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Bio-larvicidal and Cytotoxic Activity of forest Streptomyces Isolate GA9 Against Some Mosquito Larvae

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ABSTRACT

An antibiotic-producing *Streptomyces spp.* was isolated from forest soil and assessed for its bio-larvicidal and cytotoxic activity against various mosquito species (*Aedes spp, Anopheles spp, Culex spp,* and *Mansonia spp*) and Brime shrimps. Active biocides were extracted using a modified solid state fermentation process. Mosquito larvae were exposed to different concentrations of the ethanolic extract of the Streptomyces exudates for 24 hours. The extract's cytotoxicity was also evaluated using Brime shrimp. The results revealed a significant mortality rate among all four important vector mosquitoes following treatment with the extract. The LC_{50} and LC_{90} values of the extract were 0.40 mg/ml and 0.80 mg/ml for *Anopheles spp*, 0.20 mg/ml and 0.60 mg/ml for *Culex spp*, 0.40 mg/ml and 0.60 mg/ml for *Aedes spp*, and 0.20 mg/ml and 0.60 mg/ml for *Mansonia spp*, respectively. The Minimum larvicidal concentration values were 0.2 mg/ml for *Aedes* spp, *Anopheles spp, Culex spp,* and *Mansonia spp.* The extract showed a low degree of cytotoxicity, with an average mortality rate of only 20% at 0.8 mg/ml of the extract, upon testing with Brime shrimp. In conclusion, the aqueous extract of forest *Streptomyces spp.* exhibited high bio-larvicidal activity against *Aedes spp, Anopheles spp, Culex spp, and Mansonia spp* larvae, suggesting its potential as an environmentally friendly approach to mosquito control. This study represents an initial step toward supplementing eco-friendly nontoxic microbe-based bioinsecticides for synthetic insecticides against medically significant mosquitoes.

***Keywords*:** Bio-larvicide, Mosquito, Streptomyces, Cytotoxicity, Biological control, and soil

Introduction

The prevalence and spread of mosquito-borne diseases are rapidly increasing globally.[1-3] Aedes, Anopheles, and Culex mosquitoes are the primary vectors of diseases like malaria, dengue fever, zika, chikungunya, West Nile fever, yellow fever, and tularemia.[1-6] Climate change, socioeconomic factors, and inter-border travelling have all contributed to the expansion and proliferation of these diseases and their vectors, posing an existential threat to the health and well-being of over four billion people across over hundred countries.[7] Despite the urgent need to curb the associated morbidity of mosquito-borne diseases and the proliferation of mosquitoes, mosquitoes have proved remarkably resilient, adapting to a wide range of habitats and developing resistance to existing insecticides.[8,9]

The situation is dire, with synthetic biocides causing toxic residue buildup and inducing insecticide resistance in mosquitos, making traditional control methods increasingly ineffective.[10] The short life cycles and large broods of mosquitoes enable rapid genetic adaptation, rendering current control strategies obsolete.[11] Though biological control methods hold promise, there are a few challenges; *Bacillus thuringiensis* is a famous insecticide-producing bacterium,[4] but its insecticide potency is limited in organically enriched water. It may not be effective against Culex mosquitoes that prefer organic reach water environments.[5]

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The need for more effective and environmentally friendly mosquito-specific insecticides has never been more urgent. Scientists have turned to explore other novel sources of highly active biological secondary metabolites that have little or no adverse health and environmental repercussions.[6-8] Naturally occurring compounds derived from free-living microorganisms, such as *Streptomyces spp*, have gained significant relevance due to their eco-friendliness and cost-effectiveness.[9-11] Actinobacteria's most researched genus, Streptomyces, is renowned for producing bioactive chemicals. These secondary metabolites are bioactive against various targets, including antioxidants, anticancer drugs, enzymes, enzyme inhibitors, and antimicrobials.[7,12] Many of these natural products have found use in the chemical, food, and pharmaceutical industries.[12] Natural compounds derived from actinobacteria vary in structure and function, and many medications made from them are effective against newly emerging and reemerging infections.[13,14] Additionally, Actinobacteria utilize natural bioactive chemicals to create many well-known antibacterial medicines used in agriculture and medicine.[15,16] More than 7,000 molecules from Actinobacteria have been listed in the Compendium of Natural Products,[16] highlighting the significance for the pharmaceutical and chemical sectors. As a result, efforts to find new Actinobacteria strains that can produce beneficial chemicals have increased in recent years.[17-21]

The use of Streptomyces secondary metabolites has not gone beyond the lab, researchers have discovered the presence of chitinase producing Streptomyces strains from soil with evidence of secondary metabolite production and bioactive traits.[19-27] A previous study[28] revealed the presence of high antimicrobial and bio-larvicidal activity of Streptomyces isolate GA9 from soil sample against clinically significant test organisms and Anopheles larvae. Despite this novel finding from the pioneering study on the use of Streptomyces secondary metabolite as alternative to synthetic pesticides, there are

however limited information on the activity of GA9 on other mosquito larvae as well as its cytotoxicity. Thus, this study aims to isolate Streptomyces, extract active secondary metabolites from *Streptomyces spp*, and examine the bio-larvicidal activity of the secondary metabolite exudate of the Streptomyces against different species of mosquito larva (*Aedes spp, Culex spp, anopheles spp* and *Mansonia spp*) and to determine the cytotoxicity spectra of the Streptomyces secondary metabolites on brime shrimps.

Method

Soil Sample collection

From March to April 2022, soil samples were taken from various locations in Kogi Central Province with longitude 7.2912 and latitude 7.6303, Kogi State, Nigeria. Strains of Streptomyces were isolated from agricultural and nonagricultural soil in different environments from multiple areas (cassava farmland, a cashew plantation, yam farmland, a garbage dump, and a grassland). After excavating around 3 cm of the soil's surface with an auger, the samples were taken from a depth of 20 cm, placed in polyethene bags, and labelled accordingly.[19] On-site measurements of the soil samples' physiological parameters (e.g., pH) were taken. A total of 15 soil samples were taken during the sampling period across all the sampling sites. After being air-dried for ten days away from direct sunlight, the obtained soil samples were transferred to the lab for microbiological screening and other analyses.[20]

Isolation of Streptomyces pure culture

Streptomyces species were isolated using the pour plate method in a modified Czepadox agar under standard conditions, following conventional microbiological protocols. A 20 g dried soil sample was added to 180 ml of distilled water and stirred for 30 seconds. The soil samples were pretreated and serially diluted up to a dilution factor of 10^{-5}, with each dilution tube vortexed (Thermo-voertex, Thermoscientific, USA) to create a uniform suspension. Duplicates of 100 ul aliquots of each dilution factor were plated and overlaid with a modified Czepadox agar (100 ug/ml of Cycloheximide and 200 ug/ml of streptomycin). The isolation media were then incubated (Sci-incubator, Unicorn, USA) at room temperature to mimic their natural environment (28–30°C) for one week. The discrete colonies were counted in colony-forming units following the descriptions of Kizito and Nwankwo (2013).[20] Using sterile wire loops to streak colonies on fresh plates of modified Czepadox agar, isolates were purified. *Streptomyces species* isolated from pure cultures were added to Czepadox slants and kept at 4°C pending use.

Characterization of the Streptomyces isolates

Based on the recommendations provided in Bergey's Manual of Systematic Bacteriology and the International Streptomyces Project, [21-23] identifying Streptomyces isolates from soil samples required a multifaceted approach that included cultural, morphological, and biochemical characteristics. The colour of the aerial and substrate mycelia, colony formation of melanoid pigment, Gram stain appearance (oil immersion objective), and spore chain morphology were all evaluated on Czepadox agar and used for classification and differentiation. Isolates were categorized according to their hue. While spore chains were classified as rectiflexibiles (straight to flexuous), retinaculiaperti (open loops, hooks, or spirals consisting of one or two turns), and spirals (tight spirals) based on their shape under light microscopy,[24] melanoid producers and non-producers were also identified. To further characterize the isolates, biochemical assays, including catalase, motility, urea hydrolysis, hydrogen sulfide (H_2S) production, indole production, methyl red, citrate utilization, and sugar utilization, were carried out.[25]

Bioactive compound assay

A modified Solid State fermentation process was used to extract the active biocides as described by Ganesan (2018).[26] The fermentation was conducted by inoculating *Streptomyces spp* on Czepadox agar for five days at 28°C. After that, three 60 ml centrifuge tubes with 20 ml of Czepadox broth medium were inoculated with Streptomyces colonies and incubated at 28°C on a rotary shaker incubator (Sci-FI incubator, Thermofisher, Germany) at 130 rpm for 72 hours. Subsequently, six 500 ml conical flasks with cotton wool plugs containing 300 ml of Czepadox broth media were inoculated with the seed cultures. The fermentation process was conducted at 28°C for two weeks at 130 rpm in a rotary incubator. The culture was subjected to Soxhlet extraction with sterile distilled water to extract the secondary metabolites. The resulting extract was stored at −20°C until required, all stock solutions were made fresh before each bioassay.

Mosquito colonization and maintenance

Mosquito larvae were recovered from the wild and identified and characterized using a standard identification key. The larva was maintained in the lab in 800 ml water containing 200 ug/ml of glucose. All larvae were starved 24 hours before bio-larvicidal activity testing and cytotoxicity. [27-29]

Bio-larvicidal activity

Varying concentrations of the extract were prepared according to the description of Karthik *et al*. (2011).[30] A modified version of the WHO protocol for larvicidal bioassays was utilized in this study. The death rate of fourth instar larvae of the following species: *Aedes spp, Culex spp, Anopheles spp*, and *Mansonia spp* was used to evaluate the toxicity of the extract.[31,32] With a wide-bore plastic transfer pipette, batches of twenty larvae were transferred to disposable test containers. Excess water was removed, and 200 ml of dechlorinated water was added. A 5 ml of each extract concentration was added to the 200 ml of water containing the 20-mosquito larva belonging to a particular mosquito spp. The LC_{50} and LC_{90} values were determined using a restricted range of four concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, and 0.8 mg/ml), yielding a 0–95% mortality rate. The latter process was repeated for other mosquitos spp. Each bioassay was conducted under the same environmental condition, and four concentrations were tested in duplicate for each assay. The corrected percentage mortality values for each concentration were recorded 24 hours post-treatment. Larvae were considered "dead" if they did not respond to pricking with an inoculating needle.

Cytotoxicity test

A brine shrimp lethality experiment using the method outlined by Arogba (2014)[33] was carried out to evaluate the cytotoxicity of Streptomyces extract. Utilizing newly hatched brine shrimp (*Artemia salina*) larvae, artificial seawater was made by dissolving 16 g of sodium chloride solution in 500 ml of distilled water served as the incubation medium. Ten brine shrimp larvae were transferred to separate vials using a Pasteur pipette after 24 hours of incubation under bright light and aeration, and some volume of synthetic seawater was added to make the volume 10 ml.[33]

To test the cytotoxicity, 100 ul of each extract concentration or the reference potassium dichromate at varied concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, and 0.8 mg/ml) were added to the vials containing ten brine shrimp larva. After 24 h of exposure, the number of dead larvae was counted to determine the percentage lethality.[33] The experiment was duplicated, and the mean percentage lethality was recorded.

Statistical analysis

Descriptive statistics and measures of central tendency were used to present and analyze the data. The Chi-square tool was used to assess the measurement of the association between larvicidal activity and mosquito larvae. A confidence level was 95% with a probability value of 0.05.[20] IBM Statistical Package for Social Science (SPSS) version 22 was used for the analysis.

Results and Discussion

The larvicidal activity of Streptomyces isolates GA 9 was investigated, and the soil pH of the samples ranged from a slightly acidic soil condition to a slightly alkaline state. Grassland soil samples had the highest pH (7.2), while cassava had the least (6.8). There were no

significant changes in the pH value of the soil with agricultural and nonagricultural sites, as represented in Figure 1.

The multiphasic microbiological approach revealed 82 bacterial isolates, 49 of which reacted positively to Gram stain. The positive catalase (34) were assessed for filament and motility, and 22 out of 34 catalase positive isolates were filamentous. Only 16 out of the filamentous isolates were non-motile. The non-motile, filamentous, catalase-positive and gram-positive bacteria were categorized as Streptomyces genera. The Streptomyces isolates were dry, discrete, raised, or depressed. Sixteen Streptomyces isolates were identified and characterized following Bergey's manual. Out of the 16 Streptomyces isolates, isolate GA 9 (Figure 2) showed evidence of brown secondary metabolite pigmentation and was selected for all bioassay and fermentation processes.

The sugar utilization spectra of the isolates illustrate diversity in the biochemical response of the isolates and the variation in the carbon sources for the isolates despite their similar cultural and morphological traits. Isolate GA 9 could not utilize arabinose and maltose as a carbon source, as represented in Table 1. All isolates had either a convex or a concave elevation. The isolates were either dry, granular, smooth or a combination of the latter. The colour of the aerial pigmentation ranged from white, cream, grey to whitish green, and the isolates edges were either entire, fuzzy regular or irregular or a combination of the latter. The reverse view revealed the substrate pigmentation ranging from cream and off-white to yellow. The spore arrangements of the isolates varied significantly, but the isolates were majorly straight and spiral. Only one isolate showed evidence of a brown diffusible pigmentation (GA 9), as represented in Table 2.

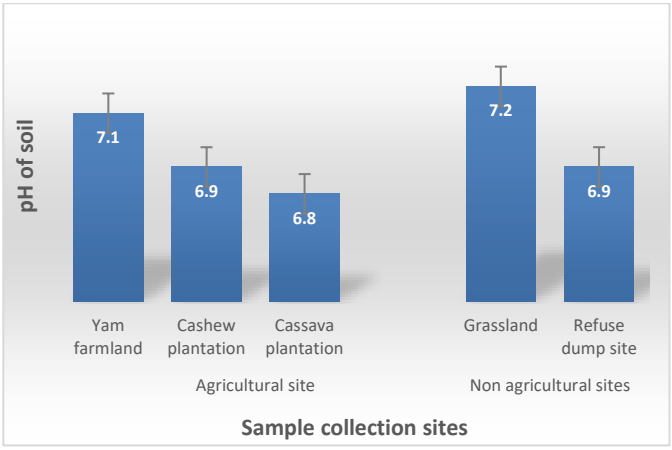

Figure 1: pH of soil samples from agricultural and nonagricultural sites

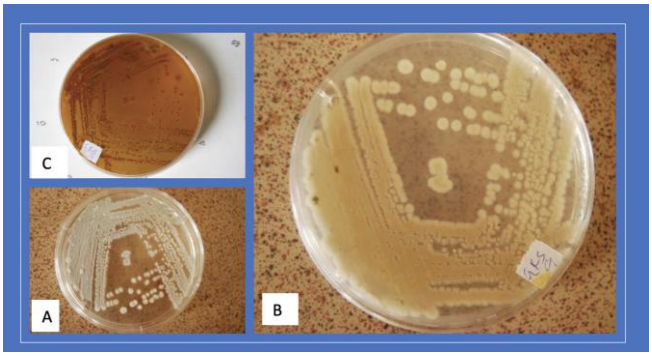

Figure 2: The cultural appearance of isolates GA 9. A: Arial view of isolate GA 9, B: Substrate view of isolate GA 9, C: Brown diffusible pigmentation.

The bio-larvicidal activity of *Streptomyces spp* GA 9 was significant across all the mosquito larva at $P < 0.05$. The larvicidal activity of the GA 9 exudate against Anopheles larva was highest at a concentration of 0.8 mg/ml at 90% Anopheles larva mortality compared to 0.2 mg/ml at 40% mortality rate. The LC_{50} of the GA 9 extract at 0.4 mg/ml was relatively lower than the LC_{90} (0.8 mg/ml). Culex and Mansonia mosquito larva had similar LC_{50} and LC_{90} (LC_{50} = 0.2 mg/ml and LC_{90} = 0.6 mg/ml). There was, however, variation in the mortality rate in relation to the concentration, as represented in Table 3. Aedes larvae were inhibited by the *Streptomyces spp* GA 9 exudates at varying concentrations. At concentrations of 0.6 mg/ml and 0.8 mg/ml, a 90% mortality rate was recorded. The extract's LC_{50} (0.4 mg/ml) had a 70% mortality rate, as shown in Table 3. The mortality rate of brine shrimps was moderate for the *Streptomyces spp* GA 9 extract. The highest concentration (0.8 mg/ml) had the highest mortality (20%). The percentage mortality for 0.2 mg/ml, 0.4 mg/ml and 0.6 mg/ml was 10%, which was comparably lower than the toxicity of the positive control with a record 100% mortality at a concentration of 0.8 mg/ml, as shown in Table 4.

The endemicity of mosquito-borne infection is still of public health concern. Over the past decades, the need for a more environmentally friendly alternative has been in full gear. However, little attention has been paid to the potential insecticidal activity of Streptomyces, despite their broad antibiotic properties.[10,39] A total of 16 Streptomyces isolates were recovered from the study through multiphasic approach, and one of the isolates (GA 9) showed significant evidence of diffusible pigmentation. The latter confirms the assertions that Streptomyces are producers of bioactive compounds with infinite biological function.[9,11] The findings of this study complement the independent report of Kizito and Nwankwo (2013).[20] Our results shed new light on the diversity and morphological features of Streptomyces genera and provide a foundation for the characterization of Streptomyces strain variants.[12, 35]

While *Streptomyces spp* GA 9 shows pigmentation, more tests are required to establish its novelty.[17,18,36] The present study investigated the larvicidal activity of Streptomyces isolates against mosquito larvae (*Aedes spp*, *Culex spp*, *Anopheles spp* and *Mansonia spp*), and the results revealed a wide range of bio-larvicidal activity across different mosquito larva. The GA 9 extract expressed high bio-larvicidal efficacy at lower concentrations against the tested mosquito larva. The findings of this study complement other reports. [26, 37-43]

Interestingly, the soil pH of the samples varied from slightly acidic to slightly alkaline conditions, which may have played a role in the growth phase of the filamentous bacteria. It was revealed that grassland soil had the highest pH value (7.2), while cassava soil had the lowest (6.8). These findings suggest that soil pH may play a critical role in the proliferation of Streptomyces isolates in the soil. There were no significant changes in the pH value of the soil with agricultural and nonagricultural sites. This finding suggests that the larvicidal activity of Streptomyces isolates may not be significantly affected by the source from which the soil samples were collected.[44]

This study revealed a wide range of morphological characteristics among the isolates. Our findings show a surprising diversity in the biochemical responses of these isolates and their sources of carbon utilization. Specifically, we found that despite their similar cultural and morphological traits, the isolates exhibited significant variation in their sugar utilization spectra. The probable reasons for the latter are unclear. Our findings underscore the need for caution when making assumptions about the metabolic capabilities of Streptomyces isolates, as their biochemical responses can vary widely.[45]

The bio-larvicidal activity of *Streptomyces spp* GA 9 was significant across all mosquito larva at a significance level of $P < 0.05$. However, a higher level of bio-larvicidal activity was observed at a concentration of 0.8 mg/ml with 90% mortality for Anopheles larva compared to 0.2 mg/ml with a 40% mortality rate. The reason for the high larvicidal activity could be attributed to the high concentration of the extract. However, the findings of this study suggest that the bio-larvicidal activity of the GA 9 extract is concentration-dependent and varies across different mosquito species.[26,41,46,47]

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Table 1: Sugar utilization of the Streptomyces isolates

NAME	XYLOSE	GLUCOSE	SUCROSE	MANNOSE	GALACTOSE	LACTOSE	FRUCTOSE	INOSITOL	ARABINOSE	MALTOSE	GAS
GRASSLAND											
GA1	+	+	+	+	+	+	-	-	+	+	+
GA 2	+	+	+	+	-	+	+	+	+	+	+
GA 9	+	+	+	+	+	+	+	+	-	-	+
YAM FARM											
YM 1	+	+	+	-	+	+	+	+	+	+	-
Y M 2	-	+	+	+	-	+	+	+	+	+	+
Y M 3	+	+	+	-	+	+	+	+	+	+	+
Y M 4	+	+	-	+	+	+	+	+	+	-	+
CASSSAVA FARM											
CSV 1	+	+	+	+	+	+	-	-	-	+	+
C SV 2	+	+	+	+	+	+	+	+	+	+	+
CSV 3	+	+	+	+	+	+	+	+	+	+	+
REFUSE DUMP											
DM 1	+	+	+	-	-	+	+	+	-	+	-
DM 2	-	+	-	+	+	+	-	+	+	+	+
DM 3	+	+	+	+	+	+	+	+	+	+	+
DM 4	+	+	-	+	+	+	+	-	+	+	+
CASHEW FARM											
CW 1	+	+	+	+	+	+	+	+	+	+	+
CW 2	+	+	+	+	+	+	+	+	+	+	+

Footnotes: +: positive, -: negative

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Table 2: Colonial morphology characteristics of *Streptomyces spp*

Isolates	Appearance	Elevation	Arial pigmentation	Edge	Substrate pigmentation	Spore arrangement	Visible diffusible pigment
Grassland							
GA 1	Granular and dry	Convex	Off white greenish	Entire-regular	Cream	Spiral	-
GA 2	Dry and smooth	Convex	White	Irregular	Cream	Straight	-
GA 9	Granular, dry and smooth	Convex	White-greenish	Fuzzy	Cream	Straight	Brown
Yam farm							
YM 1	Granular, dry and smooth	Convex	Cream-white	Entire	Cream	Straight	-
YM 2	Granular, dry and smooth	Convex	Brown with white edge	Entire	Cream	Spiral	-
YM 3	Dry and smooth	Concave	Cream	Entire	Cream	Spiral	-
YM 4	Dry and smooth	Concave	Cream	Irregular	Cream	Spiral	-
Cassava farm							
CSV 1	Smooth and dry	Convex	Cream	Irregular	Cream	Straight	-
C SV 2	Smooth and dry	Convex	Cream	Irregular	Cream	Straight	-
CSV 3	Smooth and dry	Convex	Off-white	Irregular	Cream	Straight	-
Refuse dump							
DM 1	Smooth and dry	Convex	Cream	Entire	Cream	Straigh	-
DM 2	Dry and smooth	Convex	White	Fuzzy	Cream	Straight	-
DM 3	Dry and smooth	Concave	Grey	Regular	Cream	Straight	-
DM 4	Dry and smooth	Convex	Cream	Regular	White	Straight	-
Cashew farm							
CW 1	Dry and smooth	Convex	Whitish-green	Entire	Yellow	Spiral	Brown
CW 2	Dry and rough	Convex	white	Irregular	Cream	Spiral	_

Footnote: –: absent

Table 3: Bio-larvicidal activity of *Streptomyces spp* GA 9 extract on mosquito larva

Mosquito Larva	Concentration (mg/ml)	Number of deaths ± S.E	Percentage mortality (%)
Anopheles spp	0.20	8 ± 0.23	40
	0.40	15 ± 0.29	75
	0.60	14 ± 0.46	70
	0.80	18 ± 0.35	90
	Water	0 ± 0.00	0
	LC_{50} 0.40		
	LC_{90} 0.80		
Culex spp	0.20	10 ± 0.58	50
	0.40	16 ± 1.20	80
	0.60	12 ± 1.20	90
	0.80	18 ± 1.20	70
	Water	0 ± 0.00	0
	LC_{50} 0.20		
	LC_{90} 0.60		
Aedes spp	0.20	9 ± 0.58	45
	0.40	14 ± 1.70	70
	0.60	18 ± 1.20	90
	0.80	18 ± 1.40	90
	Water	0 ± 0.00	0
	LC_{50} 0.40		
	LC_{90} 0.60		
Mansonia spp	0.20	10 ± 0.58	50
	0.40	16 ± 0.87	80
	0.60	18 ± 0.58	90
	0.80	18 ± 0.87	80
	Water	0 ± 0.00	0
	LC_{50} 0.20		
	LC_{90} 0.60		

Footnote: S.E: Standard error of mean

This study also investigated the effect of *Streptomyces spp* GA 9 exudates on Aedes larva. The results showed that the activity level at concentrations of 0.6 mg/ml and 0.8 mg/ml resulted in a 90% mortality rate, while the LC_{50} of the extract at 0.4 mg/ml had a 70% mortality rate. This demonstrates that the bio-larvicidal activity of *Streptomyces spp* GA 9 is diverse and can be a choice alternative in controlling vectors of dengue, chikungunya and zika virus. The report of this study is concordant with several other reports.[48-51] These results suggest that *Streptomyces spp* GA 9 exudates have significant bio-larvicidal activity against different mosquito species.[13,48] However, the degree of effectiveness of these exudates varies depending on the concentration and type of mosquito species, as indicated by the LC_{50} and LC_{90} values. These findings have important implications for developing bio-larvicidal agents and highlight the potential of *Streptomyces spp* GA 9 exudates as effective mosquito larvicides.[52-56]
The mortality rate of brine shrimps in response to the *Streptomyces spp* GA 9 extract was moderate, with the highest concentration (0.8 mg/ml) resulting in the highest mortality rate of 20%. Interestingly, the percentage mortality for concentrations of 0.2 mg/ml, 0.4 mg/ml, and 0.6 mg/ml was 10%, which is relatively lower than the toxicity of the positive control. The probable reason for the low level of toxicity of the extract could be due to the low concentrations of the extract. The findings of our study contradict the report of Arogba (2014),[33] who reported that biomolecule toxicity was not concentration dependent. Our findings suggest that the bio-toxicity of the *Streptomyces spp* GA 9 extract on brine shrimps is concentration-dependent, with a higher concentration resulting in a higher mortality rate. However, the observed mortality rate for the lower concentrations of the extract was not as high as that of the positive control. This indicates that while the *Streptomyces spp* GA 9 extract has a high larvicidal efficacy against mosquito larva, its bio-toxicity against brine shrimps was very low compared to the reference standard used in the study.[57,58]

Table 4: Cytotoxic activity of Streptomyces spp GA9 on brine shrimps

Concentration (mg/ml)	Number of deaths upon GA9 extract ± S.E	Percentage mortality for GA9 (%)	Number of death with Potassium di chromate ± S.E	Potassium di chromate (%)
0.20	1 ± 2.10	10	4 ± 0.21	40
0.40	1 ± 1.60	10	6 ± 0.34	60
0.60	1 ± 3.10	10	6 ± 0.16	60
0.80	2 ± 3.80	20	10 ± 0.21	100

Footnote: S.E: Standard error of mean

Conclusion

The results indicated a significant mortality rate among all four important vector mosquitoes and low cytotoxicity against brine shrimps following treatment with the extract. The LC_{50} and LC_{90} values of the extract were reported, showing a minimum larvicidal concentration of 0.2 mg/ml for *Aedes spp*, *Anopheles spp*, *Culex spp*, and *Mansonia spp*. These findings indicate that the aqueous extract of forest *Streptomyces spp* has high bio-larvicidal activity against medically significant mosquitoes, suggesting its potential as an environmentally friendly approach for mosquito control.

Furthermore, the study results contribute to developing eco-friendly nontoxic microbe-based bioinsecticides as an alternative to synthetic insecticides against medically significant mosquitoes. The findings of this study have significant implications for mosquito-borne disease control programs, especially in areas where conventional insecticides have caused resistance among mosquitoes. Further studies should be geared towards the characterization and isolation of active bio-compound with anti-insecticidal potentials

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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