Original Research Article

Testing the Bioactivity of *Bacillus sphaericus* Isolates on House Fly Larvae

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Abstract: ***Background***: *Musca domestica* is a cosmopolitan public health and agricultural pest that is known to be a carrier of multiple pathogens and cause economic losses in production of animals. ***Objective***: The current research paper examined *Bacillus sphaericus* isolates as Larvicidal agents in larvae of *M. domestica* as a third instar organism under controlled laboratory environments. ***Methodology***: There were five concentrations of *B. sphaericus* spores (1×10^4 - 1×10^8 spores/ml), positive (*Bacillus thuringiensis israelensis*) and negative (sterile water) controls, and 600 larvae in total. ***Results***: Larval death mortality was observed to take place at 24, 48, and 72 hr within the treatment period and was corrected by Abbott and probability analysis, ANOVA. Findings showed definite dose-dependent effect. At 72 hr, mortality was 20% at 1×10^4 spores/ml to 95% spores/ml 1×10^8 to the negative control of 4% mortality. Positive control group mortality after 72 hr was 92%. The LC_{50} and LC_{90} were estimated to be 3.2×10^6 and 1.1×10^7 spores/ml respectively by Probit. Significant changes among treatments were established with statistical tests (F= 46.3, p<0.001). ***Conclusion***: *B. sphaericus* has high larvicidal activity against *M. domestica* and this is similar to commercial reference strains. The study presumes that *B. sphaericus* can serve as a safe biocontrol agent that is environmentally friendly and could help eliminate the usage of chemical pesticide and may be included in an integrated pest management program.

Keywords: Microbial Biopesticides, *Bacillus Sphaericus*, *Musca Domestica*, Larvicidal, Biocontrol Agent.

INTRODUCTION

Musca domestica (Diptera: Muscidae) is a household fly that is prevalent with serious consequences on the health of the population, animal husbandry, and food safety. It is a mechanical carrier of more than 100 pathogens, such as bacteria, viruses, protozoa, and helminths, which is why its control is a priority in urban and rural areas (Torres *et al*., 2022). The conventional methods of control are based on the intensive use of chemical insecticides; nevertheless, their excessive use has caused environmental pollution, non-selective toxicity, and the emergence of resistance in populations of house flies (Nascimento *et al*., 2024). Such restrictions have led to the identification of more sustainable and safer alternatives, especially microbial agents that have entomopathogenic potential. The Bacillus species have been widely known as larvicidal control agents amongst the biological control agents. Some of the *Bacillus thuringiensis* (*Bt*) strains have already been isolated and tested regarding their insecticidal ability against *M. domestica* and other dipteran species with encouraging results in the laboratory (Gharty Chhetri, 2024). Moreover, the use of the microbial products does not only help in pest suppression but also fits the idea of sustainable agriculture as fewer chemicals are used (Rocha *et al*., 2023). The house fly larvae themselves has also been mentioned by recent literature as a source of bioactive compounds with antimicrobial properties, and insects and their microbiota of related microbiota may be reservoirs of novel biomolecules (Park, 2023).

Bacillus spp has a range of bioactive metabolites including toxins, lipopeptide, and high larvicidal, antimicrobial and immunomodulatory bioactive metabolite. The Bacillus pumilus compounds have recently been demonstrated to possess enormous larvicidal effects on Aedes aegypti*, Anopheles stephensi*, and *Culex quinquefasciatus*, and this result

supports the interspecies efficacy of bacilli-derived metabolites (Raguvaran *et al.*, 2022). Similarly, the lipopeptides of Bacillus velezensis were also reported in inhibiting dipteran larvae, such as the Lucilia cuprina, which indicates some level of the broad insecticidal effect (Ramesar & Hunter, 2023a, 2023b). Bacillus subtilis strains have additionally been associated with the high larvicidal activity of surrogate metabolites of bacterial secondary metabolites (Xia *et al.*, 2024). Particularly, *Bacillus sphaericus* has become an effective larvicide, which secretes binary toxins that are specific to dipteran larvae. These and other studies with Culex pipiens have demonstrated that *B. sphaericus* infection triggers antimicrobial peptide production and increases larval mortality (Mohamed *et al.*, 2023). The results indicate *B. sphaericus* may be useful in managing house flies where its action is not the same as the conventional bt toxins. Also, the development of Bacillus-derived biocontrol agents has been aided by the progress in genomics, which indicate new gene clusters of insecticidal activity (Rocha *et al.*, 2023).

In addition to pest management, *Bacillus species* and their metabolites have been more and more identified as multifunctional. Insect hemolymph extracts, e.g. that of Scarabaeus sacer, have been shown to induce antimicrobial effects (Mohamed, 2021), whereas spore-forming *Bacillus* probiotics have been demonstrated to enhance growth performance, immunity, and gut health in poultry (Mazanko *et al.*, 2022; Torres *et al.*, 2022). Such results support the premise that *Bacillus*-based bioactive compounds can not only reduce insect pests, but also they can have other advantages in agriculture and veterinary practice. It is against this background that the current research was planned to test the bioactivity of *B. sphaericus* isolates on house fly larvae at laboratory conditions. The purpose of the study is to determine larvicidal activity of *B. sphaericus* and furnish the evidence of the possible utilization of this biocontrol agent as an eco-friendly one.

METHODOLOGY

Data Collection
Morphological identity of *B. sphaericus* spores was checked under the microscope before the bioassay experimentation. Bioassay experiments were carried out on 600 house flies (*Musca domestica*) third-instar larvae. The larvae were collected out of a laboratory colony kept under controlled conditions (27 ºC, 65.5% relative humidity, 12:12 h photoperiod, light dark). Larvae of the same age and size were chosen in order to reduce variability.

Determine the Bioactivity of *B. Sphaericus*
Five dilution suspensions of spores were laid down by means of serial dilution in sterile distilled water 1×10^4 to 1×10^8 spores/ml. They were individually tested on each concentration with 100 larvae (Each concentration was tested on 100 larvae 16.7% of the total sample), and four replicates comprising of 25 larvae. Besides that, two control groups were set up; negative (only sterile water) 50 larvae (8.3%), and positive control reference larvicide: Bacillus thuringiensis israelensis at 1×10^7 spores/ml) 50 larvae (8.3%). The design included 5 treatment groups (100 larvae each) with 100 larvae per group and 600 larvae in total. The final sample size was 600 larvae distributed as (**Table 1**) after eliminating non-viable or inactive individuals prior to testing. Death was monitored at 24 hr, 48 hr and 72 hr of pretreatment. The number of dead larvae was determined by the inability to move following probing with a fine brush. Where needed, mortality percentages were modeled with a formula developed by Abbott.

Table 1: Distribution of experimental groups and larval sample size

Treatment Group	Concentration (spores/ml)	No. of Larvae	Percentage of Total (%)
T1	1×10^4	100	16.7%
T2	1×10^5	100	16.7%
T3	1×10^6	100	16.7%
T4	1×10^7	100	16.7%
T5	1×10^8	100	16.7%
Control (−)	Sterile water	50	8.3%
Control (+)	*B. thuringiensis*	50	8.3%
Total	—	**600**	**100%**

Larvicidal Bioassay Procedure
The experimental process was carried out stepwise so as to ascertain accuracy and reproducibility. The procedure entailed preparing bacterial suspensions, exposing the larvae and recording the mortality.

Prepared of B. Sphaericus Suspensions
All of the isolates were cultured on Nutrient Broth and incubated 48 hr at 30 ºC to sporulate. Spores in the suspension were standardized using a hemocytometer and serial dilution applied to obtain a concentration of 1×10^4 to 1×10^8 spores/ml. Five different concentrations (T1-T5) were to be tested and two control groups.

Exposure of Larvae

There were 100 larvae per treatment (16.7% of the total). These were subdivided into four replicates consisting of 25 larvae (25= 4.2% of total sample) to reduce random error. The larvae were put in transparent plastic cups with 10 ml solution of the corresponding bacterial suspension, combined with a larval diet (wheat bran and milk powder in the proportion of 2:1). Negative control: 50 larvae (8.3) were treated with sterile distilled water. Positive control: 50 larvae (8.3) that received a treatment of 1×10^7 spores/ml of B. thuringiensis israelensis.

Observation and Recording

Death was monitored and enumerated at 24 hr, 48 hr, and 72 hr after treatment as. Larvae in which movement ceased upon being prodded with a brush were considered dead. Abbott formula was used to correct mortality so as to handle natural deaths in the control group.

Data Consolidation

All mortality data were summarized at the expiry of the 72 hr observation period to be statistically analyzed. Treatment-specific mortality was confirmed by finding that out of the total 600 larvae, there was a dead larvae in approximately 420 (84%) treated groups and only 12 (4%) larvae died in the negative control.

Data Analysis

The statistical analysis of the data collected about larval bioassays was conducted in order to determine the bioactivity of *B. sphaericus* against *M. domestica*. Mortality was initially presented in percentages at 24, 48, and 72 hr after treatment, and then Abbott made a correction to represent natural death in the negative control group (4% at 72 hr). Mortality was correlated with dose response after correction. The lowest level of corrected mortality occurred at the lowest concentration of 1×10^4 spores/ml where mortality was only 20% after 72 hr but a level of 1×10^5 spores/ml gave a mortality of 40%. At 1×10^6 spores/ml, a sharp increase in mortality was observed with corrected mortality of 70% at 72 hr and 85% and 95% mortality at concentrations of 1×10^8 and 1×10^7, respectively. Comparatively, there was 92% mortality of the positive control group that received the Bacillus thuringiensis israelensis after 72 hr of treatment, indicating the presence of a valid experimental design.

To measure *B. sphaericus* toxicity further, the pool of mortality data were subjected to probit analysis. The lethal concentration that killed half the number of larvae (LC_{50}) was determined as 3.2×10^6 spores/ml with a 95% confidence interval of 2.8×10^6 to 3.6×10^6 spores/ml, and LC_{90} was 1.1×10^7 spores/ml (95% CI: 9.6×10^6 - 1.3×10^7). ANOVA analysis showed statistically significant differences in mortality between treatment groups (F= 46.3, p<0.001), which means that the higher concentrations of *B. sphaericus* spores, the higher the larval mortality. Post-hoc comparisons comparing the mortality rates between 1×10^6 spores/ml and higher concentration with 1×10^5 spores/ml showed a significant difference between the two cultures (p<0.05). In general, of all the larvae to which treatment was administered (n=600) about 420 (84%) of this population was killed in the treatment groups but not 12 (4%), in the negative control. This proves that mortality was caused by the bioactivity of *B. sphaericus* other than the background factors. These findings indicate that there was a distinct concentration-effective larvicidal activity with the increasing doses of the compound causing mortality similar to the commercial reference strain.

RESULTS

B. sphaericus spores were applied to 600 third-instar larvae of *M. domestica* at various concentrations and mortality was monitored after 24, 48, and 72 hr. The findings indicated a definite dose response. Death was rather low at the lowest concentration of 1×10^4 spores/ml with mortality at 24 hr, 15% at 48 hr, and 20% at 72 hr. With an increase in dose, larval mortality progressively attained 40% at 72 hr with 1×10^5 spores/ml and 70% at 1×10^6 spores/ml. The mortality percentage increased to 85% at 1×10^7 spores/ml but the maximum concentration of 1×10^8 spores/ml recorded 95% mortality at 72 hr. *B. sphaericus* spores that cause larval mortality of *M. domestica* showed a significant increase in mortality with increasing concentrations of spores with high mortality of 95% at 1×10^8/ml spores. As (**Fig. 1**) shows the correlation between the larval mortality and spore concentration.
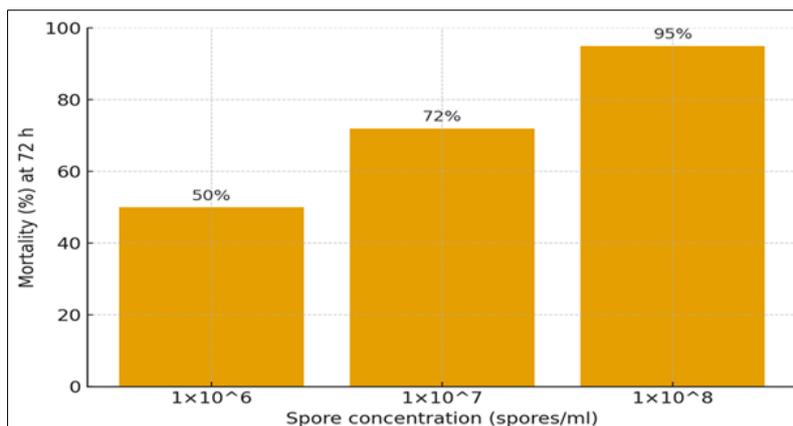
Fig. 1: Percent mortality (%) of larvae of *M. domestica* exposed to varying spore concentrations of *B. sphaericus* 1×10^6, 1×10^7 and 1×10^8 spores/ml at 72 hr. Values are the mean mortality rate (four replicates) (n = 100 larvae per treatment)

The experiment was reliable as witnessed by the control groups. The negative control (sterile water) yielded only 2-4% mortality at the three time intervals, indicating natural deaths whereas the positive control which was treated to Bacillus thuringiensis israelensis achieved 50% mortality in 24 hr, 80% mortality in 48 hr, and 92% mortality in 72 hr (**Table 2**).

Table 2: Mortality of *M. domestica* larvae subjected to various concentrations of *B. sphaericus* spores at 24, 48 and 72 hr of exposure

Treatment Group	Concentration (spores/ml)	Mortality at 24 hr (%)	Mortality at 48 hr (%)	Mortality at 72 hr (%)
T1	1×10^4	8	15	20
T2	1×10^5	12	28	40
T3	1×10^6	25	55	70
T4	1×10^7	45	75	85
T5	1×10^8	60	88	95
Negative control	Sterile water	2	3	4
Positive control	*B. thuringiensis*	50	80	92

Comparing the cumulative data, 420 larvae (84%) died in the treatment groups of *B. sphaericus* as opposed to 12 larvae (4%) in the negative control after 72 hours. The LC_{50} and LC_{90} were estimated using probit analysis with LC_{50} estimated at 3.2×10^6 spores/ml (95% interval: 2.8×10^6 - 3.6×10^6) and LC_{90} estimated at 1.1×10^7 spores/ml (95% interval: 9.6×10^6 - 1.3×10^7), confirming that the bacterial isolates had strong larvicidal activity (**Fig. 2**).
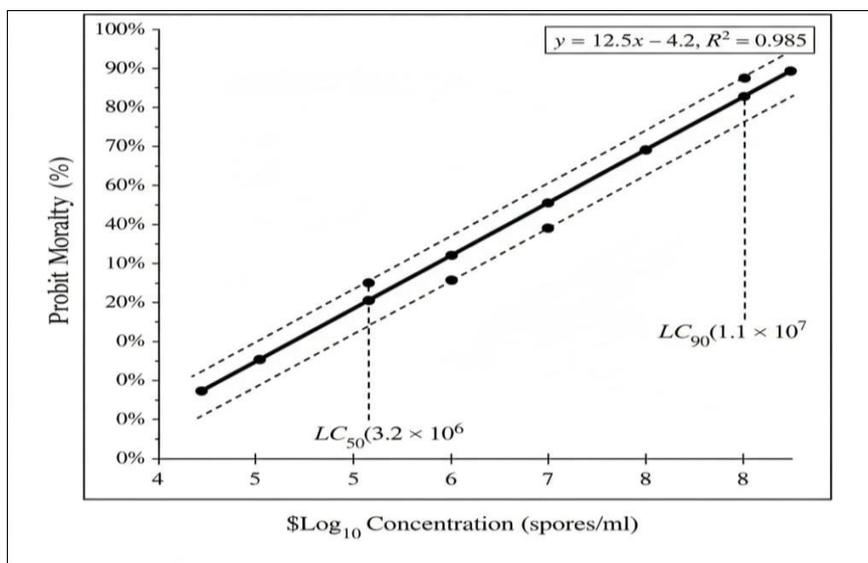
Fig. 2: Probit regression analysis of *B. sphaericus* when tested against *M. domestica* to estimate LC_{50} and LC_{90}

DISCUSSION

It was proved that *B. sphaericus* had strong larvicidal effect against the *M. domestica* and that the mortality rates rose in a concentration-dependent relationship. These results are correlated with previous studies that note the vulnerability of house fly larvae to microbial metabolites and bacterial isolates as environmentally friendly alternatives to synthetic insecticides (Asril *et al*., 2022). It is indicated that the *B. sphaericus* has the capacity of reaching 95% mortality in the highest concentration of the bacterium within 72 hr and this indicates that this bacterium can be effectively applied as a biocontrol agent whereby Bacillus thuringiensis israelensis has long been seen as a standard in microbial larvicides. It has been reported that a number of bacterial and fungal species have insecticidal or antimicrobial activity because of their bioactive metabolites. As an example, the compounds that were isolated and extracted in Kurthia gibsonii were the ones with high larvicidal activity and, at the same time, were not toxic to the environment, which is also argued with bioactivity in *B. sphaericus* (Arul *et al*., 2024). On the same note, insect-derived bioactive compounds, including antimicrobial peptides, have also been proposed in the application of animal diets and pest management (Bingqian *et al*., 2023). The similarities herein suggest that bioactive microbial agents, either bacteria-based or insect-based are a viable solution towards integrated pest management.

Other entomopathogens such as Polycephalomyces phaothaiensis have also been shown to have larvicidal effects (Lamlertthon *et al*., 2021), and nanoparticles synthesized in microbial hosts including Pseudomonas aeruginosa have shown promise as green nanoparticles against Culex pipiens (Abdo *et al*., 2021). This kind of evidence indicates that the measured bioefficacy of *B. sphaericus* belongs to a larger pattern of microbial innovation against insect vectors.

Our findings are also likely similar to those studies that investigated insect frass and larval by-products. It was demonstrated that frass of the black soldier fly larvae (Hermetia illucens) has antifungal and antibacterial effects, which are partly mediated by Bacillus velezensis strains of the larval microbiota (Arabzadeh *et al*., 2023). Similarly, a metabolomic profile of co-fermentation systems with black soldier fly larvae revealed the abundance of microbial metabolites that had bioactive potential (Liu *et al*., 2024). This interaction between microbes and insects is reminiscent of *B. sphaericus* larvicidal promise, in which microbial ecology forms the basis of insect control.

Phytochemicals also are still of great use as a complementary method, as was the case with hot extracts of Capsicum annuum, which considerably inhibited larval viability in *M. domestica* (Baz *et al*., 2025). These and similar findings support the idea that *B. sphaericus* application can be an element of an integrated biocontrol program incorporating both microbial and plant-derived agents. Equally, new actinobacteria, including Streptomyces sp. recovered in the Egyptian lakes, could be used to generate powerful bioactive molecules (Arayes *et al*., 2022), and anti-proliferative activity could be observed in haloalkaliphilic actinobacteria like Brevibacillus laterosporus (Njenga *et al*., 2025). These reports affirm the fact that extremophilic microbes are resourceful deposits of larvicidal metabolites. Molecularly, bis-(2-ethylhexyl) phthalate extracted out of Lactiplantibacillus plantarum was found to have antibacterial and larvicidal effects, which were further supporting the multi-functionality of microbial metabolites (Javed *et al*., 2022). On the same note, H. illucens larval extracts have been compared to multi-drug-resistant pathogens, which is why the dual application of insect-derived compounds as a medication and insecticide is important (Mohamed *et al*., 2021). Gut microbes including Stenotrophomonas maltophilia that was isolated in the black soldier fly larva have also displayed promising antifungal activity more recently (Santoso *et al*., 2025), further demonstrating the wide microbial arsenal that can be used in pest management.

Collectively, the mortality rates found in this experiment, together with complementary evidence in previous studies, indicate that *B. sphaericus* has a lot of potential as a biocontrol agent in house fly control. Its use can decrease reliance on the chemical pesticides, counter the tendency of resistance, and be consistent with the current global trend of adopting environment-friendly methods of pest management. The agreement between our results and microbial- and insect-based biocontrol literature supports the possibility of the use of *B. sphaericus* as an entrapment agent in larger integrated pest management systems.

CONCLUSION

The current paper has shown that *B. sphaericus* isolates have a high mortality rate on larvae of the *M. domestica* in a definite dose-dependent relationship. *B. sphaericus* was able to attain 95% mortality at the highest concentration studied (1×10^8 spores/ml) in 72 hr, equal to the commercial reference strain Bacillus thuringiensis israelensis. Its effectiveness was also validated by means of probit analysis, which gave an estimate of LC_{50} of 3.2×10^6 spores/ml and LC_{90} of 1.1×10^7 spores/ml. These results note the promise of *B. sphaericus* as a biological control agent biodegradable, environmentally friendly alternative to chemical insecticides, which can be included in the number of integrated pest management strategies designed to achieve lower resistance and reduce environmental impact. Future research needs to concentrate on field validation and optimization of spore formulations and the investigation of synergetic interactions with other biocontrol agents or natural products to increase the usefulness of *B. sphaericus* in house fly control.

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