COMPATIBILITY OF CHITOSAN AND LIGNOSULPHONATE WITH ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (HYPOCREALES: CLAVICIPITACEAE)

Abstract

Insect pests cause substantial yield losses in agriculture, prompting widespread use of chemical pesticides that threaten environmental and human health. Biological control using *Beauveria bassiana* offers a sustainable alternative, but its efficacy is often limited by environmental stresses and formulation challenges. Encapsulating *B. bassiana* conidia with biopolymers helps protect the fungus from environmental stresses and improves shelf life by acting as a physical barrier. This study evaluated the compatibility of biopolymers, chitosan (0.1 %, 0.25 %, 0.5%, 1 % and 2.5 %) and lignosulphonate (1 %, 2.5 %, 5 %, 7.5 % and 10 %) with *B. bassiana* by assessing mycelial growth, spore production and virulence against *Spodoptera litura* larvae. Low chitosan concentrations (0.1–0.5 %) maintained high radial growth (up to 99.60 %), sporulation (2.00×10^8 spores/mL) and larval mortality (88.33 %). However, at the highest concentration (2.5 %), chitosan significantly reduced *B. bassiana* performance, with radial mycelial growth declining to 90.21%, spore production dropping to 1.33×10^8 spores/mL and larval mortality decreasing to 75.00 %. Lignosulphonate demonstrated superior compatibility, with mycelial growth above 98.8 % and larval mortality above 85 % at all concentrations. The control (without any added biopolymers) achieved 100 % mycelial growth, 2.00×10^8 spores/mL and 91.67 % larval mortality. These results highlight lignosulphonate and low-dose chitosan as promising encapsulating agents for stable, effective *B. bassiana* formulations.

Keywords: Biological Control, *Beauveria bassiana,* Compatibility, Chitosan, Lignosulphonate

1. INTRODUCTION

Insect pests represent a significant global threat to agricultural productivity, capable of devastating crops and causing substantial economic losses while jeopardizing food security. Worldwide, insect pests are estimated to reduce crop yields by 18–20 %, resulting in annual economic losses of approximately $470 billion (Bihal *et al*., 2023). In India, insect pests cause an average annual yield loss of 15.7% in major crops, with associated economic losses reaching US $36.0 billion (Dhaliwal *et al*., 2015; Kumar *et al*., 2021). To address these threats, farmers have increasingly relied on chemical pesticides for pest management. Insecticides account for nearly 51% of total pesticide use in India, where agricultural systems spend an estimated Rs. 1,200 crores annually on chemical insecticides to control insect pests (Jayaraj *et al*., 2016; Agarwal and Pandey, 2017). However, this heavy dependence on synthetic pesticides has led to widespread environmental contamination, the presence of harmful residues in food and serious health risks for humans and wildlife, including endocrine disruption, cancer, reproductive disorders and heightened vulnerability among children (Ahmad *et al*., 2024). Moreover, pesticides negatively affect plant physiology, disrupt pollinator populations and degrade soil health, all of which further undermine the sustainability of agricultural systems (Wan *et al*., 2025). These impacts highlight the urgent need for more sustainable pest management strategies to safeguard both agricultural productivity and ecosystem health. Biological control using entomopathogenic fungi has

emerged as a safer and more sustainable alternative. An entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is increasingly recognized as a highly effective biocontrol agent due to its strong virulence against many insect pests, offering a sustainable alternative to synthetic pesticides (Zhang *et al*., 2020). Its use supports both environmental health and food security (Kulu *et al*., 2015). However, practical application in agriculture is limited by its sensitivity to temperature and UV light, short shelf life and low persistence, which complicate storage, transport and field use (Parra, 2014; Singh *et al*., 2016; Mohamed *et al*., 2024). Encapsulating *B. bassiana* conidia with biopolymers helps protect the fungus from environmental stresses and improves shelf life by acting as a physical barrier (Herlinda *et al*., 2018). To ensure effective and safe bioinsecticide products, it is essential that chosen biopolymers are compatible with *B. bassiana* and do not harm its growth or activity (Felizatti *et al*., 2021). Thus, compatibility studies are a key step in developing stable and sustainable biopolymer-based pest management solutions. This experimental approach aimed to understand whether the selected encapsulating agents could be effectively used in microencapsulation without compromising the entomopathogenic potential of *B. bassiana*, thus aiding in the development of stable and efficient biocontrol formulations.

2. MATERIALS AND METHODS

To evaluate the in vitro compatibility of chitosan and lignosulphonate with *B. bassiana*, stock solutions of each biopolymer were prepared in sterile distilled water at 1 % (w/v) and further diluted as needed. For chitosan, working solutions were made to achieve final concentrations of 0.1 %, 0.25 %, 0.5 %, 1 % and 2.5 % (w/v) in molten, cooled potato dextrose agar (PDA), while for lignosulphonate, the corresponding concentrations were 1 %, 2.5 %, 5 %, 7.5 % and 10 % (w/v). Approximately 12–15 mL of each amended PDA was aseptically poured into sterile Petri plates then plates were turned clock wise and anti-clock wise for even distribution of the media and allowed to solidify. As a control, PDA without any biopolymer was also prepared. From a 7- day old *B. bassiana* culture, 5 mm mycelial discs were cut and aseptically placed at the center of each plate. Each treatment, including the control, was replicated three times. After sealing, all plates were incubated at $25 \pm 2°C$ in a biological oxygen demand (BOD) incubator. Fungal growth was monitored by measuring the radial expansion of the mycelium by comparing treated plates with the control plates.

After 15 days of fungal inoculation, the sporulated cultures of *B. bassiana* were harvested from each Petri plate by adding 50 mL of sterile distilled water. The fungal mat was gently scraped using a sterile spatula to ensure uniform suspension of conidia. The resulting spore suspension was thoroughly mixed and filtered through a double layer of sterile muslin cloth to remove mycelial debris and ensure a homogeneous spore solution. The concentration of spores (conidia) in the suspension was then quantified using a Neubauer haemocytometer under a compound microscope. Each treatment, including the control, was replicated three times. The spore count was expressed as the number of conidia per millilitre (spores mL^{-1}) and this data was used to estimate the total spore production from individual Petri plates.

Subsequently, the prepared spore suspensions were used for bioassay experiments to evaluate the virulence of *B. bassiana* in the presence of two potential encapsulating agents viz., chitosan and lignosulphonate. These bioassays were designed to assess the compatibility of the polymers with the fungal pathogen, particularly focusing on their influence on the

Comment [h1]: Make italic

pathogenicity of *B. bassiana* against *Spodoptera litura*, a major lepidopteran pest. The bioassays were conducted using the leaf dip method, wherein castor leaves were dipped in the respective spore suspensions (treated with or without polymers), air-dried. 20 insects per replication were taken and fed to early instar larvae of *S. litura* under controlled laboratory conditions with three replications per treatment. Larval mortality was recorded at regular intervals post-treatment to determine any possible inhibitory or synergistic effects of chitosan and lignosulphonate on the infectivity and virulence of *B. bassiana*.

3. RESULTS AND DISCUSSION

Table 1: Effect of chitosan and lignosulphonate on mycelial growth, sporulation and insecticidal activity of *Beauveria bassiana* against *Spodoptera litura*

Treatments	Mycelial growth (%)*	Spores per ml (n x 10⁸)**	Larval mortality (%) (*S. litura*)*
Chitosan- 0.1 %	99.22 (87.06)[ab]	2.00 (1.73)	88.33 (70.09)[abc]
Chitosan - 0.25 %	98.83 (84.98)[ab]	2.00 (1.73)	88.33 (70.09)[abc]
Chitosan -0.5 %	99.60 (87.90)[ab]	2.00 (1.73)	88.33 (70.09)[ab]
Chitosan -1 %	98.04 (82.01)[b]	1.67 (1.63)	85.00 (67.38)[b]
Chitosan -2.5 %	90.21 (71.77)[c]	1.33 (1.52)	75.00 (59.98)[c]
Lignosulphonate- 1 %	99.22 (85.82)[ab]	2.00 (1.73)	88.33 (70.09)[abc]
Lignosulphonate - 2.5 %	99.22 (85.83)[ab]	2.00 (1.73)	88.33 (70.09)[abc]
Lignosulphonate - 5 %	98.82 (84.96)[ab]	2.00 (1.73)	86.67 (68.67)[abcd]
Lignosulphonate - 7.5 %	98.83 (84.98)[ab]	1.67 (1.63)	85.00 (67.38)[bcd]
Lignosulphonate - 10 %	98.84 (86.40)[ab]	1.67 (1.63)	85.00 (67.38)[bcd]
CONTROL	100.00 (90.00)[a]	2.00 (1.73)	91.67 (73.37)[a]
SEm (±)	2.28	0.06	1.72
CD (*P*=0.01)	6.72	NS	5.08

*Values mentioned in parenthesis are arc sine converted mean values. ** Values mentioned in parenthesis are square root transformed mean values. Means followed by same letter indicates no significant difference between the treatment according to Duncan's Multiple Range Test (*P*=0.01)

3.1 Effect of chitosan and lignosulphonate on mycelial growth, sporulation of *B. bassiana*

The compatibility of *Beauveria bassiana* with different concentrations of chitosan and lignosulphonate was evaluated based on mycelial growth and spore production.

In terms of mycelial growth, the untreated control recorded the highest value at 100 %, which was statistically on par with most treatments including chitosan at 0.1 %, 0.25 % and 0.5 %, which recorded 99.22 %, 98.83 % and 99.60 % growth respectively. These three concentrations of chitosan did not significantly differ from the control, indicating high compatibility. Chitosan at 1 % showed slightly reduced growth (98.04 %) but was still statistically comparable with the control and lower concentrations. However, a notable reduction in mycelial growth was observed with chitosan at 2.5 %, which recorded 90.21% growth and was significantly lower than the control and the other lower concentrations, indicating a potential inhibitory effect at higher concentration. Lignosulphonate at all tested concentrations (1 % to 10 %) exhibited good compatibility with *B. bassiana*, with mycelial growth ranging from 98.82 % to 99.22 %. All lignosulphonate treatments were statistically on

Comment [h2]: Add comma after growth

par with the control and with each other, suggesting that lignosulphonate, regardless of concentration, does not hinder fungal growth. Regarding sporulation, the control treatment yielded 2.00×10^8 spores/ml. Similar sporulation levels were maintained across most treatments, including chitosan at 0.1 %, 0.25 % and 0.5 % and lignosulphonate at 1 %, 2.5 % and 5 % all recording 2.00×10^8 spores/ml. Chitosan at 1 % and lignosulphonate at 7.5 % and 10 % showed slightly reduced sporulation (1.67×10^8 spores/ml), though still relatively close to the control. The lowest spore count was observed with chitosan at 2.5 %, which recorded 1.33×10^8 spores/ml, indicating a significant reduction compared to the control and other treatments.

> **Comment [h3]:** Discuss the present findings with few recent reviews, if avial

3.2. Effect of chitosan and lignosulphonate on insecticidal efficacy of *B. bassiana* against *S. litura*

The larval mortality of *S. litura* treated with *B. bassiana* in combination with different concentrations of biopolymers showed variable results. The control treatment resulted in the highest larval mortality at 91.67 %, which was statistically on par with several other treatments. Chitosan at 0.1 %, 0.25 % and 0.5 % each caused 88.33 % larval mortality, which was not significantly different from the control, indicating that these concentrations did not negatively affect the insecticidal efficacy of *B. bassiana*. Similarly, lignosulphonate at 1 % and 2.5 % also resulted in 88.33 % mortality, again on par with the control. Lignosulphonate at 5 % caused slightly lower mortality at 86.67 %, and although numerically less, it was still statistically comparable with the control and other effective treatments. Chitosan at 1 % and lignosulphonate at 7.5 % and 10 % each recorded 85.00 % mortality, also on par with the better-performing treatments. However, chitosan at 2.5 % resulted in only 75.00 % larval mortality, which was significantly lower than the control and other adjuvant combinations. This suggests that chitosan at 2.5 % may reduce the virulence of *B. bassiana* against *S. litura*. These observations align with the findings of Palma-Guerrero *et al*. (2010), who reported that low concentrations of chitosan stimulated or did not inhibit fungal growth and conidiation in *B. bassiana*. Consistently, in this study, chitosan at 0.1 % to 0.5 % supported optimal fungal performance and insecticidal activity, highlighting its potential as a suitable formulation additive at appropriate concentrations.

> **Comment [h4]:** Discussion part is po so try to discuss with few more and rece reviews

4. CONCLUSION

This study demonstrates that both chitosan and lignosulphonate are compatible with *B. bassiana* at lower concentrations, supporting robust mycelial growth, high spore production and strong insecticidal activity against *S. litura* larvae. Lignosulphonate showed excellent compatibility across all tested concentrations, while chitosan was effective up to 1 %, beyond which fungal growth and efficacy declined. These findings highlight lignosulphonate and chitosan as promising encapsulating agents for developing stable, efficient and environmentally friendly *B. bassiana*-based biocontrol formulations, offering a sustainable alternative to chemical pesticides in integrated pest management.

REFERENCES

Agarwal, P., & Pandey, D. (2017). Impact of Pesticide: An Overview. Trends in Biosciences, 10(6), 1341-1344.

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil, K., ... & Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon.

Bihal, R., Al-Khayri, J. M., Banu, A. N., Kudesia, N., Ahmed, F. K., Sarkar, R., Arora, A., & Abd-Elsalam, K. A. (2023). Entomopathogenic Fungi: An Eco-Friendly Synthesis of Sustainable Nanoparticles and Their Nanopesticide Properties. Microorganisms, 11(6), 1617. https://doi.org/10.3390/microorganisms11061617

Dhaliwal, G. S., Jindal, V., & Mohindru, B. (2015). Crop losses due to insect pests: Global and Indian Scenario. Indian Journal of Entomology, 77, 165-168.

Felizatti, A. P., Manzano, R. M., Rodrigues, I. M. W., da Silva, M. F. D. G. F., Fernandes, J. B., & Forim, M. R. (2021). Encapsulation of B. bassiana in Biopolymers: Improving Microbiology of Insect Pest Control. Frontiers in Microbiology, 12, 704-812.

Herlinda, S., Rizkie, L., Suwandi, Susilawati, Lakitan, B., & Verawaty, M. H. (2018). Effects of high temperature and ultraviolet-C irradiance on conidial viability and density of B. bassiana and Metarhizium anisopliae isolated from soils of lowland ecosystems in Indonesia. Eurasian Journal of Analytical Chemistry, 13, 209–216.

Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary Toxicology, 9(3-4), 90-100.

Kulu, I. P., Abadi, A. L., Afandhi, A., & Nooraidawati, N. (2015). Morphological and molecular identification of B. bassiana as entomopathogen agent from Central Kalimantan peatland, Indonesia.

Kumar, J., Murali Baskaran, R. K., Jain, S. K., Sivalingam, P. N., Dixit, A., Mallikarjuna, J., & Ghosh, P. K. (2021). Biotic stresses of agricultural crops in India: re-visiting national status and mitigation strategies. Current Science, 120(2), 264-265.

Mohamed, H. F., Sweilem, M. A. E., El-Sharkawy, R. M., & El-khawaga, O. E. A. A. (2024). Impact of Temperature and Ultra-Violet Radiation on the effectiveness of Beauveria bassiana inverted emulsion formulation on Spodoptera littoralis. Benha Journal of Applied Sciences, 9(8), 21-27.

Palma-Guerrero, J., Larriba, E., Güerri-Agulló, B., Jansson, H. B., Salinas, J., & Lopez-Llorca, L. V. (2010). Chitosan increases conidiation in fungal pathogens of invertebrates. *Applied microbiology and biotechnology*, *87*, 2237-2245.

Parra, J. R. P. (2014). Biological control in Brazil: an overview. Scientia Agricola, 71, 420-429.

Singh, H. B., Sarma, B. K., & Keswani, C. (2016). Agriculturally important microorganisms. Singapore: Springer Engineering Journal, 169(1-3), 1-10.

Wan, N. F., Fu, L., Dainese, M., Kiær, L. P., Hu, Y. Q., Xin, F., ... & Scherber, C. (2025). Pesticides have negative effects on non-target organisms. Nature Communications, 16(1), 1360.

Zhang, L., Fasoyin, O. E., Molnár, I., & Xu, Y. (2020). Secondary metabolites from hypocrealean entomopathogenic fungi: Novel bioactive compounds. Natural Product Reports, 37(9), 1181–1206.

Comment [h5]: Check all the referen as per journal guideline