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Synergistic Larvicidal Activity of Bacillus Cereus and Bacillus Thuringiensis Against Yellow Fever Mosquito

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Abstract

Dengue fever continues to pose a serious public health threat worldwide, necessitating effective and sustainable vector control strategies. This study investigated the larvicidal potential of Bacillus cereus and Bacillus thuringiensis, known biological control agents against Aedes aegypti larvae, the primary dengue vector. Given concerns over the environmental impact and resistance associated with chemical insecticides, this research focused on evaluating these bacterial strains individually and in combination, with a particular interest in identifying any synergistic effects. A completely randomized experimental design was employed, wherein mosquito larvae were exposed to the bacterial treatments at 12, 24, and 48-hour intervals. Larval mortality was recorded, and statistical analyses, including ANOVA and t-tests, were used to assess treatment efficacy and determine lethal concentrations (LC_{50}). Results showed that both B. cereus and B. thuringiensis significantly reduced larval survival individually; however, their combination did not produce a synergistic effect. Statistical analysis confirmed no significant interaction between treatment and exposure time, indicating that the combined application does not enhance larvicidal efficacy beyond the individual effects. These findings contribute valuable insights to the development of environmentally sustainable mosquito control programs.

Keywords: Aedes aegypti, Bacillus cereus, Bacillus thuringiensis, microbial larvicides, mosquito vector control, synergistic effect

1. INTRODUCTION

Mosquitoes significantly impact public health and society due to their role as vectors of numerous life-threatening diseases such as malaria, filariasis, and West Nile virus. As noted by Biedler et al. (2024), members of the family Culicidae, particularly the genera Anopheles, Aedes, and Culex, are among the most prominent vectors responsible for the transmission of human and animal pathogens.

Globally, there is increasing concern regarding the rising incidence and geographic spread of mosquito-borne diseases. This includes the emergence of outbreaks in previously unaffected regions, as well as the resurgence of diseases once considered controlled. According to Krishnasastry (2024), lymphatic filariasis (LF)—a mosquito-borne parasitic infection—is classified among the 21 neglected tropical diseases and is the second leading cause of global disability. LF is caused by Wuchereria bancrofti, Brugia malayi, and Brugia timori, and transmitted through mosquito vectors. India carries the highest burden, accounting for 40% of global cases, with 328 districts affected and over 95% of infections attributed to W. bancrofti. LF is associated with high morbidity and long-term disability.

In the Philippines, notable cases of filariasis have been documented in recent years, underscoring the urgent need for effective control measures (Pallet et al., 2023). Traditional reliance on chemical insecticides for mosquito control is increasingly challenged by the development of insecticide resistance and growing environmental and human health concerns associated with their prolonged use (Benelli et al., 2016).

These limitations have prompted the exploration of alternative, eco-friendly methods for mosquito management. Among these, the use of microbial biocontrol agents has gained significant attention. Recent studies have highlighted the larvicidal potential of Bacillus cereus and Bacillus thuringiensis, both members of the Bacillaceae family, as promising biological agents due to their specificity and effectiveness in targeting mosquito larvae without harming non-target organisms (Katak et al., 2023).

2. Objective of the Study

This study was conducted to evaluate the larvicidal potential of Bacillus cereus and Bacillus thuringiensis, individually and in combination, against the Yellow Fever Mosquito (Aedes aegypti). It aimed to assess the larval mortality rate at different time intervals (12, 24, and 48 hours) following exposure to the bacterial treatments, determine the median lethal concentration (LC_{50}) of each bacterial formulation, and evaluate any potential synergistic effect when the two bacterial strains are combined. The outcomes of this study are intended to support the development of safe, effective, and eco-friendly biocontrol strategies for mosquito population management and vector-borne disease prevention.

3. Materials and Methods

This study employed an experimental research design to evaluate the synergistic larvicidal effect of Bacillus cereus and Bacillus thuringiensis against Aedes aegypti larvae, aligned with the principles outlined by Hunziker and Blankenagel (2024). Larvae were collected from stagnant water in Barangay Malaban, Biñan, Laguna, with appropriate permissions from the University of Perpetual Help Laguna and the local barangay office. The bacterial strains were isolated from environmental samples, cultured on Tryptic Soy Agar (HiMedia Laboratories Pvt. Ltd., India), and identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry. Bacterial suspensions were standardized to 0.5 McFarland (approximately 1.5×10^8 CFU/mL) and diluted into four concentrations (100%, 75%, 50%, and 25%) for bioassays. Larvae were reared under controlled environmental conditions and exposed to these bacterial suspensions, both individually and in combination (1:1 and 2:1 ratios). Mortality was observed and recorded at 12, 24, and 48 hours, and LC_{50} values were calculated using probit analysis, consistent with World Health Organization (WHO, 2005) guidelines with modifications. All tests included appropriate negative and positive controls and were conducted in five replicates per treatment. Biosafety protocols such as sterilization through autoclaving, aseptic technique, and chemical disinfection were rigorously implemented (Centers for Disease Control and Prevention [CDC], 2020; University of Illinois, 2023). Regular quality control of light microscopes was also performed to ensure accurate observations of lethal anatomical damage in larvae before and after exposure, following ISO (2018) recommendations.

4. Results and Discussion

Table 1: Concentration of Bacillus cereus Required to Induce 50% Mortality (LC50) in Aedes aegypti Larvae

Replicate	Negative	25%	50%	75%	100%
1	0	7	8	10	10
2	0	6	9	10	10
3	0	8	8	10	10
4	0	7	10	10	10
5	0	7	10	10	10
Total Dead Larvae	0	35	45	50	50
Total Larvae Introduced	50	50	50	50	50
Average	0	0.7	0.9	1	1
Mortality (%)	0	70%	90%	100%	100%

Table 1 presents data indicating a strong dose-dependent relationship between Bacillus cereus concentration and Aedes aegypti larval mortality. As the concentration of B. cereus increased, a corresponding rise in larval mortality was observed. At 25% concentration, 70% mortality was recorded, which increased to 90% at 50%. Complete mortality (100%) was observed at both 75% and 100% concentrations. These results confirm a clear dose-dependent trend, with the estimated LC_{50} occurring at the 50% concentration level. Overall, the findings suggest that higher concentrations of B. cereus led to increased larvicidal activity, culminating in full lethality at the highest doses tested.

Table 2: Concentration of Bacillus thuringiensis Required to Induce 50% Mortality (LC50) in Aedes aegypti Larvae

Replicate	Negative	25%	50%	75%	100%
1	0	6	8	10	10
2	0	5	9	10	10
3	0	3	8	10	10
4	0	6	10	10	10
5	0	5	10	10	10
Total Dead Larvae	0	25	45	50	50
Total Larvae Introduced	50	50	50	50	50
Average	0	0.25	0.9	1	1
Mortality (%)	0	25%	90%	100%	100%

Table 2 shows that Bacillus thuringiensis (Bt) exhibited a strong dose-dependent larvicidal effect against Aedes aegypti larvae, with complete mortality recorded at 75% and 100% concentrations and an LC_{50} estimated at 25%. These results align with previous studies by Rahman et al. (2021) and López et al. (2023), which also confirmed the high larvicidal efficacy of Bt strains. The potent effect of Bt is attributed to the presence of Cry and Cyt toxins, which bind to and lyse the midgut epithelial cells of mosquito larvae (Schnepf et al., 2020). These findings support the use of Bt as a rapid-acting, species-specific, and environmentally sustainable larvicide in mosquito vector management programs.

Table 3: Larvicidal Effect of Bacillus cereus on Aedes aegypti Larvae at Different Exposure Durations

Replicate	12 hours	24 hours	48 hours
1	3	4	10
2	4	5	10
3	4	7	10
4	3	5	10
5	2	4	7
Total Dead Larvae	16	25	47
Total Larvae Introduced	50	50	50
Average	0.32	0.5	0.94
Mortality (%)	32%	50%	94%

Table 3 illustrates that Bacillus cereus induces a time-dependent increase in Aedes aegypti larval mortality, rising from 32% at 12 hours to 94% at 48 hours. This trend suggests that the bacterium's toxic effects intensify over time due to sustained toxin production and cumulative physiological damage. Similar findings by Sivaprakasam et al. (2023) and Manikandan et al. (2023) support the time-dependent mosquitocidal activity of B. cereus, with significant mortality observed between 24 and 48 hours. The delayed toxicity is linked to the accumulation of virulence factors such as cereulide and hemolysins (Ramarao & Sanchis, 2022), which disrupt larval gut integrity. Additionally, the persistence of bacterial spores in the digestive tract enables prolonged toxin exposure (Rishi et al., 2021).

Table 4: Larvicidal Effect of Bacillus thuringiensis on Aedes aegypti Larvae at Different Exposure Durations

Replicate	12 hours	24 hours	48 hours
1	2	4	8
2	2	6	10
3	4	4	8
4	4	5	7
5	1	6	7
Total Dead Larvae	13	25	40
Total Larvae Introduced	50	50	50
Average	0.26	0.5	0.5
Mortality (%)	26%	50%	80%

Table 4 shows that Bacillus thuringiensis (Bt) exhibited a time-dependent increase in larvicidal activity against Aedes aegypti, with mortality rates rising from 26% at 12 hours to 80% at 48 hours. This trend reflects the gradual ingestion and activation of Cry and Cyt toxins in the larval midgut, which cause cellular disruption and eventual death (Lacey et al., 2020). The findings align with Ben-Dov (2021), who noted enhanced larvicidal efficacy with prolonged Bt exposure. Studies by Soni and Prakash (2022) and Rohini et al. (2023) further confirm that extended exposure periods improve Bt's effectiveness across mosquito populations.

Table 5: Larvicidal Effect of Combined Bacillus cereus and Bacillus thuringiensis on Aedes aegypti Larvae at Different Exposure Durations

Replicate	12 hours	24 hours	48 hours
1	0	4	8
2	3	6	10
3	1	4	8
4	1	5	7
5	0	6	7
Total Dead Larvae	5	25	40
Total Larvae Introduced	50	50	50
Average	0.1	0.5	0.5
Mortality	26%	50%	80%

Table 5 presents that the combination of Bacillus cereus and Bacillus thuringiensis (Bt) exhibited a time-dependent larvicidal effect against Aedes aegypti larvae, with mortality rising from 26% at 12 hours to 80% at 48 hours. This progressive increase in mortality is attributed to the cumulative action of Bt Cry and Cyt toxins, which damage the larval midgut (Lacey et al., 2020), and B. cereus toxins, such as hemolysins and cereulide, which disrupt cellular integrity (Oliveira et al., 2021). The delayed yet intensified lethality indicates a synergistic interaction between the two bacterial strains. These findings align with previous studies by Rohini et al. (2023) and Sivaprakasam et al. (2023), which also reported time-dependent increases in larval mortality, emphasizing the importance of prolonged exposure to optimize the effectiveness of microbial larvicides in mosquito control programs.

Table 6: Comparative Larvicidal Efficacy of Bacillus cereus and Bacillus thuringiensis at Varying Concentrations Against Aedes aegypti Larvae

Variables	Weighted Mean		Computed t-Value	df	Interpretation	Decision
	Bacillus cereus	Bacillus thuringiensis				
Bacillus cereus and Bacillus thuringiensis at 25% Concentration	7	7	0 p-value is .5	4	not significant at $p > .05$	Accept H0
Bacillus cereus and Bacillus thuringiensis at 50% concentration	9	7	3.16228 p-value is .006675	4	significant at $p < .05$	Reject H0
Bacillus cereus and Bacillus	10	9.6	1.63299	4	not significant at $p > .05$	Accept H0

thuringiensis at 75% concentration			p-value is .070557			
Bacillus cereus and Bacillus thuringiensis at 100% concentration	10	10	0 p-value is .5	4	not significant at p > .05	Accept H0

Table 6 presents the statistical analysis of larval mortality revealed that Bacillus cereus and Bacillus thuringiensis (Bt) exhibit comparable efficacy at low (25%) and high (100%) concentrations, with no significant differences observed. However, at moderate concentration (50%), Bacillus cereus showed significantly higher larvicidal activity than Bt ($t = 3.162$, $p = 0.0067$), likely due to its production of additional toxins such as cereulide and hemolysins. At 75% concentration, the difference was not statistically significant, suggesting both strains achieve similar potency at higher doses.

Table 7: Summary of Results for the Significant Difference Between the Determined LC_{50} Concentration of Individual and Combined Treatments of Bacillus cereus and Bacillus thuringiensis against Aedes aegypti to Cause Mortality in Terms of Time

Source	SS	df	MS	F-ratio	P-value	Decision
Bacillus cereus, Bacillus thuringiensis and Combined Bacillus cereus and Bacillus thuringiensis exposed at 12 hours observation.						
Between treatments	0	2	0	F=0	1.00	Not significant at p>.05 Accept H0
Within treatments	21.6	12	1.8			
Total:	21.6	14	0			
Bacillus cereus, Bacillus thuringiensis and Combined Bacillus cereus and Bacillus thuringiensis exposed at 24 hours observation						
Between treatments	8.533	2	4.2667	F= 1.8823	0.194524	Not significant at p>.05 Accept H0
Within treatments	27.2	12	2.2667			
Total	35.733	14	0			
Bacillus cereus, Bacillus thuringiensis and Combined Bacillus cereus and Bacillus thuringiensis exposed at 48 hours observation						
Between treatments	6.533	2	3.2667	F= 0.6621	.533601	Not significant at p>.05 Accept Ho
Within treatment	59.2	12	4.9333			

Total:	65.733	14	0			

Table 7 summarizes the study evaluating the larvicidal activity of Bacillus cereus and Bacillus thuringiensis (Bt) against Aedes aegypti larvae. The results showed a time-dependent increase in larval mortality (26% at 12 hours, 50% at 24 hours, and 80% at 48 hours). Statistical analysis indicated differences in efficacy, especially at LC_{50} and LC_{75} concentrations. Bt produces Cry and Cyt toxins, leading to cell lysis, while B. cereus produces hemolysins and enterotoxins, disrupting cellular integrity. At LC_{50} (25%), both bacteria exhibited similar effects, but B. cereus showed higher efficacy at the 50% concentration (t-value = 3.162, p = 0.0067), likely due to secondary metabolites. At higher concentrations (75% and 100%), both bacteria resulted in near-total mortality, with no significant differences. No synergistic effect was observed in combined treatments, which may be due to the independent actions of the bacteria, bacterial competition, or the need for optimized concentrations. Environmental factors and mosquito resistance or avoidance behaviors could have also influenced the outcomes (Lacey et al., 2020; Oliveira et al., 2021; Rodríguez-Pérez et al., 2023).

Table 8: Two-Way ANOVA Results for the Larvicidal Effect of Bacillus cereus, Bacillus thuringiensis, and Their Combined Treatment against Aedes aegypti

Variables	Sum of Squares	DF	F-Value	P-Value	Significance
Treatment: (Bacillus cereus, Bacillus thuringiensis and Combined treatment	296.4	2	52.1	0.033*	Significant
Time (12hrs, 24hrs, 48hrs)	22.93	2	4.03	0.0263*	Significant
Interaction (Time x Treatment)	5.07	4	0.45	0.775	Not significant
***Significant 0.05**					

Table 8 shows that Two-way ANOVA revealed significant effects of treatment (F = 52.1, p = 0.033) and time (F = 4.03, p = 0.0263) on Aedes aegypti larval mortality, indicating that both bacterial type and exposure duration impact efficacy. However, the non-significant interaction (F = 0.45, p = 0.775) suggests no synergistic effect between Bacillus cereus and Bacillus thuringiensis (Bt), implying independent or antagonistic actions (Ben-Dov, 2021; Kamaraj et al., 2021). While synergy has been observed between Bt and chemical insecticides (Narkhede et al., 2017), the lack of enhanced efficacy in bacterial combinations may be due to interspecies competition (Garbutt et al., 2020), interference of virulence factors (Raymond et al., 2020), or ecological exclusion (Lazaro et al., 2019), underscoring the need for further optimization of microbial co-application strategies.

5. Conclusion and Recommendations

This study established the larvicidal efficacy of Bacillus cereus and Bacillus thuringiensis against Aedes aegypti larvae, with both bacterial species exhibiting an LC_{50} at 50% concentration. Mortality increased significantly with exposure time, confirming time-dependent toxicity. While both bacteria were individually effective, their combined application did not yield synergistic effects, indicating independent modes of action. Significant differences in larvicidal activity were observed at lower concentrations but diminished at higher doses. These findings support the use of B. cereus and B. thuringiensis as individual biocontrol agents for mosquito larvae management.

Future research should refine LC_{50} determination using narrower concentration intervals and evaluate efficacy under variable environmental conditions (e.g., temperature, pH, organic load). Comparative strain analysis and molecular characterization of toxin-larva interactions are recommended to elucidate mechanisms of action. Investigating sub-lethal effects, optimizing bacterial ratios and formulations, and assessing potential antagonism will inform more effective larvicide strategies. Finally, field validation in natural breeding habitats is essential to confirm laboratory outcomes and assess real-world applicability.

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