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Cladobotryum mycophilum as Potential Biocontrol Agent

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Abstract: A study was conducted to explore the efficacy of potential biocontrol agent *Cladobotryum mycophilum* against different phytopathogenic fungi. The growth rates of 24 isolates of *C. mycophilum* were determined, and their antagonistic activity was analysed in vitro and in vivo against *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Fusarium oxysporum* f.sp. *cucumerinum*, *Fusarium solani*, *Phytophthora parasitica*, *Phytophthora capsici*, *Pythium aphanidermatum* and *Mycosphaerella melonis*. Most isolates grow rapidly, reaching the opposite end of the Petri dish within 72–96 h. Under dual-culture assays, *C. mycophilum* showed antagonistic activity in vitro against all phytopathogenic fungi tested, with mycelial growth inhibition ranging from 30 to 90% against all the different phytopathogens tested. Similarly, of all the selected isolates, CL60A, CL17A and CL18A significantly ($p < 0.05$) reduced the disease incidence and severity in the plant assays compared to the controls for the different pathosystems studied. Based on these results, we conclude that *C. mycophilum* can be considered as a potential biological control agent in agriculture. This is the first study of *Cladobotryum mycophilum* as a biological control agent for different diseases caused by highly relevant phytopathogens in horticulture.

Keywords: biological control; Cladobotryum; Mycosphaerella; Pythium; Phytophthora; Fusarium; Botrytis

1. Introduction

Cobweb, caused by a mycopathogenic fungus (Oudem.) W. Gams and Hooz (teleomorph *Hypomyces odoratus* G.R.W. Arnold), is one of the most serious diseases affecting white button mushroom (*Agaricus bisporus* (Lange) Imbach) cultures worldwide [1–4]. Its occurrence generally reduces the production and quality, and thus generates economic losses because of mainly cap spotting, the reduction of crop surface and the necessity of early crop termination when the disease becomes epidemic [5]. The main symptoms of cobweb are characterised by the occurrence of a white fluffy mycelium over the surface of the casing layer and attacks on primordia and developing fruitbodies. The pathogen can also cause two types of cap spotting that may even appear post-harvest, affecting the profitability of the product [6]. Recently, *C. mycophilum* has been identified as the causal agent of cobweb in cultivated king oyster mushroom (*Pleurotus eryngii* (DC.: Fr.) Quél.) in Spain and Korea [7–9] and oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in Spain [10]. Methods to control cobweb include strict hygiene measures to prevent dispersion of the conidia and the application of fungicides, mainly prochloraz and metrafenone [11].

Fungal associations with other fungi are termed fungicolous fungi [12,13]. The aggressive fungicolous taxa, such as *Gliocladium* spp. and *Trichoderma* spp., have led to numerous studies on these fungi as biological control agents [14]. Most species of the *Cladobotryum* genus are characterised by rapid growth and colonisation of the parasitic basidiomycete [4]. Considering their high mycoparasitic capacity, these species are potential candidates for use as biological control agents against key agricultural phytopathogens. However, few in vitro studies have shown the antagonistic activity of *Cladobotryum* against phytopathogenic fungi. In this context, Bastos et al. [15] describe *C. amazonense* as a new hyperparasite of *Crinipellis perniciosa*, that causes 'witches' broom disease' in the cocoa tree. Marzuki et al. [16] assess a 74.8 and 74.7% decrease in the mycelial growth of *Ganoderma lucidum* and *G. boninense*, respectively, at the hand of *Cladobotryum semicirculare*, and showed the in vitro antagonistic activity of *C. semicirculare* isolates against *G. boninense* and other fungal pathogens, including *Fusarium* sp and *Phoma* sp., assessing 55 and 60% rates of inhibition, respectively. Similarly, Ramos [17] evaluated the in vitro and in vivo antagonistic activity of *C. pinarense, C. semicirculare* and *C. virescens* in the control of the phytopathogens *Alternaria brassicicola*, *Corynespora cassiicola* and *Fusarium chlamydosporum*. The findings showed that the three *Cladobotryum* species had an antagonistic effect on the pathogens studied, with no harm to plants or fruits, highlighting a fungicidal effect on filtered extracts of the three antagonistic isolates.

Biological control of diseases caused by phytopathogenic fungi has not been described for *Cladobotryum mycophilum*. Therefore, the main objective of this study is to determine: (a) The growth rate of 24 *C. mycophilum* isolates; (b) the in vitro antagonistic activity of such isolates against eight phytopathogens of interest in agriculture; (c) the in vivo capacity for controlling the development of the disease caused by such phytopathogens in different pathosystems under greenhouse conditions.

2. Materials and Methods

2.1. Fungal Isolates

A total of 24 isolates previously identified as *C. mycophilum* used in this study were obtained from *A. bisporus* crops showing cobweb disease symptoms [3]. The phytopathogenic fungi used were: *Botrytis cinerea* Pers (BOT), *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis and Shoem (FORL), *Fusarium oxysporum* f.sp. *cucumerinum* Owen (FOC), *Fusarium solani* (Mart.) Sacc (FL), *Phytophthora parasitica* Dastur var. nicotianae (Breda de Haan) Tucker (PH), *Phytophthora capsici* Leonian (PC), *Pythium aphanidermatum* (Edson) Fitzp (PY) and *Mycosphaerella melonis* (Pass.)(MM).

Plants infected with the pathogens were collected from different crops in the province of Almeria (Spain) during 2016–2018. Selected stem and leaf sections with active lesions were cut, and tissue pieces from the boundaries between healthy and discoloured areas were planted on potato dextrose agar. Pathogenicity tests were carried out to confirm their pathogenic capacity.

Fungal isolate was grown on PDA for 5 or 15 days at (25–27) ± 2 °C under dark conditions. Spore suspensions of *Cladobotryum* and phytopathogens isolates were prepared by flooding plates of 10-day-old cultures with sterile distilled water, scraping with a sterile glass rod and filtering and adjusted to a concentration of 1×10^6 spores/mL with a Neubauer haemocytometer.

2.2. Growth Conditions of the Cladobotryum Isolates in PDA

Twenty-four isolates of *C. mycophilum* were cultured at 25 °C for 7 d in PDA. Mycelium discs (5 mm diameter) were obtained from the edges of the PDA plates and inoculated into new PDA at 0.5 cm from the edge. The plates were incubated for 4–7 d at 25 °C. The colony diameters were recorded every day during culturing. The experiment was completely randomised with five replicates.

2.3. Screening Test of Antagonistic Isolates

The 24 isolates of *C. mycophilum* were screened for their antagonism in vitro against eight phytopathogens by adopting the confrontation assay of Santos et al. [18].

Petri dishes (9 cm diameter) containing 17 mL of PDA (Bioxon, Becton Dickinson, Mexico) were prepared. In this test, 0.5-cm plugs of mycelium with active growth of each isolate were placed at the ends of Petri dishes with a distance of 8 cm between the two fungi, antagonist-phytopathogen.

A plug of PDA medium was used as control treatment while the pathogen plug was placed at the other side. The plates were then assessed by measuring the distances between pathogen and fungal cultures. Results were transformed into percentages of mycelium growth inhibition. These tests were carried out in quintuplicate.

2.4. Greenhouse Evaluation of Selected Antagonists of C. mycophilum on the Severity of Eight Phytopathogens

Biocontrol activity of selected *Cladobotryum mycophilum* isolates on different pathosystems was determined: *B. cinerea*-tomato, *F. oxysporum* f. sp. *radicis-lycopersici*-tomato, *Fusarium oxysporum* f.sp. *cucumerinum*-cucumber, *Fusarium solani*-tomato, *Phytophthora parasitica*-tomato, *Phytophthora capsica*-pepper, *Pythium aphanidermatum*-melon and *Mycosphaerella* melonis-melon. All seeds were disinfected with 2% hypochlorite for 3 min and washed liberally with tap water to eliminate residues. Subsequently, the seeds were pregerminated in darkness in a humid room at 25 °C and transplanted to 500 mL pots into a commercial peat mix, with one seed per pot. Simultaneously to sowing, 5 mL of water (T0) or 5 mL spore suspension of each isolate of *Cladobotryum* was placed in each pot at 1×10^6 propagules/plant.

To prepare *Phytophthora parasitica*, *Phytophthora capsici* and *Pythium aphanidermatum* inocula, the procedure described by Diánez et al. was followed [19]. To obtain the inoculum, isolates were grown on PDA for 7 d at 25°C. Then, plates were incubated under fluorescent light for 3 d at 25 °C to induce sporangium formation. Twenty-four hours before inoculation, fungal colonies were covered with 20 mL of sterilized tap water and incubated under light overnight. During the inoculation day, Petri dishes were placed at 4 °C for 30 min, followed by 60 min at room temperature to enhance zoospore release from sporangia. The flooding water, containing zoospores and mycelium was filtered through two layers of cheesecloth. The concentration of the zoospore suspension was adjusted to approx. 10^4 zoospores mL^{-1} using a haemocytometer. The inoculum was used immediately. Inocula of the other phytopathogens were prepared by scraping and subsequent filtration, as previously indicated.

The phytopathogen was inoculated when the plant had a second true leaf and was performed using a sterile micropipette, by pouring the zoospores/conidias suspension (5 mL) uniformly over the surface of the peat in each pot at a concentration of 10^4 propagules/mL. In the case of *B. cinerea* and *M. melonis*, the pathogen was applied by spraying five times at the same concentration throughout the plant. Previously, the first true leaf had been cut to facilitate pathogen entry.

All pathogenicity tests were performed under greenhouse conditions and at different seasons (for *B. cinerea* the experiment were performed in winter; for the rest of the phytopathogens in spring) to provide the ideal environmental conditions for each pathogen. The experimental units consisted of four repetitions with 24 plants per pathosystem. The experiment were conducted using completely randomized block designs.

Symptom severity was rated periodically, and 30–60 days after inoculation final disease severity index was estimated according to the following scale: 0 = healthy plant; 1 = symptoms beginning; 2 = moderate symptoms; 3 = severely affected plant; and 4 = dead plant. Similarly, possible symptoms caused by the application of different *C. mycophilum* isolates without pathogen were observed in melon, tomato, pepper and cucumber plants.

2.5. Statistical Analysis

Data were analysed with analysis of variance (ANOVA), carried out using the Statgraphics Centurion ver. (XVI) program to evaluate the parameter values differences. Differences between treatments were determined by Duncan's multiple range test at 5% of significance level.

3. Results

3.1. Colony Growth of Cladobotryum Isolates

In Figure 1, the results obtained from the mycelial growth of 24 isolates of *C. mycophilum* are shown. The colony growth of most isolates was fast, reaching the opposite end of the Petri dish within 72–96 h. The colour of the colonies ranged from yellowish to pink and turned purple after a few days, except in isolate CL1381A, whose mycelium colour was greyish white. No isolates were discarded in determining the antagonistic activity of *Cladobotryum* against phytopathogens.

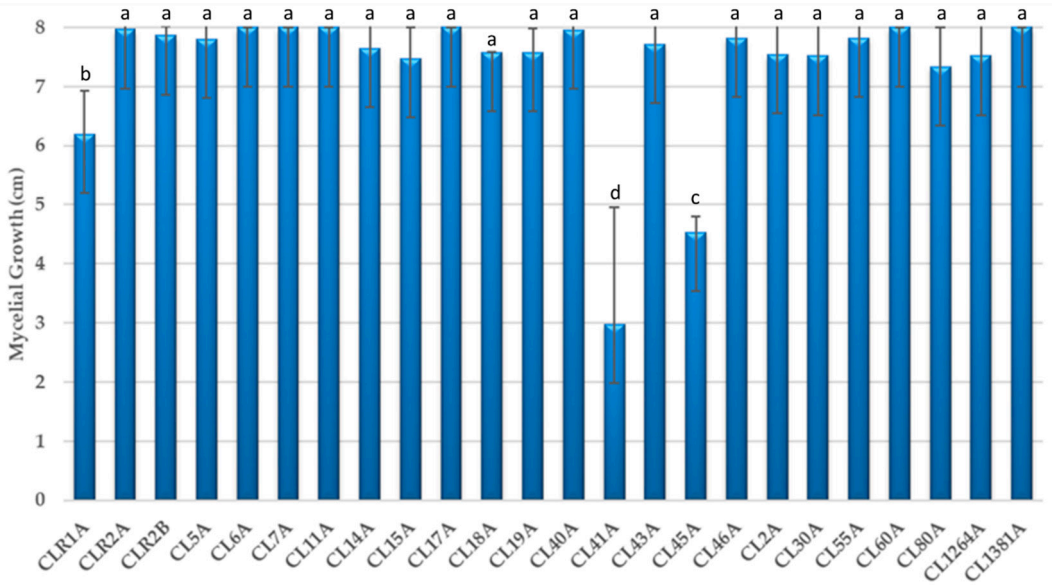

Figure 1. Mycelial growth of 24 isolates of *C. mycophilum* in vitro after 4 days. Mean standard deviation is expressed in error bar (n = 5). For each isolate, columns marked with different letters indicate a significant difference according to analyses of variance ($p < 0.05$).

3.2. Effects of Cladobotryum Isolates on the Radial Growth of Phytopathogens

In Table 1, the results obtained from the microbial antagonism for all phytopathogens tested are observed. All *C. mycophilum* isolates have shown in vitro antagonistic activity against all phytopathogenic fungi tested. The highest inhibition percentages were detected for *Phytophthora parasitica*, which reached values higher than 90% for 12 *Cladobotryum* isolates. In contrast, the lowest inhibition values were detected for *Pythium aphanidermatum*, whose maximum failed to exceed 60% for the CL17A isolate. For the other phytopathogens, mycelial growth inhibition ranged from 30 to 85%. Three isolates were selected for the in vivo tests, CL60A, CL17A and CL18A, because they reached the highest values for the highest number of phytopathogens.

Table 1. Antagonistic potential of *Cladobotryum* isolates against eight phytopathogens in dual culture on PDA medium. % mycelial inhibition was calculated as PIRG = (R1 − R2) ÷ R1 × 100, where: PIRG: percentage inhibition of radial mycelia growth of the pathogen, R1: radial growth of pathogen in control plates, R2: radial growth of pathogen in dual culture plates. Means with the same letter are not significantly different (LSD) according to ANOVA test ($p < 0.05$).

Isolates	**% Inhibition of Mycelial Growth Plant Pathogens**							
	Botrytis cinerea	**FORL**	***Fusarium solani***	**FOC**	***Phytophthora parasitica***	***Mycosphaerella melonis***	***Phytophthora capsici***	***Pythium aphanidermatum***
CLR1A	68.25 ± 1.89 ^{cdefg}	72 ± 2.44 ^{bc}	76 ± 2.05 ^{cdefg}	73.75 ± 3.19 ^{abc}	81.25 ± 10.72 ^{cdefg}	86.5 ± 7.68 ^{ab}	66.5 ± 15.72 ^{efg}	49.25 ± 1.42 ^{defg}
CLR2A	68.5 ± 1.63 ^{cdefg}	71.25 ± 3.75 ^{bc}	77.5 ± 0.88 ^{cdef}	72.75 ± 2.05 ^{abc}	89.25 ± 0.68 ^{abcdef}	80.5 ± 3.38 ^{abc}	77 ± 2.88 ^{abcd}	48 ± 4.29 ^{efghi}
CLR2B	68.75 ± 3.53 ^{cdefg}	71.5 ± 1.05 ^{bc}	79.25 ± 1.12 ^{bcd}	72.25 ± 2.40 ^{abc}	89.5 ± 2.09 ^{abcde}	72 ± 1.68 ^{cd}	74 ± 1.85 ^{bcde}	51.5 ± 2.05 ^{cdef}
CL5A	58.75 ± 12.37 ^{hi}	47.75 ± 6.09 ^{ef}	70.25 ± 6.52 ^{h}	46.5 ± 7.97 ^{g}	71.75 ± 11.20 ^{h}	60.62 ± 11.39 ^{e}	63 ± 14.19 ^{fgh}	45.75 ± 3.25 ^{ghijk}
CL6A	73.5 ± 3.23 ^{abc}	76.25 ± 2.34 ^{ab}	82.5 ± 2.17 ^{ab}	78.25 ± 4.20 ^{a}	91 ± 2.85 ^{ab}	79.25 ± 3.26 ^{abc}	77.25 ± 1.63 ^{abcd}	53.25 ± 5.19 ^{bcde}
CL7A	76.25 ± 1.97 ^{ab}	73.25 ± 1.12 ^{ab}	80 ± 1.25 ^{abc}	76 ± 1.63 ^{ab}	91 ± 1.05 ^{ab}	78.25 ± 4.11 ^{bc}	79.75 ± 3.24 ^{ab}	53.5 ± 2.40 ^{bcd}
CL11A	74.5 ± 2.87 ^{abc}	75.75 ± 1.90 ^{ab}	79.75 ± 1.05 ^{abcd}	73 ± 2.44 ^{abc}	92 ± 4.73 ^{ab}	87.5 ± 4.76 ^{ab}	77.75 ± 2.24 ^{abc}	55.25 ± 4.54 ^{abc}
CL14A	79.25 ± 12.00 ^{a}	73.75 ± 3.95 ^{ab}	74.75 ± 3.69 ^{efg}	75.75 ± 1.68 ^{ab}	91 ± 2.71 ^{ab}	86.75 ± 1.90 ^{ab}	80.75 ± 1.43 ^{ab}	49 ± 9.28 ^{defg}
CL15A	62.75 ± 6.33 ^{gh}	46.75 ± 9.04 ^{ef}	74.75 ± 4.37 ^{efg}	59.75 ± 4.28 ^{f}	80.31 ± 14.10 ^{bcdef}	54 ± 16.52 ^{ef}	65.25 ± 14.43 ^{fg}	43.25 ± 4.72 ^{ijkl}
CL17A	73.25 ± 0.68 ^{abc}	80 ± 0.88 ^{a}	83 ± 2.09 ^{ab}	79 ± 0.56 ^{a}	93.75 ± 1.25 ^{a}	85.25 ± 1.37 ^{ab}	83.25 ± 3.60 ^{a}	59.25 ± 2.43 ^{a}
CL18A	72.5 ± 1.76 ^{bcd}	76.5 ± 1.63 ^{ab}	79.5 ± 4.11 ^{abcd}	76.25 ± 1.98 ^{a}	91.75 ± 0.68 ^{ab}	85.5 ± 2.59 ^{ab}	81.25 ± 1.53 ^{ab}	58.25 ± 1.42 ^{ab}
CL19A	64.25 ± 1.67 ^{fgh}	32.18 ± 2.13 ^{h}	74.5 ± 2.88 ^{efg}	30.83 ± 1.91 ^{h}	72.5 ± 5.00 ^{gh}	38.25 ± 2.44 ^{g}	55.25 ± 10.73 ^{h}	36.25 ± 8.14 ^{m}
CL40A	71.5 ± 1.04 ^{bcde}	48.5 ± 2.40 ^{ef}	79.75 ± 2.24 ^{abcd}	75.25 ± 1.63 ^{ab}	90 ± 3.75 ^{abcd}	62.5 ± 5.80 ^{de}	74.25 ± 1.12 ^{bcde}	48.5 ± 2.40 ^{defghi}
CL41A	47.25 ± 10.24 ^{j}	42 ± 12.39 ^{fg}	72 ± 3.60 ^{gh}	32.75 ± 1.63 ^{h}	66.67 ± 18.76 ^{fgh}	46.75 ± 14.16 ^{fg}	62.75 ± 8.45 ^{fgh}	21 ± 1.62 ^{n}
CL43A	70 ± 2.16 ^{bcdef}	61.25 ± 18.54 ^{d}	79 ± 3.58 ^{bcd}	60 ± 20.54 ^{ef}	92 ± 2.27 ^{ab}	80.25 ± 3.79 ^{abc}	76 ± 6.93 ^{abcd}	53.5 ± 7.72 ^{bcd}
CL45A	53 ± 5.49 ^{ij}	51 ± 3.79 ^{e}	70.25 ± 2.71 ^{h}	67 ± 6.47 ^{cde}	80.75 ± 5.90 ^{defgh}	64 ± 5.89 ^{de}	67 ± 3.81 ^{efg}	38.5 ± 4.08 ^{lm}
CL46A	65.75 ± 3.37 ^{efg}	53.5 ± 9.82 ^{e}	76.5 ± 5.89 ^{cdef}	64 ± 2.85 ^{def}	86.25 ± 9.80 ^{abcdef}	54.75 ± 10.66 ^{ef}	60 ± 11.28 ^{gh}	40.5 ± 4.80 ^{klm}
CL2A	71.5 ± 3.46 ^{bcde}	72 ± 1.43 ^{bc}	77.75 ± 1.37 ^{cdef}	74 ± 1.85 ^{ab}	90.75 ± 2.74 ^{ab}	88.75 ± 4.59 ^{a}	79.25 ± 3.49 ^{abc}	48.75 ± 4.05 ^{defgh}
CL30A	68 ± 1.89 ^{cdefg}	75 ± 1.25 ^{ab}	79.25 ± 1.43 ^{bcd}	75.5 ± 2.09 ^{ab}	90.5 ± 0.68 ^{abc}	82 ± 3.38 ^{abc}	78.75 ± 1.53 ^{abc}	51.5 ± 1.04 ^{cdef}
CL55A	70.5 ± 8.50 ^{bcdef}	70.25 ± 2.85 ^{bc}	78.25 ± 6.29 ^{cde}	72.75 ± 3.35 ^{abc}	87.75 ± 1.05 ^{abcdef}	77.75 ± 3.99 ^{bc}	76 ± 2.05 ^{abcd}	47 ± 3.81 ^{fghij}
CL60A	76.25 ± 1.97 ^{ab}	76.5 ± 3.47 ^{ab}	83.5 ± 4.18 ^{a}	76.75 ± 1.12 ^{a}	91.5 ± 1.85 ^{ab}	86.75 ± 1.90 ^{ab}	80 ± 2.34 ^{ab}	57.75 ± 1.85 ^{ab}
CL80A	68.75 ± 4.33 ^{cdefg}	35.5 ± 5.20 ^{gh}	75.75 ± 1.43 ^{defg}	69 ± 6.34 ^{bcd}	88.75 ± 11.39 ^{abcdef}	61 ± 22.73 ^{e}	71 ± 1.63 ^{cdef}	46.25 ± 4.23 ^{fghij}
CL1264	70.25 ± 1.85 ^{bcdef}	74 ± 1.85 ^{ab}	73.75 ± 4.15 ^{fgh}	75.25 ± 1.37 ^{ab}	93.25 ± 2.44 ^{ab}	80.75 ± 3.71 ^{abc}	77 ± 1.90 ^{abcd}	42.25 ± 4.08 ^{jkl}
CL1381	66.25 ± 1.25 ^{defg}	65 ± 2.05 ^{cd}	74.75 ± 1.05 ^{efg}	46.75 ± 2.05 ^{g}	80.25 ± 3.47 ^{efgh}	62.25 ± 2.05 ^{de}	69 ± 2.05 ^{def}	43.5 ± 2.23 ^{hijkl}
p	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

3.3. Biological Control of Cladobotryum Isolates against Several Diseases

The severity of the disease caused by the different phytopathogens tested decreased after applying the three isolates, CL60A, CL17A and CL18A (Figure 2). Statistically significant differences between all treatments and experimental controls were observed ($p < 0.05$). No symptom was observed after applying CL60A, CL17A or CL18A in any plant tested (Figure 2), thus showing a plant growth-promoting effect for different parameters (data not shown).

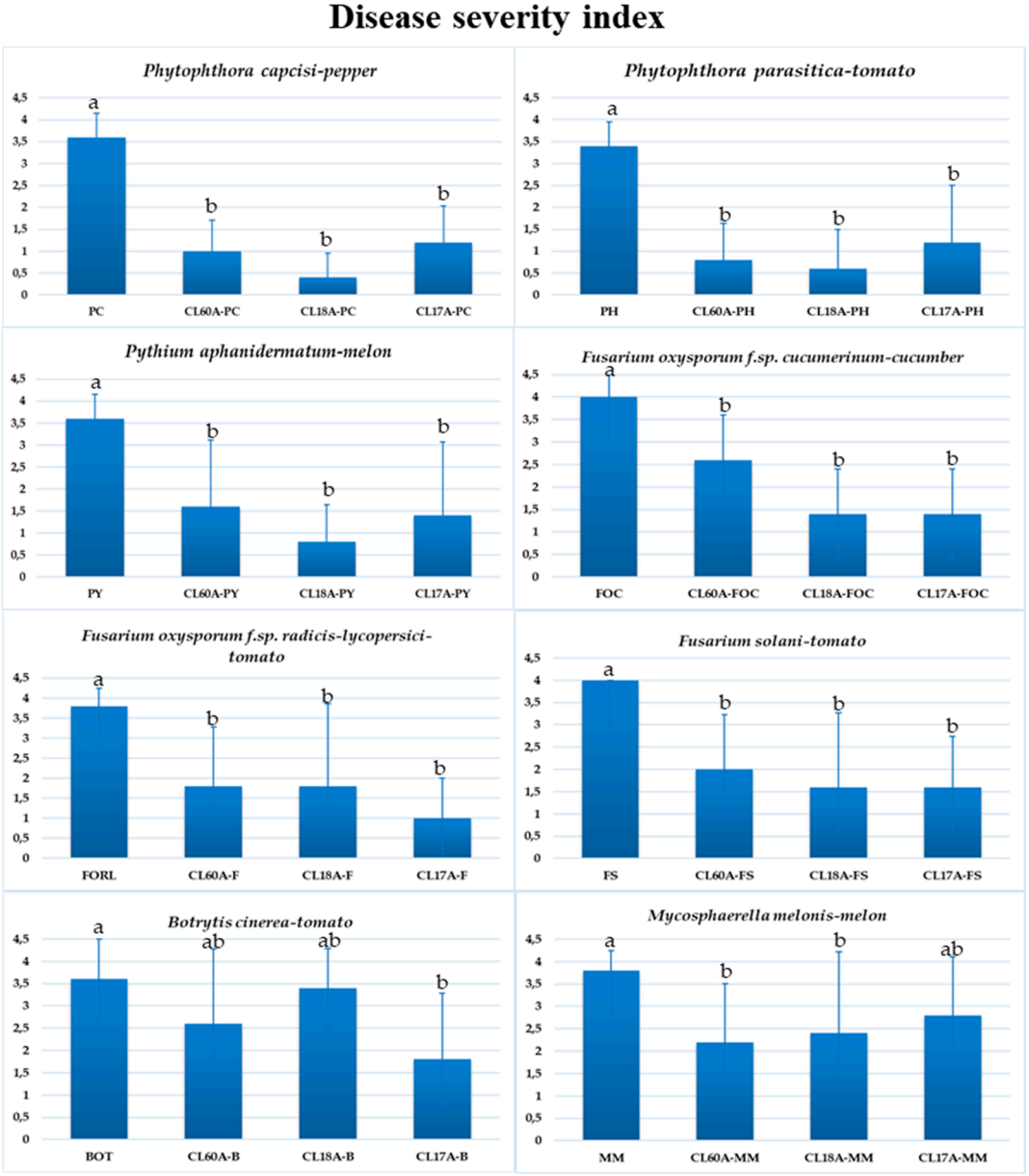

Figure 2. Disease severity of phytopathogens in plants was rated 30–60 days after inoculation based on a 0–4 scale: where 0 = no visible disease symptoms and 4 = plant dead. Mean standard deviation is expressed in error bar (24 plants/repetition). Means with the same letter are not significantly different (LSD) according to ANOVA test ($p < 0.05$).

Despite the lack of significant differences in the biological control of CL60A, CL17A and CL18A for different oomycetes, CL18A has reduced the disease by 77.8, 82.3, and 88.9% for *Pythium aphanidermatum*, *Phytophthora capsici* and *Phytophthora parasitica*, respectively. In the case of Fusaria, the disease reduction shown by the different isolates ranged from 35 to 60% for *F. solani* and *F. oxysporum* f.sp. *cucumerinum*,

and from 52.7 to 73.7% for *F. oxysporum* f. sp. *radicis-lycopersici*. Lower values were detected after applying the three antagonistic isolates against *B. cinerea* and *M. melonis*. Thus, significant differences were only observed when applying CL17A, which reduced the disease severity by 50% for *B. cinerea* and by 37 to 42.1% in the control of *Mycosphaerella melonis*.

4. Discussion

Numerous studies have reported using mycopathogenic fungi to control pests and diseases. Thus, *Ampelomyces quisqualis*, a mycoparasite specific to *Erysiphales*, has been studied as a biocontrol agent [20], and *Sphaerellopsis filum* has been used for the control of plant rust disease caused by *Puccinia graminis* subsp. *graminícola* [21], among other examples. However, the aggressive fungicolous taxa most studied and used in agriculture is *Trichoderma*, the genus by excellence [22–24].

In this study, we analysed the capacity of *Cladobotryum* isolates to control different phytopathogens that cause severe damage in many crops in vitro and in vivo to assess whether we can consider *Cladobotryum* as a potential biological control agent. All 24 *C. mycophilum* isolates have shown high percentages of in vitro inhibition of the phytopathogens tested, as a result of high competition for nutrients and space because of the rapid growth of *Cladobotryum* (Figure 3). These values are lower in the case of *P. aphanidermatum* because this phytopathogen grows even faster, but antagonism exceeds 50% for nine of the isolates tested. No inhibition halos for the production of metabolites with fungistatic activity were observed, and the mycelium of *Cladobotryum* grew normally over that of the phytopathogens, albeit showing mycelial degradation under the microscope. The production of metabolites identified in these isolates has not been studied, but this genus is known to cause hyphal lysis by producing a wide variety of secondary metabolites with marked antifungal as well as antibacterial activities as well as repressive effects on cancer cells [25–27]. One of these fungal metabolites is cladobotryal, a *Cladobotryum varium* isolate with antifungal activity [28].

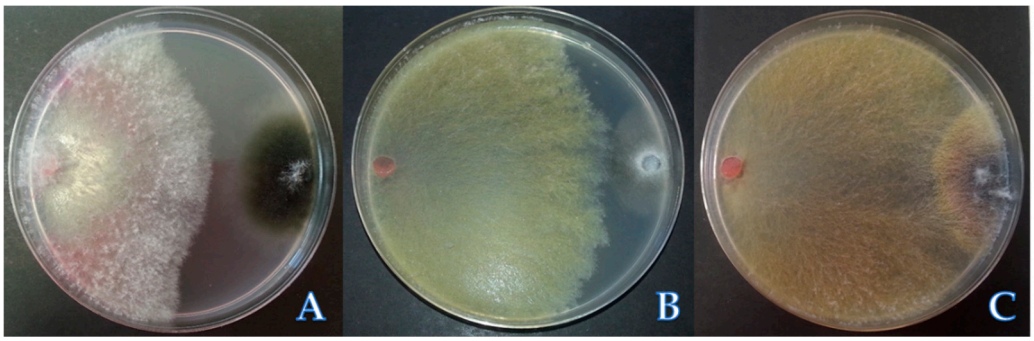

Figure 3. Antagonistic effects of *Cladobotryum* isolates against *M. melonis* (**A**), *P. capsici* (**B**) and *F. oxysporum* f.sp. *lycopersici* (**C**) in dual culture. C show overgrowth of *Cladobotryum* covering the *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) colony after 7 days of inoculation in dual culture.

In addition, very promising results have been obtained in in vivo tests. The best results have been achieved with the three oomycetes tested, with high percentages of inhibition of disease development. Bastos et al. [15] demonstrated that filtered cultures of *C. amazonense* are highly toxic to *Phytophthora palmivora* spores; they reduce the disease level under field conditions. Similarly, tests conducted by Bastos [29] confirm the inhibition of *Crinipellis perniciosa, Microcyclus ulei* and *Hemileia vastatrix* spore germination, as well as the growth of other fungi and of *Pseudomonas solanacearum*. In turn, Goh et al. [30] found similar results when using *Cladobotryum semicirculare* as a fungicolous antagonist, which inhibited *Rigidoporus microporus* mycelial growth by 79% in a direct antagonism test as well as heat stable antifungal *Cladobotryum semicirculare* filtrates. The authors also observed a reduction in disease severity of white root disease in rubber trees (47 to 50%) by the application of *Cladobotryum semicirculare*.

To our knowledge, no phytopathogenic fungal control test has been described, neither in vitro nor in vivo, for *Cladobotryum mycophilum*. Despite the good results, these studies must be continued to

determine appropriate doses and modes of application to pathogens and culture environments, and to identify the type of bioactive molecules responsible for such control.

Currently, there is a greater awareness of food safety, and consumers demand more sustainable systems that do not harm the environment and biodiversity. One of the biggest challenges we face in agricultural production is the reduction of risks and negative impacts of phytosanitary products and fertilisers on human health and the environment, while maintaining agricultural productivity and profitability. This is only possible by using new technologies in integrated production systems that reduce waste and minimise environmental impacts. New mycopathogenic microorganisms that can be used as tools for fungal disease control and plant probiotic microorganisms are needed to design new control strategies that help meet present and future needs of agricultural production systems.

5. Conclusions

All *Cladobotryum mycophilum* isolates have shown the ability to inhibit the mycelial development of eight phytopathogens. In this study, three potential *Cladobotryum* isolates have been selected for their high capacity to control diseases caused by phytopathogens, both airborne and soil pathogens. This is the first report of this mycopathogen as a beneficial fungus for agriculture.

Author Contributions: F.D. and M.S. conceived and designed the experiments; B.S.-M. and A.M.-G. performed the experiments; F.J.G. and F.D. analysed the data; M.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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