Screening of filamentous fungi for the formulation of bioinsecticides in organic agriculture

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ABSTRACT

The prospecting of filamentous fungi for the formulation of bioinsecticides is an emerging field in sustainable agriculture, especially in organic agriculture, which seeks effective, alternative and eco-friendly biological pesticides. Thus, continuous research and development of new biological products have been essential to overcome challenges of formulation and market acceptance of these alternative pesticides, thereby broadening the applicability and efficacy of bioinsecticides in organic agriculture. In this context, this study aimed to screen filamentous fungi with potential bioinsecticidal effects for the control of pest insects in organic agriculture. The collection and monitoring of filamentous fungi were carried in an area of organic corn cultivation of the Guandu Agroecological Group, located in Santa Maria, Rio Grande do Sul. Microbial agents were collected from soil samples and dead insects, followed with the isolation of these microbes and subculturing steps, obtaining 54 isolated fungi. According to the results, it was observed that some filamentous fungi (six agents named as F1, F3, F5, F7, F15, F17, F37 and F50) show promising potential for the development of bioinsecticides. Therefore, the screening of filamentous fungi showed to be a valuable tool for organic agriculture, which can encourage technological innovations in the scientific community and agri-food area.

Keywords: Bioinseticides. Microbial screening. Sustainable management. Pathogenic effect.

1 INTRODUCTION

Organic agriculture is becoming increasingly popular worldwide due to its sustainable cultivation practices and avoidance of synthetic chemicals[1]. However, organic crop production faces significant challenges, particularly in pest control, due to the restrictions on conventional chemical insecticides. To address this issue, bioinsecticides are emerging as promising alternatives for pest control, aligning with the principles of organic agriculture. These products, derived from natural sources such as filamentous fungi, offer an effective and eco-friendly solution for protecting organic crops[2]. The formulation of these bioinsecticides involves a crucial process called microbial screening as an initial step, which plays a crucial role in identifying biological agents capable of effectively combating the pests that affect organic crops[3]. This approach focuses on identifying, isolating, and characterizing microbial strains with the potential for efficient biocontrol. It is worth noting that the search and isolation of filamentous fungi for discovering new biocontrol agents is a complex and time-consuming process, but essential for sustainable agriculture progress. In this context, the objective of this study was to carry out the screening and isolation of filamentous fungi to obtain promising bioactive agents for the formulation of bioinsecticides aimed at organic agriculture.

2 MATERIAL & METHODS

Filamentous fungi were monitored and collected in an area of organic corn cultivation owned by the Guandu Agroecological Group, located in Santa Maria (Rio Grande do Sul, Brazil). Monitoring and collection of filamentous fungi were conducted in an area of organic corn cultivation belonging to the Guandu Agroecological Group, with the approximate geographical coordinates: latitude 29° 40’ 15’’ South and longitude 53° 52’ 24’’ West. The collection took place in areas without the application of biological products prior to the collection. After a sweep of the area, the collection of dead insects in the adult phase and soil samples from distinct points within the organic cultivation area occurred.

The isolation of the agents was carried out directly from the cadavers of the insects[4]. Regarding the soil samples, plating was performed on Petri dishes[5].The plates were transferred to a Biochemical Oxygen Demand (BOD) type incubator oven at a temperature of 25°C until the formation of cultures on the surface of the cultivation medium, followed by constant subculturing until the formation of pure isolates. The isolates were subjected to submerged state fermentation (SSF) using 250 mL Erlenmeyer flasks, containing 125 mL of Potato Dextrose (PD) culture medium[6]. During the vacuum filtration of the fermented broths, the biomass was extracted from the culture broth[7].

The concentration of spores (conidia and blastospores) was determined using a Neubauer chamber (hemocytometer)[8]. The pH of the samples was determined using a benchtop pH meter (Servylab, mPA210) at room temperature (25 °C). The specific density of each sample was measured with a high-precision automatic densimeter DDM 2911 Plus (Rudolph, DDM 2911 Plus, USA) through a touchscreen interface with the injection of 3 mL of each sample into the equipment at a temperature of 20 °C. The surface tension measurement was carried out with modifications[9]. For this purpose, a DAS 25E goniometer (Krüss GmbH, Hamburg, Germany) was used.

3 RESULTS & DISCUSSION

During the screening phase for filamentous fungi, 54 fungi were obtained and constitute the set of pre-selected isolates. In the preliminary phenotypic identification analysis at the genus level, the fungi belong to *Beauveria bassiana*, *Cordyceps fumosorosea*, *Fusarium* spp., *Metarhizium anisopliae*, *Trichoderma* spp., *Trichoderma asperelloides*, *Trichoderma hamatum*, and *Trichoderma virens* (Table 1). The fungi isolated from *Trichoderma* spp. were 25 (46.3%), *Beauveria bassiana* were 14 (25.9%), *Metarhizium anisopliae* were 7 (13%), *Cordyceps fumosorosea* were 6 (11.1%), and *Fusarium* spp. were 2 (3.7%). Out of the 54 fungi, 46 were isolated from soil and 8 fungi originated from insects.

Table 1 Cultural characteristics of the 54 isolates regarding origin, genus, pH, biomass (g L^{-1}), spores (m L^{-1}), specific density (g cm^{-3}), and surface tension (mN m^{-1}).

Isolate	Origin	Fungus	pH	Biomass (g L^{-1})	Concentration (spores mL^{-1})	Specific Density (g cm^{-3})	Surface Tension (mN m^{-1})
F1	Soil	*Metarhizium anisopliae*	4.49	1.457	1.95×10^{6}	1.009441	39.26 ±0.38
F2	Soil	*Fusarium* spp.	4.89	2.247	4.45×10^{6}	1.006119	56.85±0.85
F3	Soil	*Trichoderma virens*	4.31	1.608	4.95×10^{6}	1.008927	36.89±0.55
F4	Soil	*Trichoderma virens*	4.68	1.236	1.55×10^{6}	1.008477	58.04±0.91
F5	Soil	*Beauveria bassiana*	6.29	1.708	1.25×10^{6}	0.984257	39.93±0.14
F6	Soil	*Beauveria bassiana*	4.80	1.674	1.90×10^{6}	1.008048	44.72±0.07
F7	Soil	*Beauveria bassiana*	4.27	1.903	5.40×10^{6}	1.008197	56.57±0.58
F8	Soil	*Trichoderma asperelloides*	4.32	1.179	6.00×10^{6}	1.008164	55.19±0.53
F9	Soil	*Trichoderma asperelloides*	4.81	1.170	6.40×10^{6}	1.007823	58.44±0.81
F10	Soil	*Trichoderma* spp.	5.08	0.260	2.55×10^{6}	1.022885	59.14±0.97
F11	Soil	*Trichoderma* spp.	6.57	1.679	7.50×10^{5}	1.006295	50.71±0.58
F12	Soil	*Cordyceps fumosorosea*	6.49	1.508	2.20×10^{6}	1.008991	56.39±0.61
F13	Soil	*Beauveria bassiana*	7.26	1.836	6.00×10^{5}	1.006955	52.21±0.69
F14	Soil	*Cordyceps fumosorosea*	5.29	0.472	1.55×10^{6}	1.006940	56.76±0.61
F15	Soil	*Cordyceps fumosorosea*	6.61	1.601	2.90×10^{6}	1.008045	35.77±0.27
F16	Soil	*Trichoderma hamatum*	4.30	1.226	7.65×10^{6}	1.008125	56.85±0.97
F17	Soil	*Trichoderma* spp.	4.48	0.485	1.12×10^{8}	1.018200	52.96±0.38
F18	Soil	*Beauveria bassiana*	4.27	0.729	6.10×10^{6}	1.012320	56.85±0.86
F19	Insect	*Beauveria bassiana*	4.22	0.827	9.35×10^{6}	1.011417	53.87±0.86
F20	Insect	*Beauveria bassiana*	4.25	0.783	7.85×10^{6}	1.009821	57.17±0.86
F21	Insect	*Cordyceps fumosorosea*	4.32	0.726	5.55×10^{6}	1.011328	56.80±0.88
F22	Insect	*Beauveria bassiana*	5.30	0.446	2.85×10^{6}	1.021734	54.06±0.71
F23	Insect	*Trichoderma asperelloides*	4.94	1.041	4.20×10^{6}	1.016729	54.91±0.82
F24	Soil	*Trichoderma asperelloides*	5.27	1.419	1.45×10^{6}	1.007692	56.55±1.73
F25	Soil	*Metarhizium anisopliae*	5.98	1.496	9.50×10^{5}	1.007831	44.62±0.56
F26	Soil	*Metarhizium anisopliae*	5.89	1.773	9.00×10^{5}	1.007164	61.51±0.81
F27	Soil	*Trichoderma* spp.	4.70	1.439	6.60×10^{6}	1.007332	41.33±1.19
F28	Soil	*Trichoderma* spp.	4.33	1.627	1.10×10^{6}	1.013125	58.13±0.64
F29	Soil	*Metarhizium anisopliae*	5.18	2.402	2.45×10^{6}	1.007638	42.86±0.19
F30	Soil	*Trichoderma* spp.	4.81	0.879	1.20×10^{6}	1.019848	63.91±1.43
F31	Soil	*Trichoderma asperelloides*	4.77	1.512	1.65×10^{6}	1.007333	49.12±2.25
F32	Soil	*Trichoderma asperelloides*	4.71	0.934	8.00×10^{6}	1.018724	64.19±1.62
F33	Soil	*Cordyceps fumosorosea*	5.81	1.967	6.00×10^{5}	1.006895	65.87±0.48
F34	Soil	*Metarhizium anisopliae*	5.31	1.395	8.60×10^{6}	1.007309	63.98±1.56
F35	Soil	*Trichoderma* spp.	5.45	1.642	3.05×10^{6}	1.009011	42.67±0.19
F36	Soil	*Trichoderma* spp.	5.72	0.458	1.35×10^{6}	1.020527	56.13±0.76
F37	Soil	*Beauveria bassiana*	6.79	1.924	9.00×10^{5}	1.003234	56.28±0.38
F38	Soil	*Beauveria bassiana*	6.15	1.575	7.00×10^{5}	1.007596	51.48±0.87
F39	Soil	*Trichoderma* spp.	5.27	1.349	1.40×10^{6}	1.007940	64.19±0.30
F40	Soil	*Cordyceps fumosorosea*	6.26	1.231	4.18×10^{7}	1.007632	60.09±0.54
F41	Soil	*Beauveria bassiana*	6.96	1.507	1.25×10^{7}	1.007300	59.40±1.44
F42	Insect	*Trichoderma asperelloides*	7.12	1.761	1.50×10^{6}	1.006485	52.59±2.14
F43	Insect	*Trichoderma* spp.	5.43	0.267	1.70×10^{6}	1.020203	55.58±0.58
F44	Insect	*Fusarium* spp.	7.79	0.332	7.00×10^{6}	1.019109	51.36±0.90
F45	Soil	*Trichoderma virens*	5.54	0.302	1.70×10^{6}	1.020447	56.54±1.96
F46	Soil	*Trichoderma asperelloides*	7.08	1.830	2.50×10^{6}	1.006927	54.84±0.98
F47	Soil	*Beauveria bassiana*	6.97	1.466	6.00×10^{6}	1.006508	51.19±0.78
F48	Soil	*Beauveria bassiana*	5.56	1.094	5.60×10^{6}	1.002354	58.00±2.05
F49	Soil	*Metarhizium anisopliae*	6.56	1.392	6.00×10^{6}	1.007094	56.63±5.01
F50	Soil	*Trichoderma* spp.	6.82	1.447	1.18×10^{7}	0.967489	62.15±3.75
F51	Soil	*Trichoderma* spp.	4.41	0.931	3.10×10^{6}	1.010589	61.27±0.87
F52	Soil	*Metarhizium anisopliae*	5.02	1.285	1.70×10^{6}	1.005783	54.80±1.05
F53	Soil	*Beauveria bassiana*	5.64	0.600	4.50×10^{5}	1.012004	56.29±0.63
F54	Soil	*Trichoderma asperelloides*	6.93	1.340	3.15×10^{6}	1.007071	58.69±3.36

Additionally, in Table 1, the pH, biomass (g L^{-1}), spores (m L^{-1}), specific density (g cm^{-3}), and surface tension (mN m^{-1}) of the isolates are seen. The concentration, determined by spore counting under a microscope indicated up to 1.2×10^{8} spores mL^{-1} (isolate F17). Biomass production ranged from 2.402 g L^{-1} (isolate F29) to 0.260 g L^{-1} (isolate F10). The pH of the samples was

slightly higher in samples F13, F41, F42, F44, F46, F47 in the range of 7-8 (mildly alkaline/neutral), and the isolates F7 and F8 had a pH of 4.3 (acidic). The specific density ranged from 0,967489 g cm^{-3} (F50) to 1,022885 g cm^{-3} (F10). The surface tension values varied from 35,77 mN m^{-1} to 65,87 mN m^{-1}. Based on the methodological design described in this study, it is possible to explore the parameters performed for the characterization of the isolates.

It was observed that isolate F17 had a high spore concentration of 1.12×10^8, making it a promising candidate for reducing pest populations.[10] Studies have shown that a concentration of 1.10×10^8 of *Metarhizium anisopliae* and *Beauveria bassiana* achieved a mortality rate of *Spodoptera frugiperda* of 80% and 90%, respectively.[11] Regarding the production of fungal biomass, among the isolates analyzed, F7 and F37 belonging to the fungi *Beauveria bassiana* reached fungal biomass rates of 1.9 g L^{-1}. Regarding pH, most entomopathogenic fungi develop in the pH range of 5,0 to 7,0.[12] The values found in the study are close to the ideal range; the pH of the isolates ranges from 5,0 to 8,5.

Regarding the results for surface tension and specific density, Table 1 indicated heterogeneous results for the isolates. In the application of biological products, the surface tension of the product is a crucial factor for the effectiveness of the distribution and adherence of the product on insects. The cuticle of an insect is an outer layer made primarily of chitin, which serves to coat the insect's body, being responsible for rigidity and structural support.[13] Therefore, the surface tension of the bioproduct directly influences its ability to adhere to the insect's cuticle. Products with low surface tension are more easily spread and cover the insect's surface, increasing the efficacy of contact. This fact can be analyzed in isolates F1, F3, F5, and F15, which obtained surface tensions below 40 mN m^{-1}, making them promising fermentative broths for better penetration and efficient spreading over the insect cuticles. Simultaneously, the specific density significantly influences the efficacy of the fermented broths in controlling the investigated pest insect.[14] Higher specific densities indicate a greater concentration of compounds present in the solution, making uniform spreading over the insects difficult and leading to the potential formation of irregular droplet coverage on the chitin of the target pest insects. On the other hand, low specific density values form a thin and uniform layer, ensuring a more homogeneous and efficient coverage on the desired target. Thus, the balance between specific density and surface tension is crucial for a quality fermentative broth. It is observed that isolate F50 exhibited low values of specific density, being efficient in optimizing the adherence of conidia to the insect chitin surface and facilitating the formation of a uniform layer for effective infection in pest insects.

4 CONCLUSION

This study shows that filamentous fungi F1, F3, F5, F7, F17, F37 and F50 have the potential to be developed as bioinsecticides, which can be an effective tool in controlling pest insects in organic agriculture systems. This discovery contributes to sustainable pest control strategies and is relevant for both the scientific community and organic farmers. By researching the effectiveness of these filamentous fungi as bioinsecticides, we can reduce our reliance on chemical pesticides and promote healthier and more sustainable agricultural practices that favor ecological balance.

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