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Original Article

LABORATORY EVALUATION OF LEMONGRASS OIL-BASED SILVER NANOPARTICLES COMBINED WITH BORIC ACID TOXIC BAIT AGAINST *AEDES AEGYPTI*

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ABSTRACT

Background: Recently, the eco-friendly plant-based synthesis of Silver Nanoparticles (AgNPs) has gained prominence as a promising approach for mosquito control. Combining Lgeo-AgNPs with Boric Acid Toxic Bait (BATB) represents a novel approach exploiting adult mosquitoes' feeding behaviour and utilizing boric acid as a potent toxin.

Material & Methods: Lemongrass essential oil-based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) were evaluated

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against *Aedes aegypti* mosquitoes. *Ae. aegypti* mosquitoes, with 15 females per 200mL paper cup, were subjected to various combinations of Lgeo-AgNPs and BATBs in bioassay treatments. Bioassays were carried out at 24.6°C ± 1°C, 60% ± 5% relative humidity, and a 14L:10D photoperiod. Mosquito mortality /grounded / knockdown was recorded every 3, 6, 12, and 24 hours. Each experiment was quadruplicated and repeated thrice.

Results: A multiple comparisons test, such as the Least Significant Difference (LSD), was conducted to assess mean differences among various exposure durations (e.g., 3 hrs, 6 hrs, 12 hrs, and 24 hrs) for the "mortality /grounded / knockdown count" variable. The results revealed significant mean variations across all exposure time intervals ($p < 0.001$), indicating a substantial impact of exposure duration on mosquito mortality rates. The combination of 0.25% Lgeo-AgNPs with 1% BATB demonstrated significant efficacy, resulting in rapid knockdown and mortality of *Ae. aegypti* mosquitoes within a short exposure time.

Conclusion: A combination of 0.25% Lgeo-AgNPs with 1% BATB was effective against *Ae. aegypti* adults. Further optimization and long-term evaluation of this formulation are recommended to establish sustainable adulticidal mosquito control strategies for mitigating the transmission of vector-borne diseases.

Keywords: *Aedes aegypti*, Boric acid Toxic Bait, Lemongrass essential oil, Nanoparticles

INTRODUCTION

With its worldwide distribution, *Aedes aegypti* (L.) (Diptera: Culicidae) is crucial in transmitting arboviral diseases such as dengue, chikungunya, yellow fever, and Zika. The success of this species can be attributed to its behavioural plasticity, rapid development, desiccation-resistant eggs, resistance to typical insecticides, preference for urban environments, proximity to humans, and a tendency to bite humans during daylight hours while seeking resting places in moist vegetation and indoors[1]. Ensuring mosquito populations remain below the tolerable threshold necessary to prevent or control arboviral transmission is a critical challenge in combating arboviral disease prevention. A growing number of studies aimed at controlling Aedes species by mass-trapping using diverse approaches to lure, capture and eliminate, or to use mosquitoes to disseminate control agents to decrease their population[2]. The World Health Organization has launched the Global Arbovirus Initiative, recognizing the increasing global threat posed by arboviruses transmitted by Aedes mosquitoes. This initiative aims to monitor, prevent, and respond to the growing risk of arbovirus disease by fostering collaboration among key partners, strengthening capacity-building, conducting research, and enhancing preparedness and response strategies[3].

Control strategies for managing *Aedes* mosquitoes and preventing arboviral transmission include source reduction, larviciding, and adulticiding measures[4]. Developing effective adulticidal strategies becomes imperative in addressing the challenges of mosquito-borne diseases. However, adulticidal chemical control strategies have diminished effectiveness due to growing obstacles, such as insecticide resistance and the widespread availability of potential developmental sites in urban areas. These factors have contributed to increased vector dispersal and the global spread of disease epidemics[1]. Moreover, despite various existing vector control tools, they have yet to achieve complete success. Consequently, this lack of effectiveness has resulted in multiple issues, such as insecticide resistance, resurging mosquito populations, adverse effects on humans and non-target organisms, and disruption of natural ecosystems. Alternative approaches are necessary to overcome these challenges and improve the efficacy of mosquito control measures[5].

The initial studies by Xue and Barnard[6] on boric acid as an active ingredient of the Attractive Toxic Sugar Baits (ATSB) provided valuable insights into their effectiveness for attracting and controlling *Ae. albopictus* Skuse populations. This has contributed to our understanding of boric acid toxic bait (BATB) that may have potential as a mosquito control agent. The ATSB research has led to active and exciting areas of research on targeted sugar baits for mosquito control[7-8]. Here, we attempted to evaluate Lemongrass essential oil-based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) as an effective toxic substance against *Ae. aegypti*. This innovative approach, specifically targeting the elimination of adult mosquito populations, has the potential to become a successful tool in effectively controlling mosquito-borne diseases and addressing the public health concerns associated with such diseases.

MATERIAL AND METHODS

***Mosquitoes*:** Three- to five-day-old adult *Ae. aegypti mosquitoes (Orlando strain) were used in* the experiment. The mosquitoes were reared at AMCD (Anastasia Mosquito Control District) insectary under controlled conditions at a temperature of 26.6°C ± 1°C, 70% ± 10% relative humidity, and a 14L:10D photoperiod.

Chemicals: Lemongrass essential oil was obtained from Majestic Pure Cosmeceuticals (San Diego, CA, USA). Silver nitrate was obtained from Sigma Aldrich (St. Louis, MO, USA). Tween 20 was used to emulsify lemongrass essential oil into the silver nitrate solution and was obtained from Sigma Aldrich (St. Louis, MO, USA).

Treatment solution: Lemongrass essential oil-based Silver nanoparticles (Lgeo-AgNPs) were synthesized via lemongrass essential oil (% v/v) emulsified into an equal volume of *Tween 20* and added dropwise to a 0.31 mMol $AgNO_3$ solution. The reactant mixture was heated under constant agitation at 100°C for one hour. Nanoparticle formation was confirmed by a visual change of colour followed by subsequent characterization. The synthesised Lgeo-AgNPs solution was mixed with boric acid (BA) as bait in different concentrations, as specified in Table 1.

Experiment: *Aedes aegypti* mosquitoes were placed in 200mL paper cups (15 female mosquitoes per cup). Several experimental combinations of Lgeo-AgNPs and BATBs were prepared and used for bioassay treatments. The control solution consisted of a 10% sucrose solution. The cups were covered with nylon mosquito netting, and a cotton ball saturated with treatment solution was placed on top of each cup to feed at *ad libitum*. Bioassays were conducted at room temperature of 24.6°C ± 1°C, 60% ± 5% relative humidity, and a 14L:10D photoperiod. The dead and grounded mosquitoes were recorded at 3hr, 6hr, 12hr and 24hrs intervals. Each experiment was in quadruplicate and replicated three times.

Fig. 1. Dead mosquito showing an engorged abdomen due to a dyed blue colour after exposure of blue dyed toxic baits.

An additional experiment was conducted to confirm that 90% of dead female mosquitoes took in the Lgeo-AgNPs and boric acid toxic baits by observing their abdomens with blue food dye (Fig. 1).

Data Analysis. A comprehensive result analysis was conducted in the study evaluating the effectiveness of lemongrass oil-based silver nanoparticles combined with boric acid toxic bait against *Ae. aegypti* mosquitoes. The analysis involved performing normality testing, ANOVA, the Duncan test, multiple comparisons, and descriptive statistics to assess the treatment's efficacy. Firstly, normality testing was performed using the Kolmogorov-Smirnov test, which indicated that most experiments (Experiments Nos: 1, 2, 3, 7, 5, 6, 12, 8, 9, 11, and 13) followed a normal distribution ($p > 0.05$). However, experiments No. 4 and 14 (control group) demonstrated non-normal distribution patterns ($p < 0 .05$).

RESULTS

Analysis of variance (ANOVA) of the data, it was found that both the "Experiments" and "Exposure time" factors had a significant effect on the "mortality count" variable ($p < 0.001$). Additionally, a significant interaction effect was observed between the factors "Experiments" and "Exposure time" ($p < 0.001$), indicating that the combined impact of these factors influenced mosquito mortality (Fig.2).

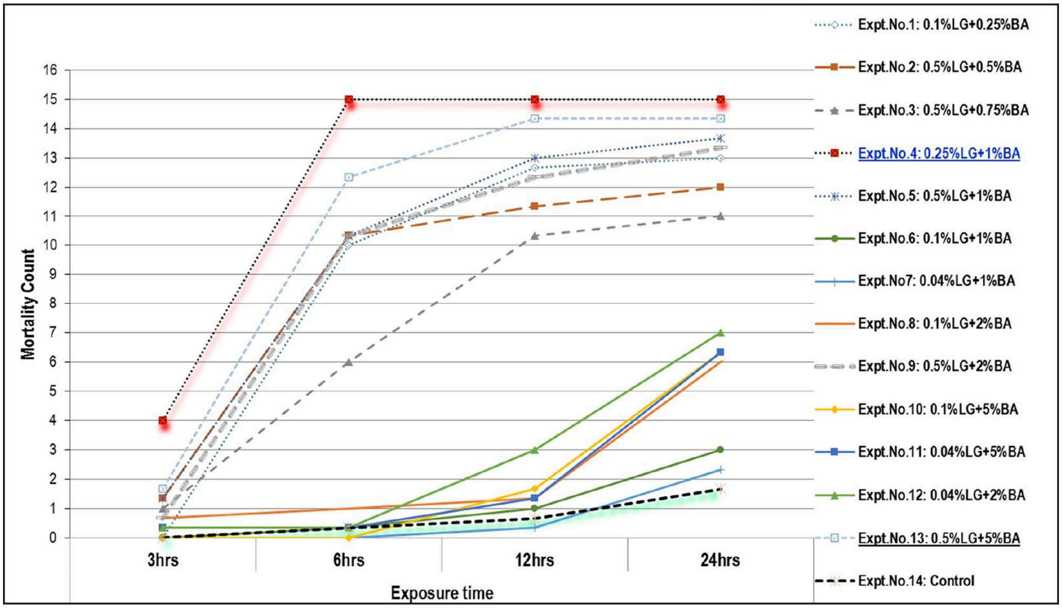

Fig. 2. Efficacy of Lgeo-AgNPs combined with BATB against *Aedes aegypti* (n=15)

The Duncan test was then utilized to identify homogeneous subsets based on the mean values of the "mortality count" variable. The test revealed six subsets with distinct mean values: Subset 1: Control and Expt. No.7 (0.04%Lgeo-AgNPs+1%BA) had similar mean values ($p = 0.051$). Subset 2: Expt. No. 6, 10, 11, and 8 showed identical mean values within the subset ($p > 0.05$). Subset 3: Expt. No.12 (0.04%Lgeo-AgNPs+2%BA) and Expt. No.3 (0.5%Lgeo-AgNPs+0.75%BA) displayed similar mean values ($p > 0.05$). Subset 4: Expt. No. 2, 1, 9, and 5 demonstrated similar mean values within the subset ($p > 0 .05$). Subset 5: Expt. No.13 (0.5%Lgeo-AgNPs+5%BA) showed noticeable mean values compared to all other subsets ($p < 0.05$). Subset 6: Expt. No.4 (0.25%Lgeo-AgNPs+1%BA) stood out prominently with distinct mean values that significantly differed from all other subsets ($p < 0.05$).

A multiple comparisons test (e.g., Least Significant Difference - LSD) was performed to compare the means of different exposure times (hrs) for the "mortality count" variable. The results indicated significant mean differences between all pairs of exposure time intervals (3hrs, 6hrs, 12hrs, and 24hrs) ($p < 0.001$) (Table 1). This finding suggests that the exposure time significantly affected the mosquito mortality rate.

Additional tests with blue food dye with the Lgeo-AgNPs and boric acid toxic baits showed that the dead adult mosquitoes caused by the different toxic baits were not the result of starvation or contact poisoning after exposure to such low concentrations of Lemongrass oils.

Finally, descriptive statistics were calculated, providing mean and standard deviation values for the "mortality count" variable, grouped by the factors "Experiments" and "Exposure time." The analysis offered an overview of observed mortality counts' central tendency and variability across different experimental conditions and exposure times.

Table 1. Evaluation of Lemongrass Essential oil- based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) against *Aedes aegypti* (n=15)

Exp vs Mortality count	Exposure Time								
	3hrs		6hrs		12hrs		24hrs		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Expt.No.1: 0.1%Lgeo-AgNPs+0.25%BA	0.00	0.00	10.00	2.65	12.67	1.15	13.00	1.00	.000
Expt.No.2: 0.5% Lgeo-AgNPs +0.5%BA	1.33	1.53	10.33	4.62	11.33	3.06	12.00	2.65	.010
Expt.No.3: 0.5% Lgeo-AgNPs +0.75%BA	1.00	1.00	6.00	0.00	10.33	1.53	11.00	1.73	.000
Expt.No.4: 0.25% Lgeo-AgNPs +1%BA	4.00	2.00	15.00	0.00					.000
Expt.No.5: 0.5% Lgeo-AgNPs +1%BA	1.33	0.58	10.33	2.52	13.00	1.00	13.67	1.15	.000
Expt.No.6: 0.1% Lgeo-AgNPs +1%BA	0.00	0.00	0.33	0.58	1.00	1.73	3.00	1.00	.031
Expt.No.7: 0.04% Lgeo-AgNPs +1%BA	0.00	0.00	0.00	0.00	0.33	0.58	2.33	1.15	.006
Expt.No.8: 0.1% Lgeo-AgNPs +2%BA	0.67	1.15	1.00	1.00	1.33	2.31	6.00	1.73	.012
Expt.No.9: 0.5% Lgeo-AgNPs +2%BA	0.67	1.15	10.33	4.51	12.33	2.52	13.33	1.53	.002
Expt.No.10: 0.1% Lgeo-AgNPs +5%BA	0.00	0.00	0.00	0.00	1.67	2.08	6.33	3.21	.011

Exp vs Mortality count	Exposure Time								
	3hrs		6hrs		12hrs		24hrs		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Expt.No.11: 0.04% Lgeo-AgNPs +5%BA	0.33	0.58	0.33	0.58	1.33	0.58	6.33	1.53	.000
Expt.No.12: 0.04% Lgeo-AgNPs +2%BA	0.33	0.58	0.33	0.58	3.00	2.00	7.00	0.00	.000
Expt.No.13: 0.5% Lgeo-AgNPs +5%BA	1.67	2.08	12.33	4.62	14.33	0.58	14.33	0.58	.001
Expt.No.14: Control 10% Sucrose solution	0.00	0.00	0.33	0.58	0.67	1.15	1.67	1.53	.274
P-value	**.005**		**.000**		**.000**		**.000**		

In summary, the comprehensive result analysis demonstrates the effectiveness of lemongrass essential oil-based silver nanoparticles combined with boric acid bait in controlling *Ae. aegypti* mosquitoes. The study revealed standard distribution patterns for most experiments, significant effects of experimental factors and exposure time on mosquito mortality, distinct subsets based on mean values, important differences between exposure time intervals. It provided descriptive statistics to understand the mortality count - the standout performance of Expt. No.4 (0.25%Lgeo-AgNPs+1%BA) within a shorter exposure time frame highlights its potential as a promising approach in mosquito control. Further research can focus on optimizing the formulation and understanding its mechanisms to enhance mosquito control strategies and mitigate the spread of mosquito-borne diseases.

DISCUSSION

One of the latest alternatives to conventional insecticides is plant-based metal nanoparticles, which have demonstrated toxicity against all life stages of mosquitoes at different concentration levels. The unique properties of nanoparticles are a high surface area-to-volume ratio, facilitating the efficient delivery of active ingredients to the target site[5]. These nanoparticles' size, shape, and stability are influenced by the concentration of the plant extract or metabolite and the concentration of metal ions in the substrate[9]. These nanoparticles have been screened for various mosquito control activities, including ovicidal, larvicidal, pupicidal, adulticidal, and repellent properties[10]. The phytochemical compounds can disrupt the receptor site of the nervous system through different metabolic pathways, such as monoterpenes acting on the Na+/K+ ion channel, while flavonoids target as Acetylcholinesterase inhibitors[11]. The plant essential oils significantly inhibited both cytochrome P450 and glutathione S-transferase activities, suggesting that inhibiting detoxification contributes to the enhancement or synergism of plant crucial oils for permethrin and pyrethroids[12-14]. This innovative and environmentally friendly approach involves synthesizing plant-based metallic nanoparticles that contain bioactive phytochemicals that are safe, biodegradable, and hold promise as a strategy for controlling mosquito-borne diseases[15-18]. However, most studies have focused on the larvicidal efficacy of metal nanoparticles, with few studies examining their adulticidal potential[19]. The growing challenges posed by mosquito-borne diseases have generated our interest in exploring effective control strategies.

Our study findings demonstrated that the combination of 0.25% Lgeo-AgNPs and 1% BATB was the most effective, leading to 100% mosquito mortality within 6 hours of exposure. This combination proved highly effective in controlling *Ae. aegypti* mosquitoes, highlighting the importance of optimising the concentration of nanoparticles and toxic bait for effective mosquito control.

Comparisons with other published studies on Attractive Toxic Sugar Bait (ATSB) ingredients and mosquito species revealed varying results. Xue and Barnard6 tested 1% boric acid in a 10% sucrose solution for *Ae. albopictus*, achieving ≥98% mortality after 48 hours. Xue *et al.*[20] performed semi-field trials with *Ae. albopictus* and *Culex nigripalpus*, using 1% boric acid in a 5% sucrose solution, resulting in ≥96% mortality after 48 hours. Muller *et al*.[21] conducted outdoor field trials using a mixture of nectarines, red wine, brown sugar, red food dye, spinosad, and BaitStab sprayed on plants, showing 91% mortality for *Anopheles sergentii* and 67% for *Ae. caspius* after 30 days. These studies demonstrate the varied effectiveness of different formulations and ingredients against different mosquito species in laboratory and field conditions. Studies of Kumar *et al*.[22] have reported that the Toxic Sugar Baits (TSB) induced complete mortality of *Ae. aegypti* and *An. stephensi* at a 4% concentration of boric acid. Meanwhile, *An. culicifacies*, 100% mortality was attained at a 3% concentration of TSB solution. Moreover, the TSB solution containing 2% boric acid resulted in 99.1% mortality in *An. culicifacies*, with approximately 95% mortality observed in both *An. stephensi* and *Ae. aegypti.*

The findings of our study demonstrate significant efficacy within a short exposure time of 6 hours and a higher percentage of mosquito mortality using the specific combination of 0.25% Lgeo-AgNPs and 1% BATB in laboratory conditions, marking a noteworthy advancement in mosquito control strategies. However, a deeper understanding of the mechanism by which Lgeo-AgNPs and BATBs induce mortality is required, and further research is needed to validate their effectiveness. Field assessments are essential to evaluate this approach's efficiency, feasibility, and potential impact on adulticidal mosquito control strategies. Additionally, a comprehensive investigation is necessary to evaluate the long-term effects on non-target organisms and assess the environmental implications of this approach.

CONCLUSION

This study extensively evaluated the impact of lemongrass essential oil-based silver nanoparticles (Lgeo-AgNPs) combined with boric acid (BA) bait on the mortality of *Ae. aegypti* mosquitoes. The analysis of variance (ANOVA) results indicated that both the experimental conditions and exposure time significantly influenced mosquito mortality. A notable interaction effect between these factors further emphasized their combined impact on mortality rates.

The Duncan test identified six homogeneous subsets of mean mortality counts, with Experiment No. 4 (0.25% Lgeo-AgNPs + 1% BA) standing out as significantly different and more effective compared to other subsets. Additionally, a multiple comparisons test revealed significant differences in mortality across various exposure times (3, 6, 12, and 24 hours), reinforcing the importance of exposure duration in mosquito control strategies.

Descriptive statistics provided a comprehensive overview of mortality counts' central tendency and variability across different experimental setups and exposure times. The findings highlight the standout performance of Experiment No. 4 within a shorter exposure time frame, showcasing its potential as a promising approach for mosquito control.

Furthermore, additional tests confirmed that the observed mosquito mortality was due to the toxic baits rather than starvation or contact poisoning. This reinforces the efficacy of Lgeo-AgNPs combined with boric acid in mosquito control.

In conclusion, this study demonstrates the significant potential of Lgeo-AgNPs combined with boric acid bait in controlling *Ae. aegypti* mosquitoes. The results underscore the importance of optimizing nanoparticle concentration and exposure time for effective mosquito control. Future research should focus on understanding the mechanisms underlying the observed mortality, optimizing formulations, and conducting field assessments to validate these findings. Additionally, evaluating the long-term effects on non-target organisms and environmental implications is crucial for developing sustainable mosquito control strategies.

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