	475	2001	1203
8%	706	1602	552
10%	636	1351	1017

TABLE 2. Hatch Rate of Eggs Produced by *Aedes aegypti* at Different Concentrations

Concentration (%)	Pineapple Extract (%)	Bromelain Extract (%)	Sugar Solution (%)
0%	89	189	189
1%	114	119	61
2%	265	114	29
4%	93	36	20
6%	84	16	32
8%	62	77	53
10%	197	34	26

TABLE 3. Longevity of Adult *Aedes aegypti* Exposed to Different Concentrations

Concentration (%)	Pineapple Extract (days)	Bromelain Extract (days)	Sugar Solution (days)
0%	13	13	13
1%	9	19	34
2%	9	20	34
4%	13	24	33
6%	11	45	42
8%	13	45	45
10%	13	38	47

to varying concentrations of pineapple extract and bromelain enzyme. The results show that bromelain at higher concentrations (6% and 8%) had the most substantial inhibitory effect on egg production, with the total number of eggs laid by female mosquitoes significantly reduced compared to those in the control and sugar solution groups. It is consistent with previous studies that have demonstrated the ability of proteolytic enzymes like bromelain to disrupt cellular processes critical to reproduction(Page *et al.*, 2014; Fani and Sri, 2019; Chakraborty *et al.*, 2021), such as oogenesis and egg maturation. The decline in egg production with increasing bromelain concentration suggests that this enzyme interferes with the hormonal regulation of reproduction in *Aedes aegypti*.

Moreover, the pineapple extract, while less potent than bromelain, also showed some reduction in egg production, particularly at higher concentrations. It can be attributed to the presence of other bioactive compounds in pineapple, such as serotonin, which is known to stimulate uterine contractions and could potentially induce premature egg expulsion in mosquitoes(Norville, Sweeney and Elliott, 2010). Although the mechanism of action remains unclear, it is hypothesized that the compounds in pineapple extract may interact with the mosquito's neuroendocrine system, leading to disrupted reproductive cycles. The sugar solution, used as a control, did not significantly reduce egg production. Mosquitoes fed on sugar solution produced more eggs at lower concentrations, likely due to the increased availability of carbohydrates, which are essential for energy during egg development(Barredo and DeGennaro, 2020; Maestas, Lee and Choi, 2023).

This figure displays a plot of the average number of *Aedes aegypti* eggs produced when exposed to three different types of solutions: pineapple juice (Ananas comosus), bromelain extract from pineapple, and sugar solution. The plot shows variations in the number of eggs produced at different concentrations of each solution (0%, 1%, 2%, 4%, 6%, 8%, and 10%). The data indicate that the bromelain extract produced the highest number of eggs, while the sugar solution resulted in the lowest egg count (Figure 1A). Figure 1B illustrates the average percentage of *Aedes aegypti* eggs that successfully hatched when subjected to the same three solutions (pineapple juice, bromelain extract, and sugar solution) at various concentrations. The plot shows that the hatching rate was highest in the pineapple juice group, with a lower percentage in the bromelain extract and sugar solution groups. Figure 1C presents a plot of the average lifespan of *Aedes aegypti* mosquitoes that were exposed to different solutions and concentrations. The results indicate explicit differences in the longevity of mosquitoes depending on the solution consumed. The sugar solution resulted in the expanded lifespan, followed by the bromelain extract, while the pineapple juice group had the shortest average lifespan.

The ANOVA results show a significant effect of both treatment and concentration on the total number of eggs produced by *Aedes aegypti* mosquitoes ($p < 0.001$). The interaction between treatment and concentration is also significant, indicating that the effect of the treatments depends on the concentration used. Similarly, both treatment and concentration significantly affect the hatch rate of mosquito eggs ($p < 0.001$), with a notable interaction effect. It suggests that higher concentrations of bromelain or pineapple extract have a

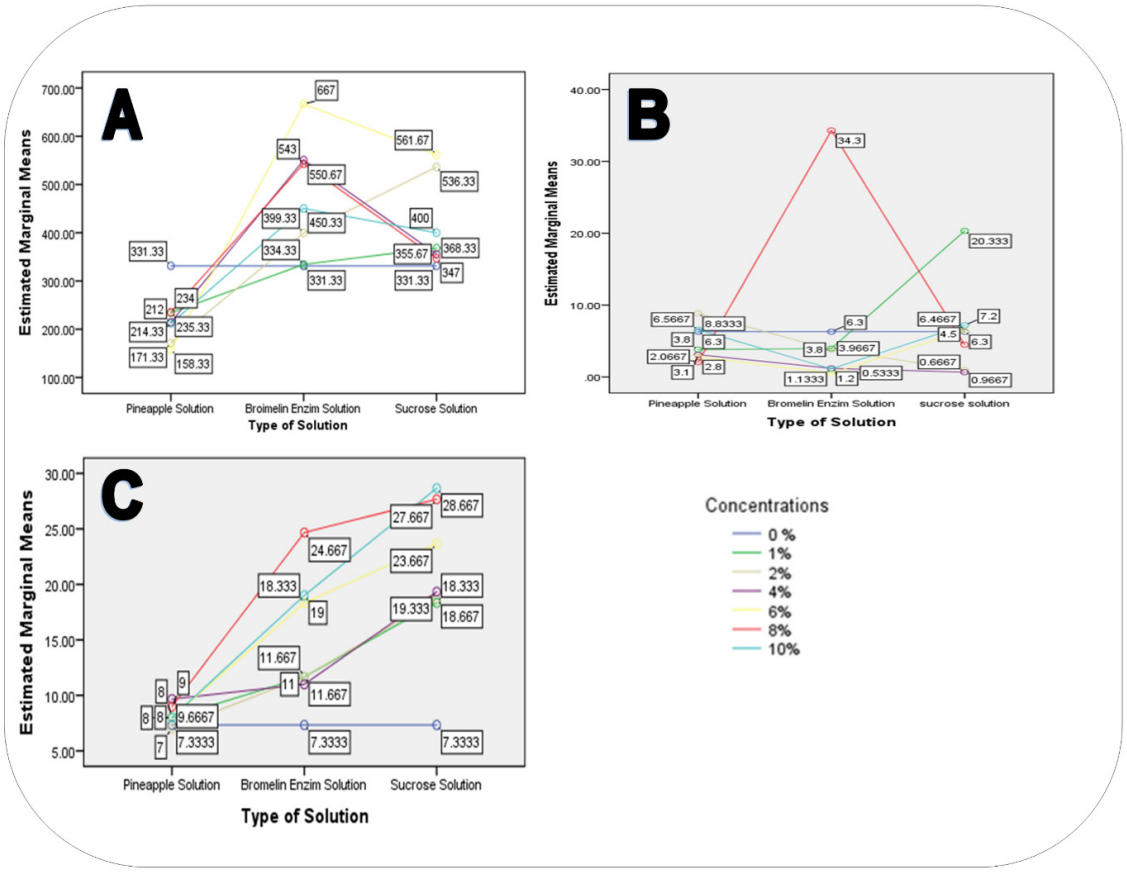

FIGURE 1. Concentration of 3 Types of Solution (Pineapple, Bromeline enzyme and Sucrose against *Aedes aegypti*. A. Average Number of Eggs; B. Average Number of Eggs Hatched; C. Average Length of Life of Hatched Eggs

TABLE 4. Two-Way ANOVA for the Effects of Pineapple Extract, Bromelain, and Sugar Solution on Egg Production, Hatch Rate, and Longevity of *Aedes aegypti*

Parameters	Sources of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)		
Egg Production	Treatment	12480.45	2	6240.23	35.67	< 0.001
		3125.67	6	520.95	12.89	< 0.001
	Treatment x	2789.34	12	232.45	8.56	< 0.001
	Error	1745.23	63	27.70		
Hatch Rate	Treatment	9823.12	2	4911.56	42.45	< 0.001
		2190.58	6	365.10	9.34	< 0.001
	Treatment x	1887.40	12	157.28	6.78	< 0.001
	Error	1460.80	63	23.18		
Longevity	Treatment	5432.90	2	2716.45	31.21	< 0.001
		1842.67	6	307.11	7.43	< 0.001
	Treatment x	1523.44	12	126.95	4.65	< 0.001
	Error	2040.22	63	32.39		

more pronounced impact on egg viability. The longevity of mosquitoes is significantly affected by both treatment and concentration ($p < 0.001$), and the interaction effect between treatment and concentration is also statistically significant. It means that the lifespan of the mosquitoes was reduced differently depending on the concentration of the treatment they were exposed to.

Hatch rate is a critical measure of reproductive success, as it reflects the viability of the eggs laid(Assersohn *et al.*, 2021). In this study, the percentage of eggs that successfully hatched varied significantly across the treatment groups. Bromelain extract showed a pronounced negative effect on egg viability, with hatch rates dropping as low as 16% at the highest concentrations. It supports the hypothesis that bromelain not only affects egg production but also compromises the structural integrity of the eggs, potentially through its proteolytic activity(Varilla *et al.*, 2021; Ávalos-Flores *et al.*, 2022). Bromelain may break down essential proteins in the eggshell, rendering the eggs non-viable(Tsopmo, Tsige and Udenigwe, 2019; Lavanya *et al.*, 2020). In *contrast*, eggs laid by mosquitoes exposed to pineapple extract exhibited more variability in hatch rates. While some concentrations showed reduced hatchability, others, particularly the 10% concentration, saw a hatch rate comparable to the control. It suggests that while pineapple extract may influence egg production, its effect on egg viability is less consistent than that of bromelain. The sugar solution, as expected, maintained relatively high hatch rates across all concentrations, further highlighting the inhibitory effects of bromelain and pineapple extract.

Longevity is an essential factor in determining the success of vector control strategies, as longer-lived mosquitoes are more likely to spread disease(Shaw and Catteruccia, 2019). In this study, mosquitoes exposed to bromelain had the shortest life span, particularly at higher concentrations. It could be attributed to the enzyme's systemic effects, which may weaken the mosquitoes' overall health and immune response(Vicente-Crespo, 2021). Pineapple extract, on the other hand, had a less pronounced effect on longevity, with mosquitoes living slightly longer than those exposed to bromelain. It suggests that while both treatments affect reproductive parameters, bromelain is more effective at reducing mosquito life span. Interestingly, mosquitoes fed on sugar solution had the longest life span, particularly at higher concentrations. It aligns with previous research that has shown the importance of sugar feeding in maintaining mosquito longevity. In natural settings, sugar serves as a critical energy source for mosquitoes, particularly males. They do not feed on blood(Airs, Kudrna and Bartholomay, 2019). The results of this study highlight the importance of sugar availability in sustaining mosquito populations and suggest that limiting sugar sources in the environment could further enhance vector control efforts.

One of the limitations of this study is the laboratory setting, which may not fully reflect real-world environmental conditions where *Aedes aegypti* mosquitoes thrive. The controlled environment may have limited the understanding of how factors such as fluctuating temperatures, humidity, and the presence of other ecological interactions influence the efficacy of bromelain and pineapple extract. Additionally, the study only explored short-term effects on egg production, hatch rates, and longevity, leaving the long-term implications of using bromelain as a vector control method unexplored. Future research should focus on field trials to assess the practical application of these findings in diverse ecological conditions and over extended periods. Despite these limitations, the study offers promising prospects for using bromelain as an eco-friendly alternative to chemical insecticides, contributing to sustainable dengue vector control strategies. Bromelain's ability to reduce mosquito fertility and lifespan positions it as a potential biolarvicide, which could be integrated into existing vector control programs to combat insecticide resistance and minimize environmental impact.

Conclusion

This study demonstrates the potential of *Ananas comosus* extract and bromelain enzyme as biological agents for controlling *Aedes aegypti* populations. The significant reduction

in egg production, hatch rate, and mosquito longevity observed in the bromelain treatment group indicates that this natural compound could be an effective alternative to chemical insecticides in vector control programs. Future studies should focus on field trials to assess the practical application of these findings and explore the long-term effects of using botanical extracts for mosquito population management.

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