Membrane separation process (MSP) for the potentialization of biological pesticides to control *Spodoptera frugiperda* (J.E. Smith, [1797]) (Lepidoptera: Noctuidae)

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ABSTRACT

One of the main challenges in purifying biopesticides is the selective separation of target compounds from other components present in the fermented broth. This scenario requires the optimization of purification techniques that can effectively isolate bioactive compounds, minimizing loss of activity and maintaining their stability. The membrane separation process (MSP) holds considerable promise for the purification and formulation of biopesticides, offering an efficient approach to isolating and concentrating bioactive compounds from complex mixtures. Accordingly, the purpose of this study was to perform MSP on distinct fermented broths of bioagents obtained by bioscreening to evaluate the potential control of *Spodoptera frugiperda*. The collected biomaterials were superficially sterilized, placed in Petri dishes with Potato Dextrose Agar (PDA), and went through the submerged fermentation process at 28 ºC and 120 rpm for seven days. Subsequently, the broth was centrifuged at 4000 rpm and 10 ºC for 10 min and filtered using a vacuum pump. Finally, the assays were subjected to MSP with a 0.45 µm nylon membrane. The control rate for *S. frugiperda* was up to 40%. Appropriately, the MSP indicated promising results as a breakthrough technology for the biopesticides formulation process, based on optimizing and improving the control potential of harmful pests.

Keywords: Bioscreening. Fermented broth. Food security. Pest management. Purification.

1 INTRODUCTION

One of the main challenges in the field of biopesticides is the purification process required to isolate secondary metabolites from crude extracts. Secondary metabolites, which often have bioactive properties, are synthesized by microorganisms or plants and play a crucial role in the effectiveness of biopesticides. Nonetheless, obtaining these secondary metabolites in pure form presents several obstacles, such as low concentrations of these substances in the crude extract, requiring efficient concentration techniques to increase yields (GUPTA et al., 2023).

Contextually, the membrane separation process (MSP) emerges as an effective and widely applied technique. The basic mechanism of PSM technology consists of a highly effective sieve effect, in which the separation process occurs due to the distinction of particle size, in addition to other characteristics, such as shape, charge, and interactions between the membrane and the particles of the filtered biomaterial (CONIDI et al., 2018). Furthermore, the PSM procedure comprises distinct primordial mechanisms: exclusion by material size, repulsion/attraction, and hydrophobic/hydrophilic interactions retaining thicker molecules and isolating smaller molecules. Additionally, MSP offers high selectivity, enabling precise separation of target compounds and minimizing the loss of valuable components (TODERO et al., 2019).

Although MSP holds promise for the efficient purification of biopesticides, there is a need for comprehensive research to optimize and validate this approach (SANTOS et al., 2023). Appropriately, the purpose of this study was to concentrate the fermented broth of 40 bioagents isolated by bioscreening, using MSP and, subsequently, formulate bioproducts with high biopesticide potential to indicate a potential phytotoxic effect in the control of *Spodoptera frugiperda*.

2 MATERIAL & METHODS

The monitoring and collection of microbial agents were performed in agricultural cultivation areas in different areas of the Northwest and Central regions of Rio Grande do Sul and Goiás, Brazil. The insect pests, killed naturally, were obtained in areas with considerable populations of pests and which were not subjected to the application of pesticides before collection. Collection of individual pests was conducted based on scanning the cultivation area. The collected insects were sent to the Biotec Factory® Laboratory at the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil. The first step consisted of isolating the microbial agents present in the dead insects. The following methodology was adopted: (a) the insect corpses were sent to the laboratory separately in sterile tubes; (b) the insects were observed under a binocular biological microscope (40–1,600× Illumination) (TIM-107, Zeiss-Opton, Germany), at 40× resolution to verify the level of damage and the potential for propagation of the insect. fungus; (c) in locations with easily visible damage, insects were surface sterilized using 70 wt% ethanol and 0.5 wt% NaOCl (>99.0%, Sigma-Aldrich, Germany) for 3 min, with three subsequent washes with 100 mL of sterilized water and then the sporulated fungus from the insect corpse was quickly added to Petri dishes along with the culture medium; (d) dead insects with the presence of fungi were placed in culture medium in a Biochemical Oxygen Demand (BOD) Incubator, at 25 ºC, for seven days; in cases of non-germination, the dead insects were placed in Petri dishes containing selective (fermentative)

medium; (e) the fungi obtained were cultivated in PDA (Potato, Dextrose and Agar) (Sigma-Aldrich, Germany) (Sigma-Aldrich, Germany), at concentrations of 39 g of PDA /L of distilled water until pure culture was obtained. The medium was previously autoclaved at 121 ºC±1 ºC for 30 min and then placed in Petri dishes. To maintain a considerable size of the population of each bioagent, constant subcultures were conducted.

The submerged fermentation (FS) process was performed using a 250 mL Erlenmeyer flask filled with 125 mL of Potato Dextrose (PD) culture medium and sterilized in an autoclave at 121 ºC ± 1 ºC for 30 min. Fungi and bacteria were visually identified based on the morphological characterization of the bioagent and its arrangement in the Petri dish. The fungal fermentation process consisted of (g L^{-1}): glucose (≥99.5%, Sigma-Aldrich, Germany), 10.0; yeast extract (Titan Biotech Ltd, India), 7.5; peptone (Titan Biotech Ltd, India), 10.0; $(NH_4)_2SO_4$ (>99.0%, Labsynth, Brazil), 2.0; $FeSO_4 \cdot 7H_2O$ (99.0%, Labsynth, Brazil), 1.0; $MnSO_4 \cdot H_2O$ (98.0%-101.0%, ACS Científica, Brazil), 1.0; and $MgSO_4$ (98.0%, Sigma-Aldrich, Germany), 0.5. For bacterial fermentations, the methodology corresponded to (g L^{-1}): meat extract (Titan Biotech Ltd, India), 1; bacteriological peptone (Titan Biotech Ltd, India), 5; yeast extract (Titan Biotech Ltd, India), 2; and NaCl (99.0%, Dinâmica – Química Contemporânea LTDA, Brazil), 5. The conditions of the submerged fermentation process were 28 ºC and 120 rpm for seven days in an incubator with orbital shaker (New BrunswickTM Innova 44, USA).

After the fermentation process, the cells were separated by vacuum filtration in a vacuum pump (SL-61, Solab, Brazil), with 12.5 cm filters (Qualy, Jacareí, Brazil), and by centrifugation at 4000 rpm and 10 ºC for 10 min (Eppendorf, model 5804R). Finally, the tests were subjected to MSP with membrane filtration (Merck Millipore), with a pore size of 0.45 µm and a diameter of 47 mm in a PSM system coupled to a vacuum pump with a pump power of 300 W.

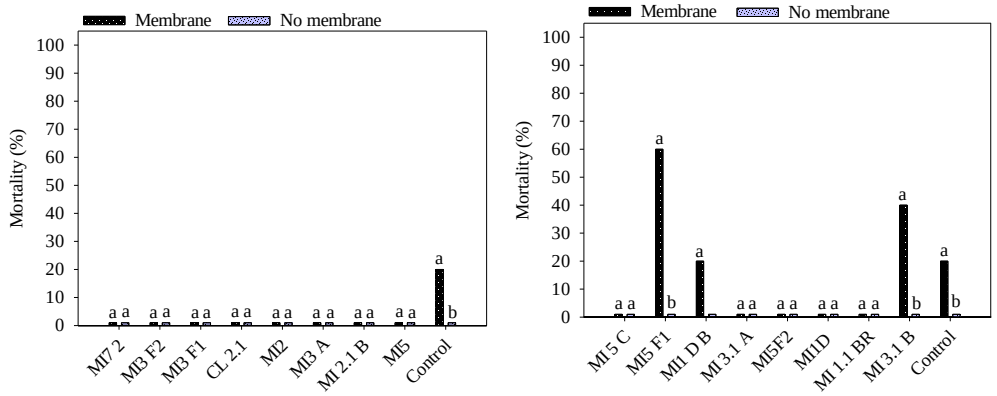
The application of the fermented solutions was conducted automatically by DeVries Generation III in a spray chamber (DeVries Manufacturing, Hollandale, United States). The system calibration for fermented solution applications was approximately 200 L ha^{-1} at a working speed of 2.7 km h^{-1}. Applications occurred in the L1/L2 larval stage. Finally, mortality assessments were investigated from 24 h to 240 h after applications. Abbott's universal equation was applied to indicate the corrected control mortality and calculate the total effect of each fermented broth, based on the formula:

$$\text{Corrected mortality} = \frac{\text{mortality in } treatament\ (\%) - \text{mortality in } control\ (\%)}{100 - \text{mortality in } control\ (\%)} \quad (1)$$

The data obtained were subjected to normality and homogeneity analysis and, when significant, to variance comparison analysis via the Tukey test, at a significance level of 95% ($p<0.05$). Therefore, statistical analysis was performed using the statistical software Sisvar 5.6.

3 RESULTS & DISCUSSION

Based on a preliminary investigation of the microbial agents collected for this study, 40 microorganisms were isolated from dead insects. The bioagents MI5F1, MI1D B, MI3.1 B, MIF, P1, OL7, and 0L5 and the control treatment demonstrated a statistical difference between the PSM and the control treatment (Figure 1). Considering this species, only 5 bioagents reported higher mortality effects for MSP: MI5F1, MI1D B, MI3.1 B, MIF, and P1. These microorganisms showed mortality rates of 60%, 20%, 40%, 50%, and 25% for PSM, respectively. The microorganisms did not express potential control under the control treatment, except MIF, which indicated a mortality rate of only 25%. Concerning the control treatment, the treatments were 20% and 0% for PSM and no membrane conditions, respectively. Finally, treatments based on the higher mortality rate in membrane treatments compared to MSP were indicated as six bioagents (OL7, OL5, SM, 15.1, BR2, and BR4). The results expressed in this study suggested mortality rates of 40%, 40%, 40%, 20%, and 80% in the membrane-free condition, respectively. These results were superior to the control rate in the MSP condition. Furthermore, this dimension details that the use of MSP was advantageous for a small number of bioagents isolated in this study, and the most significant portion did not express action potential for the control of *S. frugiperda*. Nonetheless, it is appropriate to highlight the importance of intensifying the exploration of microorganisms that indicated higher control in the MSP condition, since the MSP treatment expressed zero mortality rate.



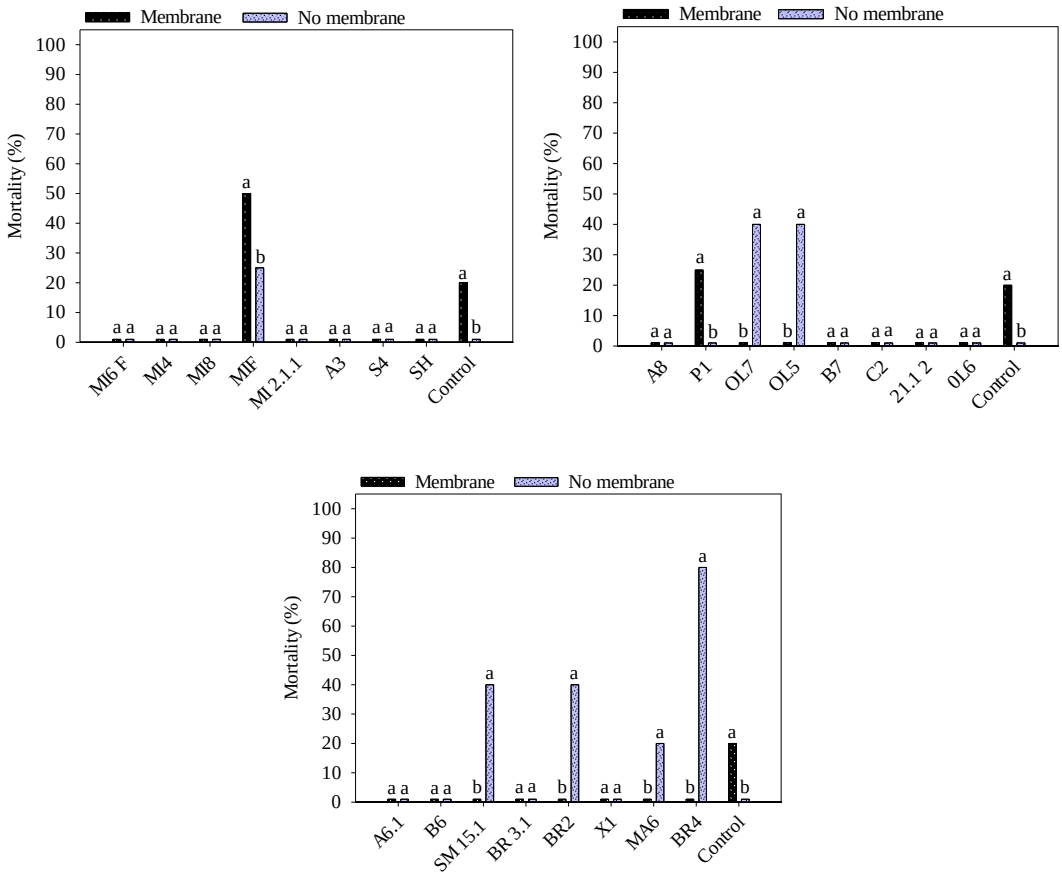


Figure 1 Abbott mortality rate (%) of 40 bioagents isolated from bioscreening for the potential control of *Spodoptera frugiperda* (J.E. Smith, [1797]) (Lepidoptera: Noctuidae) at the L1/L2 larval stage. *Averages followed by the same letter in the bars do not differ statistically from each other using the Tukey test at 5% probability.

4 CONCLUSION

This study reported the application of 40 microbial agents to control *Spodoptera frugiperda*. Three bioagents were indicated that promoted mortality effects of up to 40%. Other bioagents indicated effects of at least 20%. With further research and development, MSP has the potential to revolutionize biopesticides production, facilitating the development of safer, more effective, and environmentally friendly solutions for pest management in agriculture. Accordingly, the application of MSP for biopesticides purification holds great promise in advancing sustainable agricultural practices and addressing global pest control and food safety challenges.

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