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BIOFORMULATIONS WITH *Bacillus velezensis* S26 ENDOSPORES CONTROL FUNGAL DISEASES IN STRAWBERRIES

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ABSTRACT

Bacillus velezensis is an endospore-forming bacterium that plays a crucial role in the biological control of fungal diseases. Endospores are resistant structures that ensure the development of bioformulations with extended shelf life. Additionally, utilizing agro-industrial residues reduces manufacturing costs and preserves bacterial viability during storage. This study aimed to develop a liquid formulation containing *B. velezensis* S26 endospores using agro-industrial by-products and to assess its effectiveness in managing strawberry diseases. Four distinct formulations were prepared using liquid carriers, such as 0.85 % NaCl solution (Bioformulation 1) and combinations of composting leachate and whey (Bioformulations 2, 3, and 4). These bioproducts were foliar sprayed on strawberries cultivated in a commercial greenhouse from May to October 2023. The product Duravel® (*Bacillus amyloliquefaciens* MBI600) served as a control. The experiment followed a randomized complete block design, with three replicates and fifteen plants per plot. Disease incidence and severity were determined at fifteen-day intervals. The experiment revealed that all formulations exhibited efficacy similar to Duravel®. Therefore, bioformulations containing *B. velezensis* S26 endospores effectively controlled anthracnose and gray mold in strawberry plants.

Keywords: Agro-industrial residues. Anthracnose. Composting leachate. Gray mold. Whey.

1 INTRODUCTION

The cultivation of strawberries has been gaining prominence in Brazilian fruit growing. The country is among the top ten largest producers of this fruit worldwide[1]. However, fungal diseases, including anthracnose and gray mold, can cause significant economic losses[2]. Traditional disease management methods, which rely on synthetic fungicides, have adverse effects on the environment and pose risks to human health. Consequently, biocontrol strategies, including using antagonistic bacteria, have garnered interest. The *Bacillus* genus encompasses Gram-positive bacteria capable of inhibiting phytopathogens, stimulating plant defense mechanisms, and enhancing plant growth[3].

These bacteria also can form resistant structures called endospores. Thus, incorporating bacterial endospores into bioformulations extends the shelf life and stability of biofungicides. Nevertheless, developing effective bioformulations utilizing these beneficial bacteria requires meeting specific criteria, including resistance to environmental conditions, consistent biocontrol activity, low toxicity, long shelf life, and cost-effectiveness[4]. In this regard, agro-industrial wastes, such as whey and composting leachate, can serve as culture media and carriers in formulations, offering an appropriate environment for microorganisms' sustenance[5].

The present study aimed to develop a liquid formulation containing *Bacillus velezensis* S26 endospores using composting leachate from grape pomace and whey and assess its effectiveness in controlling anthracnose and gray mold in strawberry plants.

2 MATERIAL & METHODS

B. velezensis S26 was isolated from the rhizospheric soil of an organic vineyard in Caxias do Sul, Rio Grande do Sul State, Brazil[6]. The bacterial pre-inoculum was prepared by transferring a loop of a colony-forming unit into a plastic flask containing 10 mL of LB liquid medium (1% peptone, 0.5% yeast extract, and 0.5% sodium chloride, pH 7.2). This culture was incubated on a rotary shaker at 130 rpm and 28 ºC. After 24 hours, the pre-inoculum was added to an Erlenmeyer flask containing 90 mL of LB liquid medium. Four flasks containing 100 mL of bacterial pre-inoculum were prepared.

Batch cultivation was carried out in a 7-L bench bioreactor BioFlo/Celligen 115 (New Brunswick, USA). After autoclaving 3.6 L of LB liquid medium with a pH adjusted to 7.0, the bioreactor was inoculated with 400 mL of bacterial pre-inoculum. Growth conditions were maintained at 28 ºC, 200 rpm, and a specific air flow of 0.2 vvm for 24 hours. After that, 2 L of culture broth was removed, and 2 L of fresh LB liquid medium supplemented with a sporulation inductor agent was added. The pH was adjusted to 8.0 and controlled using 5 moL/L NaOH or 5 mol/L H_2SO_4 through peristaltic pumps. Cultures were maintained at 37 °C, 200 rpm, and 0.2 vvm airflow for 24 hours.

Subsequently, the bacterial culture broth was distributed in 50 mL plastic tubes, and the samples were centrifuged at 10,000 rpm for 15 minutes. The resulting pellet was washed three times with sterile water. Afterward, it was resuspended to a final concentration of 1.0×10^{10} spores mL^{-1} in four distinct bioformulations employing 0.85% NaCl solution (Bioformulation 1) and

agro-industrial wastes, such as composting leachate and whey (Bioformulations 2, 3, and 4). The specific composition of these formulations remains confidential, as they are subject to patent protection under deposit number BR102024003463.

The experiment was performed in a commercial greenhouse located in Antônio Prado, Rio Grande do Sul State, Brazil. Strawberry plants cv. San Andreas, under an organic crop system and cultivated in a plastic-covered greenhouse, were subjected to the following treatments: Bioformulation 1, Bioformulation 2, Bioformulation 3, Bioformulation 4, and Duravel® (*Bacillus amyloliquefaciens* MBI600). All formulations were diluted in sterile water to a final concentration of 9.1×10^8 spores mL^{-1}, according to the recommendation of the manufacturer (BASF). A negative control was omitted to prevent potential financial losses. The experiment spanned May 2023 to October 2023. Strawberry plants were weekly hand-sprayed with 1.2 L of each formulation. The trial followed a randomized block design with three replicates containing 20 strawberry plants. The incidence of anthracnose and gray mold was evaluated at 15-day intervals. Disease incidence (DI) was calculated as follows: DI (%) = (number of infected strawberry plants / total number of strawberry plants) × 100.

The statistical analysis was performed using SPSS version 22.0 for Windows. Shapiro-Wilk and Levene's tests were employed to assess the normality of the dataset and homogeneity of variances, respectively. The means were compared using one-way ANOVA followed by Dunnett's T3 test ($P < 0.05$).

3 RESULTS & DISCUSSION

The incidence of anthracnose and gray mold in strawberry plants treated with the four bioformulations containing *B. velezensis* S26 endospores showed no statistical difference compared to the biocontrol provided by the commercial bioproduct Duravel®. Furthermore, throughout the experiment, all treatments exhibited equivalent efficacy in suppressing both strawberry diseases (Tables 1 and 2).

Table 1 Anthracnose incidence in strawberry plants weekly treated with four bioformulations containing *B. velezensis* S26 endospores and Duravel® from May to October 2023. There was no statistically significant difference among the treatments according to ANOVA followed by Dunnett's T3 test ($P < 0.05$).

Treatments	Months					
	May	June	July	August	September	October
Bioformulation 1	0.00	5.26	0.68	0.52	0.00	0.00
Bioformulation 2	0.00	0.00	1.37	0.52	1.18	2.42
Bioformulation 3	0.00	0.00	0.68	1.03	0.00	0.00
Bioformulation 4	0.00	5.26	0.00	0.52	1.18	0.00
Duravel®	0.00	15.79	0.00	1.55	3.55	1.61

Table 2 Gray mold incidence in strawberry plants weekly treated with four bioformulations containing *B. velezensis* S26 endospores and Duravel® from May to October 2023. There was no statistically significant difference among the treatments according to ANOVA followed by Dunnett's T3 test ($P < 0.05$).

Treatments	Months					
	May	June	July	August	September	October
Bioformulation 1	0.00	0.00	1.37	1.03	0.00	0.00
Bioformulation 2	0.00	1.82	0.68	0.00	0.00	0.00
Bioformulation 3	0.00	0.00	1.37	0.52	0.00	0.81
Bioformulation 4	0.00	3.64	2.05	0.00	0.59	1.61
Duravel®	0.00	0.00	6.85	1.03	1.78	1.61

In agreement with our findings, *Bacillus atrophaeus* DM6120 controlled anthracnose through soil drenching and foliar spraying.[7] *B. velezensis* XT1 also reduced the incidence and severity of gray mold in strawberry plants[8]. Additionally, treating strawberry plants with the biocontrol agents *B. amyloliquefaciens Bc2* and *Trichoderma harzianum* TR effectively suppressed anthracnose, gray mold, and powdery mildew under field conditions[9].

4 CONCLUSION

The current research has formulated economically viable and high-performing bioformulations incorporating *B. velezensis* S26 endospores. These formulations have effectively controlled anthracnose and gray mold by utilizing whey and composting leachate as liquid carriers, demonstrating efficacy comparable to Duravel®. Consequently, this study underscores the potential of *B. velezensis* S26 as a promising biocontrol agent for managing strawberry diseases.

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