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Assessment of the Potential of *Trichoderma* spp. Strains Native to Bagua (Amazonas, Peru) in the Biocontrol of Frosty Pod Rot (*Moniliophthora roreri*)

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Abstract: The use of native *Trichoderma* strains has been proposed as a sustainable alternative to control cocoa diseases. The aim of this study was to assess indigenous *Trichoderma* strains from Bagua Province, Peru, with reference to their antagonistic characteristics in vitro and their potential for in vitro biocontrol against frosty pod rot (FPR) disease. A total of 199 strains were assessed for in vitro mycoparasitism, antibiosis, and potential antagonism. The effect of four strains was evaluated in vitro using epidemiological variables, yield, and efficacy at two sites (Copallín and La Peca). Significant differences ($p < 0.05$) were reported for all variables evaluated in vitro and in vitro. Mycoparasitism ranged from 32% to 100%, antibiosis from 33.36% to 57.92%, and potential antagonism from 42.36% to 78.64%. All strains were found to affect the in vitro-assessed parameters in addition to enhancing the productive yield. The efficiency ranged from 38.99% to 71.9% in Copallín, and 45.88% to 51.16% in La Peca. The CP24-6 strain showed the highest potential for biocontrol under field conditions when considering its effect on both sites.

Keywords: biological control; field testing; mycoparasitism; *Theobroma cacao* L.

1. Introduction

In Peru, cocoa (*Theobroma cacao* L.) is the second largest perennial crop with a total of 144,200 hectares. In the region of Amazonas, this crop is one of the most representative. The province of Bagua is one of the major areas for cultivation of native fine-flavor cocoa in the region and covers 2124 hectares [1]. In 2012, 56% of the national cocoa production was reported as common cocoa and 44% as fine-flavor cocoa [1]. In 2018, the cocoa cultivation area in Peru reached 199,000 hectares [2].

The amount of land area sown with cocoa has significantly grown due to the increased demand in Latin America due the quality and safety of the beans. However, cocoa production is constantly being threatened by fungal diseases which undermine the quantity and quality of crops.

One of the most important cocoa diseases is frosty pod rot (FPR), also known as moniliasis, caused by the fungus *Moniliophthora roreri* (Cif and Par (Evans et al.)) [3,4]. This phytopathogen infects only pods at any stage of development; however, young pods are more susceptible [5]. *M. roreri* infection results in induction of internal necrosis, premature ripening, and irregular brown or chocolate-colored

spots; fungal mycelia (white stroma) growing on the brown spots have highly infective spore masses and cause significant losses in production, in some cases, more than 75% [3,6,7].

FPR has been controlled using various strategies, such as through cultural actions that have included the removal of mummified pods and the complete removal of pods during low production (purging), periodic removal of diseased pods (every seven days), timely harvesting, pruning to rehabilitate cacao trees, sucker removal, weed control, drainage management, shade regulation, and maintenance pruning [6,8–12]. Likewise, in chemical control, the use of rational application of fungicides has been recommended, where copper hydroxide, flutolanil, azoxystrobin, trifloxystrobin, tebuconazole, and propiconazole have demonstrated field efficiency against FPR [9,12,13]. In genetic control, the use of cocoa clones with resistance to *M. roreri* has been recommended; ICS-95 and CATIE R6, for instance, have been identified as resistant genotypes [14]. One frequently explored strategy involves the use of fungi with an antagonistic effect on *M. roreri* as biological control agents [15]. Several studies adopting this approach have reported the isolating several species of the genus *Trichoderma* that have demonstrated antagonism against *M. roreri* [16,17]. Generally, *T. harzianum* and *T. virens* isolates have shown high biocontrol potential against FPR [18–20].

According to Infante et al. [21], different biocontrol mechanisms are involved in the biocontrol activity of the diverse species and strains of *Trichoderma*, including competition for space and nutrients, mycoparasitism, and antibiosis. However, some studies report that among the various *Trichoderma* strains, there is variability in the expression of these biocontrol mechanisms, which necessitates in vitro and in vitro characterization to allow for the selection of isolates with greater potential for the control of phytopathogenic fungi [20,21].

With this in mind, the aim of this research was to characterize strains of *Trichoderma* native to Bagua Province, Amazonas, with respect to their in vitro antagonistic characteristics and potential for in vitro biocontrol of FPR.

2. Materials and Methods

2.1. Acquisition of Microorganisms

In the present study, 199 native *Trichoderma* spp. strains from Bagua Province were assessed. The native strains of *Trichoderma* spp. are part of a study of *Trichoderma* species diversity. These strains were obtained from soil collected in the rhizosphere of *T. cacao* grown in plantations of native fine-flavor cocoa from the districts of Aramango, Imaza, Copallín, and La Peca in the province of Bagua in the region of Amazonas, Peru (Table 1). The dilution method was used to obtain the strains [22]; and they then were submitted to determine the growth rate at 25 °C with a photoperiod of 12-h fluorescent light and 12-h darkness within 4 days. The presence of *Trichoderma* was noted by the existence of green conidia patches or cushions [23–26]. Cultures grown were taken to new petri dishes, thus obtaining monosporic culture [27]. They were finally identified morphologically using the codes of Chaverri et al. [28], Gams and Bissett [29], Kraus et al. [30], Park et al. [31], Samuels et al. [32], and Samuels et al. [33] (Appendix A).

Only a strain of *Moniliophthora roreri* was used for the in vitro experiments. This strain was isolated from native fine-flavor cocoa pods from La Peca District, Bagua Province.

Both strains of *Trichoderma* and *Moniliophthora roreri* are currently part of the fungi collection of the Laboratory of Plant Health Research (LABISANV) of the Research Institute for the Sustainable Development of Ceja de Selva (INDES-CES), National University Toribio Rodriguez of Mendoza.

Table 1. Native *Trichoderma* spp. strains from soils of the province of Bagua, evaluated in vitro against *Moniliophthora roreri*, the causal agent of frosty pod rot in Peru.

District	UTM Coordinates	Altitude (m.a.s.l.)	Strains
Copallín	787460/ 9371158	894	CP19-1; CP19-2; CP19-3
	787010/ 9371871	931	CP20-1; CP20-2; CP20-3
	786828/ 9373275	873	CP22-1; CP22-2; CP22-3
	786287/ 9373896	962	CP23-1; CP23-2; CP23-3; CP23-4; CP23-5
	786771/ 9371113	820	CP24-1; CP24-2; CP24-3; CP24-4; CP24-5; CP24-6; CP24-7; CP24-8
	787395/ 9369923	785	CP43-1; CP43-2; CP43-3; CP43-4; CP43-5
	787524/ 9369984	801	CP44-1
	787575/ 9370291	825	CP46-1
	787663/ 9370405	835	CP49-1; CP49-2
	787574/ 9370503	846	CP50-1
	787682/ 9370679	850	CP51-1; CP51-2; CP51-3
	787695/ 9370824	855	CP52-1; CP52-2
	787375/ 9371358	905	CP53-1; CP53-2; CP53-3
	787315/ 9371220	898	CP54-1
	787414/ 9371086	890	CP55-1; CP55-2; CP55-3; CP55-4
	787465/ 9371110	894	CP56-1
	787440/ 9371333	913	CP57-1; CP57-2; CP57-3; CP57-4
	787398/ 9371399	915	CP59-1; CP59-2
La Peca	781248/ 9377461	657	CP14-1; CP14-2; CP14-3; CP14-4; CP14-5; CP14-6; CP14-7
	782400/ 9382298	1001	CP39-1; CP39-2; CP39-3; CP39-4
	781206/ 9377452	647	CP15-1; CP15-2
	785913/ 9373121	1063	CP16-1; CP16-2; CP16-3; CP16-4; CP16-5; CP16-6; CP16-7; CP16-8; CP16-9; CP16-10; CP16-11; CP16-12; CP16-13; CP16-14
	783389/9380375	887	CP17-1; CP17-2; CP17-3; CP17-4; CP17-5; CP17-6; CP17-7
	782893/ 9380404	897	CP18-1; CP18-2; CP18-3
	787237/ 9376536	1111	CP25-1
	786802/ 9377593	1137	CP27-1
	785718/ 9377204	1026	CP28-1; CP28-2; CP28-3; CP28-4; CP28-5
La Peca	785213/ 9377128	1011	CP29-1; CP29-2; CP29-3
	785501/ 9377143	1033	CP30-1
	782448/ 9377142	737	CP32-1; CP32-2; CP32-3
	782412/ 9377183	731	CP33-1; CP33-2; CP33-3
	782353/ 9377313	714	CP34-1; CP34-2; CP34-3; CP34-4
	782410/ 9377234	724	CP35-1; CP35-2; CP35-3
	783488/ 9377494	764	CP36-1; CP36-2; CP36-3
	783926/ 9380799	958	CP37-1; CP37-2
	783122/ 9381573	981	CP38-1; CP38-2
	786176/ 9378005	1011	CP42-1; CP42-2; CP42-3; CP42-4; CP42-5
Aramango	783111/ 9399062	515	CP1-1; CP1-2; CP1-3; CP1-4; CP1-5; CP1-6
	786053/ 9398435	874	CP61-1; CP61-2; CP61-3; CP61-4
	786017/ 9398414	891	CP62-1; CP62-2
	786355/ 9398229	949	CP63-1; CP63-2
	785646/ 9398424	859	CP64-1
Imaza	794828/ 9437021	315	CP4-1; CP4-2; CP4-3; CP4-4; CP4-5
	793717/ 9421156	341	CP6-1; CP6-2; CP6-3; CP6-4; CP6-5; CP6-6; CP6-7
	800851/ 9427309	284	CP7-1; CP7-2; CP7-3; CP7-4; CP7-5; CP7-6; CP7-7
	801104/ 9427855	286	CP9-1; CP9-2; CP9-5
	800750/ 9427725	279	CP8-1; CP8-2; CP8-3; CP8-4
	800999/ 9427257	284	CP10-1; CP10-2; CP10-3; CP10-4; CP10-5; CP10-6
	794520/ 9422160	320	CP11-1; CP11-2; CP11-3; CP11-4; CP11-5; CP11-6; CP11-7; CP11-8; CP11-9
	800705/ 9427717	278	CP12-1; CP12-2; CP12-3; CP12-4; CP12-5
	801103/ 9427282	286	CP13-1; CP13-2; CP13-3; CP13-4; CP13-5; CP13-6
	800945/ 9427291	285	CP31-1; CP31-2; CP31-3; CP31-4; CP31-5
	794808/ 9436976	314	CP5-1; CP5-2

2.2. *In vitro* Experiments

Mycoparasitism was evaluated using the pre-colonized petri dishes method according to Evans et al. [18]. For this purpose, a 5-mm diameter punching of 10-day-old *M. roreri* colonies was placed at the edge of a 90-mm diameter petri dish containing Potato Dextrose Agar (PDA) medium. These dishes were incubated for 25 days at 30 ± 1 °C in darkness. Once the fungus colonized the medium, a 2.5 × 0.5-cm punching of *Trichoderma* inoculum, obtained from the edge of a four-day-old colony, was placed at the side opposite to the *M. roreri* inoculum. These dishes were incubated for 15 days under the same conditions as for pre-colonization. After incubation, 10 samples of 5 mm diameter were extracted from the dish, initiating the cutting of the inoculum on the side where *M. roreri* was placed and in the direction of the *Trichoderma* inoculum. The punching was disinfected in each cut and the samples obtained were placed in petri dishes with PDA medium; five samples were sown per dish, in the order in which they were cut. The dishes with the samples were incubated at 30 ± 1 °C in the dark and evaluated for seven days before characterizing the growth of *Trichoderma* or *M. roreri*. The percentage of mycoparasitism was recorded using the following formula:

$$PP = (TG \times 100)/N \quad (1)$$

where:

PP = parasitism (%);
TG = *Trichoderma* growth;
N = number of samples taken from each replicate.

Likewise, antibiosis was evaluated using the paired culture method according to Holmes et al. [34]. For this experiment, a punching of 5 mm in diameter of 10-day-old *M. roreri* was taken from a petri dish and placed at the edge of a PDA petri dish. The inoculated plates were incubated and kept in the dark for 7 days at 30 ± 1 °C to allow the colony to establish. Subsequently, a punching 5 mm in diameter was extracted from a four-day-old *Trichoderma* colony and placed at the side opposite to *M. roreri*. The inoculated petri dishes were incubated at 30 ± 1 °C under dark conditions. Five repetitions per strain and five control petri dishes were established. The control sample 5 consisted of seven-day-old non-confronted *M. roreri* colonies. Radial growth of *M. roreri* was recorded daily until one of the *Trichoderma* strains had mycelial contact with *M. roreri*. The percentage of mycelial growth inhibition was calculated using Abbott's formula [35]:

$$PA = [(RG - RGT)/RG] \times 100 \quad (2)$$

where:

PA = antibiosis (%);
RG = radial growth of non-confronted *M. roreri* (mm);
RGT = radial growth of *M. roreri*-confronted *Trichoderma* (mm).

Parasitism and antibiosis percentage data were used to determine the potential antagonism following the formula used by Reyes–Figueroa et al. [20]:

$$PA = (TM + TA)/2 \quad (3)$$

where:

PA = potential antagonism;
TM = *Trichoderma* mycoparasitism against *M. roreri* (%);
TA = *Trichoderma* antibiosis against *M. roreri* (%).

2.3. Field Experiments

Two 25-year-old cocoa plantations were selected for evaluating the effect of *Trichoderma* strains against FPR under field conditions. These plantations are dominated by native "criollo" cacao germoplasm.

One cocoa farm was located in Lluhuana Village in the district of Copallín in Bagua Province, Amazonas Region, at 835 m.a.s.l. and UTM coordinates 787662/9370405. The second cocoa farm was located in La Tranquilla Village in the La Peca District of the same province as above, at 1060 m.a.s.l., and the UTM coordinates 786024/9377170. In each plantation, 15 experimental subplots (5 treatments with 3 repetitions each) were established, each consisting of 64 trees in a square of 8 × 8 trees. The treatments were applied to all of the trees in the plot, of which 16 central trees were evaluated in a square of 4 × 4 trees according to Bateman et al. [9]. The subplots were established in a random blocks design with three repetitions, including for the control group.

In each plot, maintenance pruning, weed control, and a "purge" that consisted of the total removal of residual pods of the previous productive cycle were carried out with the purpose of preparing the plot for the evaluation of *Trichoderma*. Subsequently, new pod populations of 8–12 cm were identified and labeled according to generation.

Based on the results of mycoparasitism, antibiosis, in vitro antagonism potential, high mass propagation capacity in solid substrate, and high conidium viability, CP10-3, CP53-2, CP24-6, and CP38-2 strains were therefore selected for further field trials.

The strains were multiplied in mass individually, and using rice as matrix and solid substrate [36], the concentration and viability of conidia were quantified; incubation took place at 25 °C ±2, at 12-h fluorescent light and 12-h darkness. Posterior, the biosolution was prepared by adding to the rice substrate, which contained the quantified conidia of *Trichoderma*, 100 mm of agricultural oil, to be subsequently dissolved in pure water at a pH of 6.5. Following a blank test per tree, conidia inoculum was applied at a dose of 1×10^9 conidia mL–1 using a 20-L "Jacto" brand spray at a rate of 0.2 L per cocoa tree. The spraying was done at intervals of 15 days throughout the production cycle (5 months), starting at the peak flowering stage [37]. The incidence of cocoa pods with RPF symptoms was quantified using the following formula:

$$I = [DP/TP] \times 100 \quad (4)$$

where:

I: Incidence (%);
DP: Number of damaged pods;
TP: Total pods.

The severity of external damage to infected pods was measured and recorded as the percentage (0–100%) of pod surface covered by necrotic spots. Each infected pod was examined to determine the severity of the infection, and it was then reported as an average was per tree and per treatment.

Trichoderma strains' effect was determined using two epidemiological variables, namely crop yield and treatment efficacy (E). The former was estimated in kg of dry cocoa beans; here we took into account the change in mass during processing (dry weight is 40% of the fresh weight of the equivalent material); as a result of this, kg of dry cocoa ha−1 year, for a density of about 833 plants ha−1 was obtained for each plot. On the other hand, efficiency of the treatment was calculated using the following formula [35]:

$$E = ((FIWoT-FIWT)/FIWoT) \times 100 \quad (5)$$

where:

E = efficiency (%);
FIWoT = % of final incidence without application of *Trichoderma* spp.;
FIWT = % of final incidence with application of *Trichoderma* spp.

2.4. Statistical Analyses

Data on mycoparasitism, antibiosis, and potential antagonism were analyzed under a completely random design. Prior to analysis, mycoparasitism, antibiosis, and potential antagonism data were transformed to the arcsine square root of the ratio. The data were subjected to ANOVA with Infostat software. The mean separation test (Scott Knott, $\alpha = 0.05$) was applied when the F test was significant for treatments.

In the same way, field experiment data were analyzed under a completely random blocks design. Prior to analysis, incidence, severity, and efficiency data were transformed to the arcsine square root of the ratio. The data were subjected to ANOVA with Infostat software. The mean separation test (Scott Knott, $\alpha = 0.05$) was applied when the F test was significant for treatments.

The in vitro experiments had 199 treatments and the field experiments had four treatments plus a control treatment.

3. Results and Discussion

3.1. In Vitro Mycoparasitism of Trichoderma spp. Against FPR

Trichoderma strains showed significant differences ($p < 0.05$) in parasitism against *M. roreri*, which ranged from 32% to 100% (Table 2). Fungi of the genus *Trichoderma* have strong parasitic activity, as shown by several studies that isolated species such as *T. asperellum*, *T. harzianum*, and *T. virens* [17–19]. However, this parasitic activity can be very variable even among strains of the same species, as reported by studies such as those of Reyes–Figueroa et al. [20] in Mexico and Bailey et al. [17]. Reyes–Figueroa et al. [20] found variability in parasitism of *Trichoderma* strains from the cocoa agroecosystem in Tabasco, Mexico, with values ranging from 0% to 100%, and they reported variability among strains of the same species. Bailey et al. [17], also using the method of pre-colonized plates, found differences in the parasitism of 15 strains of *Trichoderma* isolated from pods and stems of *Theobroma* species. All strains examined in the present study had some parasitism; however, it should be noted that 25 of them reached 100% parasitic activity according to the method used, which demonstrates the strong activity of the studied strains (Table 2).

3.2. In Vitro Antibiosis of Trichoderma spp. *Against FPR*

There were significant differences ($p < 0.05$) in the antibiosis of *Trichoderma* strains against *M. roreri*. All strains showed antibiosis values ranging from 33.36% to 57.92%. Strains CP7-1, CP24-6, CP23-1, CP35-1, and CP13-4 showed the highest antibiosis values, while strains CP27-1, CP24-8, and CP11-3 showed the lowest values (Table 3).

The highest values for antibiosis are similar to those reported by Reyes–Figueroa et al. [20] who found strains with 55.5% antibiosis; however, these authors reported strains with lower antibiosis levels than those found in this research. The antibiotic action of *Trichoderma* strains has also been reported for *M. perniciosa*, the causative agent of cocoa witches' broom [17].

According to Sivasithamparam and K. Ghisalberti [38], Howell [39], and Vinale et al. [40], *Trichoderma* exerts an antibiosis mechanism through volatile and non-volatile metabolites such as 6 pentyl-α-pyrone, isonitrile, harzianolide, trichodermine atroviridina, alameticine, suzucacilline, glyovirine, heptelidic acid, viridine, azapylone, butenolide, viridiole, gliotoxin, 1-hydroxy-3-methylanthraquinone, 1,8-dihydroxy-3-methyl-anthraquinone, koninginine, trichoviridine, and harzianic acid.

3.3. In Vitro Potential Antagonism of Trichoderma spp. Against FPR

There were significant differences ($p < 0.05$) in the potential antagonism values for the *Trichoderma* strains against *M. roreri* (Table 3). The values for antagonism ranged between 42.36% and 78.643%. Strains CP24-6, CP10-3, CP42-4, and CP28-1 had the highest values while strains CP51-3, CP13-2, and CP7-4 had the lowest (Table 4).

Table 2. Mycoparasitism of 199 native strains of *Trichoderma* from Bagua Province, Amazonas, Peru, evaluated over *Moniliophthora roreri* (± = standard error; average with the same letters are not statistically different (Scott Knott, $\alpha = 0.05$)).

Strain	Mycoparasitism	Strain	Mycoparasitism	Strain	Mycoparasitism	Strain	Mycoparasitism
CP18-2	100 ± 0a	CP32-2	92 ± 4.9a	CP14-4	84 ± 9.8b	CP7-5	80 ± 6.32b
CP18-3	100 ± 0a	CP31-1	92 ± 8a	CP20-3	84 ± 7.48b	CP7-3	80 ± 11b
CP24-6	100 ± 0a	CP19-3	92 ± 8a	CP14-7	84 ± 7.48b	CP7-6	80 ± 8.94b
CP25-1	100 ± 0a	CP36-1	92 ± 4.9a	CP23-4	84 ± 7.48b	CP57-3	80 ± 11b
CP17-7	100 ± 0a	CP35-2	92 ± 4.9a	CP23-3	84 ± 7.48b	CP57-2	80 ± 8.94b
CP17-1	100 ± 0a	CP34-3	92 ± 4.9a	CP23-2	84 ± 7.48b	CP22-2	80 ± 6.32b
CP17-2	100 ± 0a	CP34-2	92 ± 4.9a	CP16-8	84 ± 7.48b	CP64-1	80 ± 11b
CP17-4	100 ± 0a	CP12-5	92 ± 4.9a	CP16-7	84 ± 7.48b	CP8-3	80 ± 8.94b
CP17-5	100 ± 0a	CP16-3	92 ± 8a	CP16-6	84 ± 7.48b	CP19-1	80 ± 12.6b
CP28-1	100 ± 0a	CP61-3	92 ± 4.9a	CP20-1	84 ± 11.7b	CP7-1	80 ± 8.94b
CP37-2	100 ± 0a	CP1-4	92 ± 3.74a	CP19-2	84 ± 4b	CP6-7	80 ± 8.94b
CP42-4	100 ± 0a	CP1-6	92 ± 4.9a	CP12-2	84 ± 7.48b	CP52-2	80 ± 12.6b
CP43-3	100 ± 0a	CP4-1	92 ± 4.9a	CP13-5	84 ± 4b	CP23-1	80 ± 8.94b
CP53-2	100 ± 0a	CP61-4	92 ± 4.9a	CP11-3	84 ± 11.2b	CP57-4	76 ± 9.8b
CP33-2	100 ± 0a	CP62-2	92 ± 4.9a	CP13-6	84 ± 4b	CP8-2	76 ± 7.48b
CP28-3	100 ± 0a	CP12-4	92 ± 4.9a	CP13-4	84 ± 7.48b	CP11-5	76 ± 9.8b
CP28-5	100 ± 0a	CP42-3	92 ± 4.9a	CP1-1	84 ± 9.8b	CP11-4	76 ± 7.48b
CP29-1	100 ± 0a	CP24-7	88 ± 7.35a	CP12-3	84 ± 7.48b	CP11-2	76 ± 11.7b
CP32-1	100 ± 0a	CP8-4	88 ± 8a	CP24-8	84 ± 9.8b	CP63-1	76 ± 11.7b
CP13-1	100 ± 0a	CP14-6	88 ± 8a	CP24-3	84 ± 7.48b	CP6-2	76 ± 11.7b
CP10-4	100 ± 0a	CP24-5	88 ± 8a	CP24-2	84 ± 9.8b	CP31-2	76 ± 7.48b
CP16-5	100 ± 0a	CP5-2	88 ± 4.9a	CP6-4	84 ± 7.48b	CP31-3	76 ± 7.48b
CP10-3	100 ± 0a	CP1-3	88 ± 8a	CP4-4	84 ± 7.48b	CP1-5	76 ± 9.8b
CP16-14	100 ± 0a	CP6-1	88 ± 8a	CP55-3	84 ± 7.48b	CP31-4	76 ± 7.48b
CP14-5	100 ± 0a	CP31-5	88 ± 8a	CP52-1	84 ± 4b	CP49-2	76 ± 7.48b
CP27-1	98 ± 2a	CP9-1	88 ± 4.9a	CP7-2	84 ± 7.48b	CP59-2	76 ± 9.8b
CP33-3	96 ± 4a	CP57-1	88 ± 8a	CP38-2	82 ± 11.1b	CP55-4	76 ± 7.48b
CP32-3	96 ± 4a	CP59-1	88 ± 8a	CP16-2	80 ± 8.94b	CP56-1	76 ± 11.7b
CP38-1	96 ± 4a	CP61-1	88 ± 5.83a	CP13-3	80 ± 8.94b	CP55-1	76 ± 7.48b
CP36-2	96 ± 4a	CP24-1	88 ± 8a	CP43-5	80 ± 8.94b	CP24-4	72 ± 10.2b
CP16-13	96 ± 4a	CP42-1	88 ± 12a	CP16-1	80 ± 6.32b	CP11-8	72 ± 12b
CP16-11	96 ± 4a	CP37-1	88 ± 8a	CP5-1	80 ± 8.94b	CP51-2	72 ± 10.2b
CP22-1	96 ± 4a	CP39-4	88 ± 8a	CP10-1	80 ± 11b	CP53-3	72 ± 8b
CP11-7	96 ± 4a	CP16-10	88 ± 8a	CP53-1	80 ± 8.94b	CP62-1	72 ± 10.2b
CP29-2	96 ± 4a	CP11-6	88 ± 4.9a	CP46-1	80 ± 8.94b	CP6-5	72 ± 10.2b
CP28-4	96 ± 4a	CP17-6	88 ± 8a	CP15-1	80 ± 8.94b	CP6-3	68 ± 8b
CP17-3	96 ± 4a	CP16-12	88 ± 8a	CP50-1	80 ± 12.6b	CP8-1	68 ± 13.6b
CP12-1	96 ± 4a	CP6-6	88 ± 4.9a	CP42-2	80 ± 11b	CP23-5	68 ± 8b
CP30-1	96 ± 4a	CP35-1	88 ± 4.9a	CP4-2	80 ± 8.94b	CP63-2	68 ± 4.9b
CP29-3	96 ± 4a	CP34-4	88 ± 8a	CP1-2	80 ± 6.32b	CP43-1	68 ± 12b
CP14-3	96 ± 4a	CP35-3	88 ± 8a	CP39-3	80 ± 11b	CP51-1	68 ± 10.2b
CP16-9	96 ± 4a	CP4-5	88 ± 8a	CP11-9	80 ± 6.32b	CP20-2	64 ± 11.7b
CP28-2	96 ± 4a	CP22-3	88 ± 8a	CP43-2	80 ± 6.32b	CP43-4	64 ± 11.7b
CP9-5	92 ± 4.9a	CP15-2	86 ± 6.78a	CP16-4	80 ± 8.94b	CP49-1	64 ± 13.3b
CP9-2	92 ± 4.9a	CP4-3	84 ± 4b	CP42-5	80 ± 11b	CP11-1	56 ± 4c
CP39-2	92 ± 4.9a	CP36-3	84 ± 7.48b	CP14-1	80 ± 8.94b	CP44-1	48 ± 15c
CP18-1	92 ± 8a	CP61-2	84 ± 7.48b	CP55-2	80 ± 6.32b	CP51-3	44 ± 7.48c
CP34-1	92 ± 4.9a	CP10-2	84 ± 11.7b	CP54-1	80 ± 11b	CP13-2	36 ± 11.7c
CP10-5	92 ± 4.9a	CP39-1	84 ± 11.7b	CP14-2	80 ± 6.32b	CP7-4	32 ± 13.6c
CP33-1	92 ± 4.9a	CP10-6	84 ± 11.7b	CP7-7	80 ± 11b		

Table 3. Antibiosis of 199 native strains of *Trichoderma* from Bagua Province, Amazonas, Peru, evaluated over *Moniliophthora roreri* (± = standard error; average with the same letters are not statistically different (Scott Knott, $\alpha = 0.05$)).

Strain	Antibiosis	Strain	Antibiosis	Strain	Antibiosis	Strain	Antibiosis
CP7-1	57.92 ± 4.43a	CP15-2	52.91 ± 1.57 [a]	CP35-3	51.367 ± 0.327a	CP50-1	48.47 ± 2.37 [a]
CP24-6	57.27 ± 1.47a	CP16-5	52.907 ± 0.341 [a]	CP31-2	51.32 ± 2.56a	CP62-1	48.44 ± 3.44 [a]
CP23-1	56.69 ± 4.99a	CP8-3	52.88 ± 2.12 [a]	CP33-3	51.25 ± 1.3a	CP9-2	48.34 ± 1.82 [a]
CP35-1	56.12 ± 2.38a	CP39-4	52.87 ± 1.43 [a]	CP59-1	51.2 ± 2.53a	CP53-3	48.33 ± 2.14 [a]
CP13-4	56.03 ± 3.28a	CP16-2	52.77 ± 1.77 [a]	CP16-11	51.166 ± 0.7a	CP55-3	48.31 ± 1.21 [a]
CP38-2	55.843 ± 0.522a	CP35-2	52.76 ± 2.58 [a]	CP6-2	51.087 ± 0.418a	CP4-1	48.2 ± 1.66 [a]
CP11-4	55.77 ± 1.55a	CP7-5	52.75 ± 1.62 [a]	CP7-6	51.07 ± 1.85a	CP17-1	48.17 ± 1.74 [a]
CP14-7	55.765 ± 0.49a	CP7-4	52.71 ± 1.67a	CP28-4	51.02 ± 1.12a	CP53-1	48.12 ± 1.97 [a]
CP10-6	55.46 ± 2.74a	CP17-3	52.66 ± 1.06a	CP13-2	50.886 ± 0.594a	CP10-1	47.934 ± 0.909a
CP10-3	55.361 ± 0.684a	CP1-6	52.6 ± 1.29a	CP11-9	50.885 ± 0.879a	CP62-2	47.71 ± 2.46 [a]
CP24-7	55.182 ± 0.249a	CP6-3	52.584 ± 0.551a	CP14-3	50.88 ± 1.99a	CP56-1	47.59 ± 1.8 [a]
CP36-3	54.94 ± 3.08a	CP8-2	52.58 ± 1.67a	CP22-2	50.86 ± 1.73a	CP31-3	47.39 ± 2.05 [a]
CP61-1	54.836 ± 0.551a	CP39-1	52.477 ± 0.995a	CP34-3	50.81 ± 3.33a	CP1-2	46.92 ± 8.09b
CP16-7	54.8 ± 1.18a	CP38-1	52.43 ± 1.25a	CP5-2	50.81 ± 1.27a	CP20-2	46.85 ± 2.56b
CP42-2	54.76 ± 1.89a	CP4-3	52.423 ± 0.383a	CP32-3	50.727 ± 0.828a	CP55-1	46.74 ± 2.64b
CP31-4	54.68 ± 2.29a	CP16-10	52.39 ± 1.47a	CP6-1	50.697 ± 0.473a	CP57-2	46.25 ± 1.62b
CP19-1	54.43 ± 1.26a	CP16-1	52.364 ± 0.914a	CP8-4	50.67 ± 2.22a	CP23-3	46.12 ± 3.44b
CP61-3	54.28 ± 1.51a	CP24-3	52.35 ± 1.42a	CP29-1	50.62 ± 2.12a	CP1-3	45.95 ± 3.73b
CP15-1	54.173 ± 0.567a	CP6-5	52.26 ± 1.25a	CP18-3	50.599 ± 0.598a	CP52-2	45.91 ± 2.83b
CP43-4	54.15 ± 1.58a	CP37-2	52.26 ± 1.44a	CP12-1	50.49 ± 1.36a	CP51-1	45.63 ± 2.14b
CP61-2	54.14 ± 1.4a	CP17-4	52.222 ± 0.803a	CP63-1	50.48 ± 2.25a	CP52-1	45.46 ± 1.22b
CP64-1	54.122 ± 0.831a	CP5-1	52.221 ± 0.886a	CP9-1	50.3 ± 1.26a	CP51-3	45.3 ± 1.65b
CP42-4	54.03 ± 2.42a	CP13-3	52.22 ± 1.82a	CP33-1	50.208 ± 0.898a	CP55-2	45.18 ± 1.69b
CP42-1	54.01 ± 2.5a	CP16-4	52.22 ± 1a	CP29-3	50.201 ± 0.861a	CP43-5	45.09 ± 2.95b
CP11-7	53.99 ± 1.18a	CP30-1	52.197 ± 0.682a	CP34-2	50.15 ± 0.925a	CP23-2	44.92 ± 2.91b
CP36-2	53.98 ± 1.03a	CP18-1	52.191 ± 0.933a	CP43-3	50.04 ± 6.41a	CP20-3	44.7 ± 3.47b
CP51-2	53.96 ± 1.13a	CP37-1	52.15 ± 2.04a	CP16-14	50.02 ± 1.05a	CP4-2	44.47 ± 2.22b
CP16-3	53.886 ± 0.825a	CP29-2	52.144 ± 0.917a	CP11-5	49.983 ± 0.636a	CP22-1	44.2 ± 3.4b
CP42-3	53.88 ± 2.2a	CP10-2	52.13 ± 3.86a	CP46-1	49.98 ± 4.72a	CP59-2	43.82 ± 1.92b
CP39-3	53.78 ± 2.2a	CP31-5	52.12 ± 1.02a	CP49-2	49.97 ± 2.9a	CP43-2	43.33 ± 2.3b
CP11-2	53.65 ± 1.54a	CP11-6	52.083 ± 0.768a	CP6-6	49.9 ± 1.31a	CP1-4	43.035 ± 0.929b
CP16-13	53.593 ± 0.595a	CP10-5	52.051 ± 0.913a	CP19-3	49.821 ± 0.736a	CP57-4	42.96 ± 2.56b
CP28-1	53.59 ± 1.95a	CP34-1	52.03 ± 1.14a	CP13-6	49.82 ± 1.88a	CP22-3	42.89 ± 1.17b
CP16-6	53.59 ± 1.92a	CP33-2	52.008 ± 0.622a	CP25-1	49.733 ± 0.668a	CP23-4	42.6 ± 2.51b
CP36-1	53.57 ± 2.03a	CP32-2	51.961 ± 0.704a	CP28-5	49.669 ± 0.902a	CP4-5	42.48 ± 3.98b
CP13-1	53.45 ± 1.55a	CP13-5	51.91 ± 1.3a	CP18-2	49.65 ± 2.49a	CP24-2	42.36 ± 1.7b
CP24-1	53.33 ± 3.08a	CP31-1	51.889 ± 0.591a	CP55-4	49.65 ± 1.35a	CP23-5	41.91 ± 2.8c
CP63-2	53.27 ± 1.52a	CP6-4	51.79 ± 0.595a	CP11-8	49.65 ± 1.32a	CP57-1	41.52 ± 1.26c
CP14-4	53.234 ± 0.976a	CP28-3	51.75 ± 1.12a	CP9-5	49.637 ± 0.989a	CP57-3	41.51 ± 2.27c
CP61-4	53.2 ± 1.76a	CP7-2	51.71 ± 1.49a	CP7-7	49.38 ± 1.37a	CP49-1	41.37 ± 3.13c
CP7-3	53.15 ± 2.52a	CP14-6	51.67 ± 1.57a	CP39-2	49.37 ± 1.05a	CP19-2	41.15 ± 3.22c
CP42-5	53.121 ± 0.785a	CP17-7	51.665 ± 0.747a	CP1-5	49.31 ± 1.83a	CP24-5	40.99 ± 2.13c
CP14-5	53.099 ± 0.342a	CP34-4	51.659 ± 0.533a	CP8-1	49.07 ± 1.03a	CP1-1	40.41 ± 3.47c
CP16-8	53.044 ± 0.928a	CP24-4	51.57 ± 1.33a	CP12-2	49.058 ± 0.869a	CP4-4	40.34 ± 1.59c
CP28-2	53.013 ± 0.52a	CP16-12	51.53 ± 1.32a	CP54-1	48.95 ± 1.15a	CP43-1	39.4 ± 3.42c
CP12-4	53.011 ± 0.451a	CP10-4	51.506 ± 0.953a	CP44-1	48.72 ± 2.87a	CP53-2	38.206 ± 0.85c
CP17-2	53.01 ± 0.682a	CP16-9	51.48 ± 1.07a	CP6-7	48.71 ± 0.867a	CP27-1	36.13 ± 1.16d
CP20-1	52.99 ± 1.06a	CP12-5	51.456 ± 0.645a	CP11-1	48.69 ± 1.91[a]	CP24-8	35.16 ± 5.7d
CP32-1	52.946 ± 0.834a	CP14-2	51.449 ± 0.962a	CP17-5	48.678 ± 0.897[a]	CP11-3	33.36 ± 1.13d
CP14-1	52.93 ± 1.42a	CP17-6	51.425 ± 0.637a	CP12-3	48.54 ± 1.25[a]		

Table 4. Potential antagonism of 199 native strains of *Trichoderma* from Bagua Province, Amazonas, Peru, evaluated over *Moniliophthora roreri* (± = standard error; average with the same letters are not statistically different (Scott Knott, $\alpha = 0.05$).

Strain	Potential Antagonism	Strain	Potential Antagonism	Strain	Potential Antagonism	Strain	Potential Antagonism
CP24-6	78.64 ± 0.735a	CP10-5	72.03 ± 2.75a	CP39-1	68.24 ± 5.53 [a]	CP53-1	64.06 ± 5.16b
CP10-3	77.68 ± 0.342a	CP34-1	72.01 ± 2.25a	CP4-3	68.21 ± 1.87 [a]	CP10-1	63.97 ± 5.88b
CP42-4	77.01 ± 1.21a	CP32-2	71.98 ± 2.71a	CP24-3	68.17 ± 3.17 [a]	CP31-2	63.66 ± 3.92b
CP28-1	76.79 ± 0.962a	CP31-1	71.94 ± 4.02a	CP10-2	68.06 ± 6.99 [a]	CP6-2	63.54 ± 5.9b
CP13-1	76.72 ± 0.773a	CP12-5	71.73 ± 2.58a	CP13-5	67.95 ± 1.94 [a]	CP1-2	63.46 ± 5.24b
CP14-5	76.55 ± 0.171a	CP24-7	71.59 ± 3.71a	CP6-4	67.89 ± 3.49 [a]	CP23-4	63.3 ± 3.14b
CP17-2	76.51 ± 0.341a	CP61-1	71.42 ± 2.94a	CP7-2	67.85 ± 3.56 [a]	CP63-1	63.24 ± 5.62b
CP32-1	76.47 ± 0.417a	CP34-3	71.4 ± 2.54a	CP1-4	67.52 ± 1.78 [a]	CP24-2	63.18 ± 4.23b
CP16-5	76.45 ± 0.787a	CP33-1	71.1 ± 2.69a	CP42-2	67.38 ± 5.42 [a]	CP57-2	63.13 ± 4.66b
CP37-2	76.13 ± 0.719a	CP34-2	71.08 ± 2.76a	CP19-1	67.21 ± 6.5 [a]	CP11-5	62.99 ± 5.01b
CP17-4	76.11 ± 0.502a	CP42-1	71 ± 6.99a	CP15-1	67.09 ± 4.64a	CP49-2	62.98 ± 2.91b
CP33-2	76 ± 0.311a	CP19-3	70.91 ± 3.92a	CP27-1	67.07 ± 1.12a	CP51-2	62.98 ± 4.86b
CP28-3	75.87 ± 0.561a	CP9-5	70.82 ± 2.49a	CP64-1	67.06 ± 5.81a	CP52-2	62.95 ± 6.37b
CP17-7	75.83 ± 0.373a	CP39-2	70.68 ± 2.84a	CP1-3	66.97 ± 3.14a	CP55-4	62.82 ± 3.58b
CP10-4	75.75 ± 0.476a	CP24-1	70.67 ± 4.1a	CP13-6	66.91 ± 2.07a	CP1-5	62.65 ± 5.66b
CP29-1	75.31 ± 1.06a	CP39-4	70.43 ± 4.6a	CP39-3	66.89 ± 5.63a	CP55-2	62.59 ± 3.89b
CP18-3	75.3 ± 0.299a	CP16-10	70.19 ± 3.36a	CP7-3	66.57 ± 6.19b	CP19-2	62.58 ± 2.68b
CP43-3	75.02 ± 3.21a	CP9-2	70.17 ± 2.19a	CP42-5	66.56 ± 5.81b	CP43-5	62.54 ± 4.73b
CP16-14	75.01 ± 0.523a	CP22-1	70.1 ± 1.67a	CP12-2	66.53 ± 3.83b	CP4-2	62.23 ± 4.79b
CP11-7	75 ± 2.28a	CP4-1	70.1 ± 2.95a	CP14-1	66.47 ± 4.62b	CP1-1	62.2 ± 5.69b
CP36-2	74.99 ± 1.84a	CP37-1	70.07 ± 4.39a	CP8-3	66.44 ± 5.03b	CP4-4	62.17 ± 4.4b
CP25-1	74.87 ± 0.334a	CP31-5	70.06 ± 3.94a	CP16-2	66.39 ± 4.5b	CP6-5	62.13 ± 4.59b
CP28-5	74.83 ± 0.451a	CP11-6	70.04 ± 2.37a	CP7-5	66.37 ± 2.49b	CP56-1	61.79 ± 5.82b
CP18-2	74.82 ± 0.662a	CP13-4	70.02 ± 4.79a	CP12-3	66.27 ± 3.17b	CP24-4	61.78 ± 4.97b
CP16-13	74.8 ± 2.26a	CP14-7	69.88 ± 3.76a	CP16-1	66.18 ± 2.93b	CP31-3	61.69 ± 4.61b
CP28-2	74.51 ± 2.18a	CP62-2	69.86 ± 1.81a	CP55-3	66.15 ± 3.64b	CP43-2	61.67 ± 3.41b
CP17-5	74.34 ± 0.449a	CP14-6	69.84 ± 3.5a	CP5-1	66.11 ± 4.45b	CP55-1	61.37 ± 4.66b
CP17-3	74.33 ± 1.88a	CP34-4	69.83 ± 3.93a	CP13-3	66.11 ± 4.36b	CP11-8	60.82 ± 5.51b
CP38-1	74.22 ± 2.38a	CP16-12	69.77 ± 3.73a	CP16-4	66.11 ± 4.67b	CP57-3	60.76 ± 5.8b
CP30-1	74.1 ± 1.82a	CP10-6	69.73 ± 5.65a	CP11-4	65.89 ± 4.23b	CP63-2	60.63 ± 2.37b
CP17-1	74.09 ± 0.87a	CP17-6	69.71 ± 4.01a	CP14-2	65.72 ± 2.78b	CP6-3	60.29 ± 3.89b
CP29-2	74.07 ± 1.79a	CP35-3	69.68 ± 4.1a	CP7-6	65.54 ± 4.52b	CP62-1	60.22 ± 5.94b
CP16-9	73.74 ± 2.23a	CP59-1	69.6 ± 3.54a	CP22-3	65.44 ± 4.01b	CP53-3	60.16 ± 3.63b
CP33-3	73.63 ± 1.51a	CP36-3	69.47 ± 3.81a	CP11-9	65.44 ± 2.79b	CP59-2	59.91 ± 4.15b
CP16-11	73.58 ± 2.01a	CP15-2	69.45 ± 3.51a	CP22-2	65.43 ± 3.54b	CP24-8	59.58 ± 5.66b
CP28-4	73.51 ± 1.91a	CP5-2	69.4 ± 2.96a	CP31-4	65.34 ± 4.17b	CP57-4	59.48 ± 5.75b
CP14-3	73.44 ± 2.08a	CP16-7	69.4 ± 3.91a	CP4-5	65.24 ± 4.79b	CP43-4	59.08 ± 5.17b
CP32-3	73.36 ± 2.28a	CP6-1	69.35 ± 4a	CP23-3	65.06 ± 4.56b	CP11-3	58.68 ± 5.86b
CP12-1	73.24 ± 2.52a	CP8-4	69.33 ± 2.94a	CP46-1	64.99 ± 5.96b	CP8-1	58.54 ± 6.95b
CP61-3	73.14 ± 2.98a	CP9-1	69.15 ± 2.33a	CP11-2	64.83 ± 6.22b	CP51-1	56.82 ± 4.66b
CP29-3	73.1 ± 1.89a	CP53-2	69.1 ± 0.425a	CP57-1	64.76 ± 3.42b	CP20-2	55.42 ± 6.47c
CP16-3	72.94 ± 4.33a	CP61-2	69.07 ± 3.43a	CP52-1	64.73 ± 1.75b	CP23-5	54.95 ± 2.67c
CP42-3	72.94 ± 3.34a	CP7-1	68.96 ± 5.09a	CP7-7	64.69 ± 6.1b	CP43-1	53.7 ± 6.33c
CP36-1	72.79 ± 1.68a	CP6-6	68.95 ± 2.53a	CP24-5	64.49 ± 3.82b	CP49-1	52.68 ± 7.04c
CP61-4	72.6 ± 3a	CP38-2	68.92 ± 5.5a	CP54-1	64.47 ± 5.37b	CP11-1	52.35 ± 2.15c
CP12-4	72.51 ± 2.42a	CP16-6	68.79 ± 3.67a	CP23-2	64.46 ± 2.47b	CP44-1	48.36 ± 7.06c
CP35-2	72.38 ± 1.38a	CP14-4	68.62 ± 4.62a	CP6-7	64.35 ± 4.32b	CP51-3	44.65 ± 4.49c
CP1-6	72.3 ± 2.65a	CP16-8	68.52 ± 4.02a	CP20-3	64.35 ± 4.14b	CP13-2	43.44 ± 5.63c
CP18-1	72.1 ± 4.19a	CP20-1	68.49 ± 6.03a	CP8-2	64.29 ± 3.73b	CP7-4	42.36 ± 6.69c
CP35-1	72.06 ± 3.15a	CP23-1	68.35 ± 6.81a	CP50-1	64.23 ± 6.69b		

Antibiosis and mycoparasitism are important antagonistic interactions when considering the selection of *Trichoderma* as a biological control agent [41]. Several studies have identified the antagonistic activity of *Trichoderma* fungi based on their antibiosis and parasitism; however, the use of both simultaneously as a source for assessing antagonistic potential was first presented by Reyes–Figueroa et al. [20]. In our research, we used their proposed formula and reported strains with higher potential antagonism than found in their report.

3.4. Field Responses of Trichoderma spp. Against FPR

Trichoderma strains demonstrated an effect on the epidemic intensity of FPR, affecting the final incidence and severity of damage. In the plot located in Copallín District, there were significant differences ($p < 0.05$) between treatments in terms of final incidence of cocoa FPR, damage severity, yield, and efficiency of strains (Table 5). The highest incidence was reported in the control treatment (15.2%); in addition, this treatment showed the lowest yield. The final incidence of each strain was variable; the lowest incidence was found in the plot treated with strain CP10-3, and the second lowest final incidence on the plot treated with the strain CP24-6, though these differences were not found to be significant. The lowest damage severity was presented in the plot treated with strain CP24-6, although no significant differences were reported with plots treated with other strains. Although there were no differences in yield between strains, the highest adjusted productive yield was found in the plot treated with CP24-6 followed by CP10-3. However, CP10-3 showed the highest control effectiveness with 71.9%, followed by CP24-6 (Table 5).

Table 5. Effect of four native strains of *Trichoderma* on epidemiological parameters of frosty pod rot (FPR), production yield, and respective efficiency. ([y] Averages with same letters are not statistically different (Tukey, $\alpha = 0.05$); [z] values not calculated due to the character of Abbott's formula).

Site	Treatment	Incidence [y] (%)	Severity (%)	Efficiency (%)	Yield (kg/ha)
Copallín	Control	15.2a	52.5a	— [z]	826.39b
	CP10-3	4.05c	30.43b	71.9a	1095.0ab
	CP53-2	8.05bc	30.6b	44.53bc	952.94ab
	CP24-6	5.62bc	27.36b	61.42ab	1115.0a
	CP38-2	8.77b	35.1b	38.99c	969.94ab
		F = 13.52	F = 11.04	F = 14.29	F = 3.97
		$p = 0.0005$	$p = 0.001$	$p = 0.0014$	$p = 0.035$
La Peca	Control	20.32a	51.43a	—	449.33b
	CP10-3	10.32b	32.05b	49.23a	866.0a
	CP53-2	9.94b	33.35b	51.16a	873.33 [a]
	CP24-6	10.96b	28.63b	45.88a	787.0a
	CP38-2	10.58b	34.10b	47.79a	823.67 [a]
		F = 28.47	F = 31.17	F = 0.5	F = 10.57
		$p < 0.0001$	$p < 0.0001$	$p = 0.69$	$p = 0.0013$

In the plot located in La Peca District, significant differences ($p < 0.05$) were found between treatments according to the four evaluated parameters (Table 5). In the same way as the Copallín plot, all *Trichoderma* strains affected the final incidence and damage severity parameters, thus showing a protective effect over cocoa pods during the production process. The control treatment presented the highest incidence (20.32%) and the lowest adjusted yield. In this site, strain CP53-2 showed the lowest final incidence of FPR, the highest efficiency, and the highest yield. As for Copallín, there were no differences between strains in La Peca with respect to yield. The CP10-3 strain did not show the same behavior shown in the Copallín plot; however, the CP24-6 strain showed the highest values in the evaluated parameters.

In this study, in vitro assessment made it possible to preselect promising strains for biological control. In vitro assessment demonstrated that the CP24-6 strain had the highest biocontrol potential under field conditions when considering its effect in both localities; this strain was also outstanding in parasitism, antibiosis, and potential antagonism under in vitro conditions, while the CP10-3 strain was the most effective only in Copallín. It is important to mention that the CP10-3 strain was isolated in the district of Imaza at 284

m.a.s.l. and evaluated at 835 m.a.s.l. in Copallín and 1060 m.a.s.l. in La Peca. On the other hand, CP24-6 was isolated in Copallín at 820 m.a.s.l. According to Krauss and Soberanis [37], native antagonistic fungi are better adapted to local conditions. In our study, the observed efficacy of *Trichoderma* spp. in field conditions is in agreement with this conclusion from Krauss and Soberanis [37] who evaluated mixtures of different strains of *Trichoderma* in the field. All of them showed efficiency, reducing FPR significantly and increasing production both in terms of the percentage of healthy pods and absolute production. On the other hand, Krauss et al. [42] evaluated *Clonostachys byssicola*, *C. rosea*, and *Trichoderma* spp. as biological control agents against FPR in field conditions. Although all treatments showed biocontrol efficacy, *Trichoderma* spp. was the most efficient and gave an increase in yield of 34% with respect to the control sample.

4. Conclusions

The native strains of *Trichoderma* spp., from the province of Bagua, Amazonas, demonstrated intraspecific variability in terms of mycoparasitism and antibiosis against FPR disease. As demonstrated using in vitro experiments, all assessed strains affected the parameters of the epidemic process in addition to improving the productive yield. The CP24-6 strain presented the greatest biocontrol potential under field conditions when considering its effect on evaluations at the two sites. In the cocoa agroecosystem of Bagua Province, fungi of the genus *Trichoderma* have antagonistic activity against FPR disease, supporting the concept that good sites for identifying antagonists are places close to the site of infection. In addition, the variability in the assessed characteristics of the strains confirms the importance of using both an in vitro and in vitro evaluation in selection. To summarize, the results reveal the potential of the native *Trichoderma* strains in the development of biological formulations for FPR control.

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Appendix A

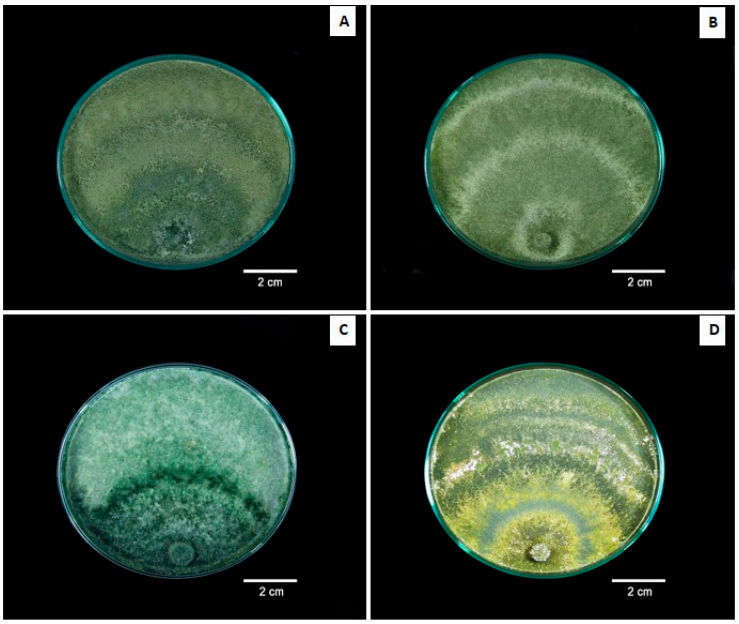

Figure A1. 15-day old *Trichoderma* colonies in potato dextrose agar (PDA) medium. (**A**) CP 10-3; (**B**) CP53-2; (**C**) CP24-6; (**D**) CP38-2.

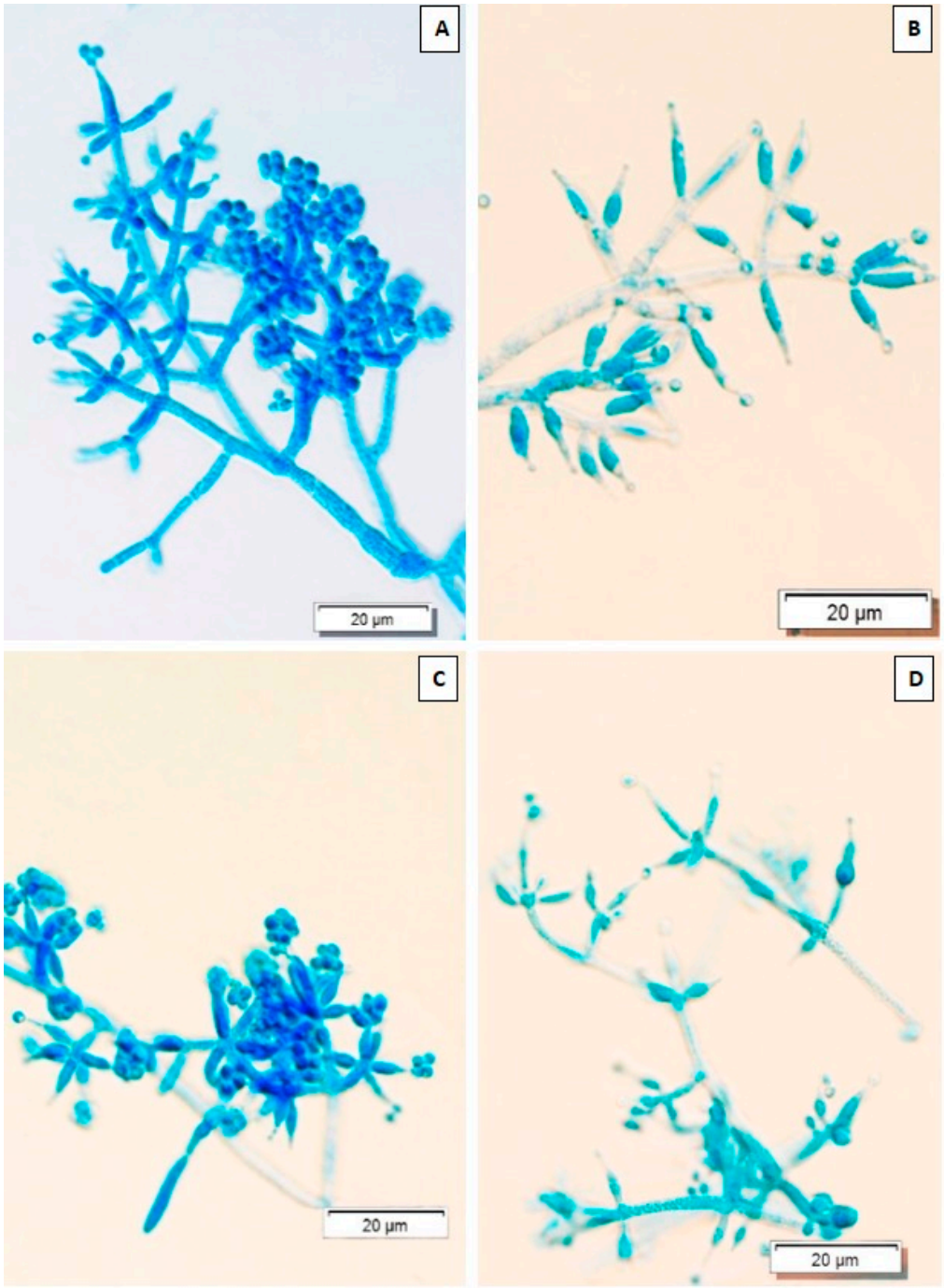

Figure A2. *Trichoderma* spp. conidia observed under the microscope. (**A**) CP 10-3; (**B**) CP53-2; (**C**) CP24-6; (**D**) CP38-2.

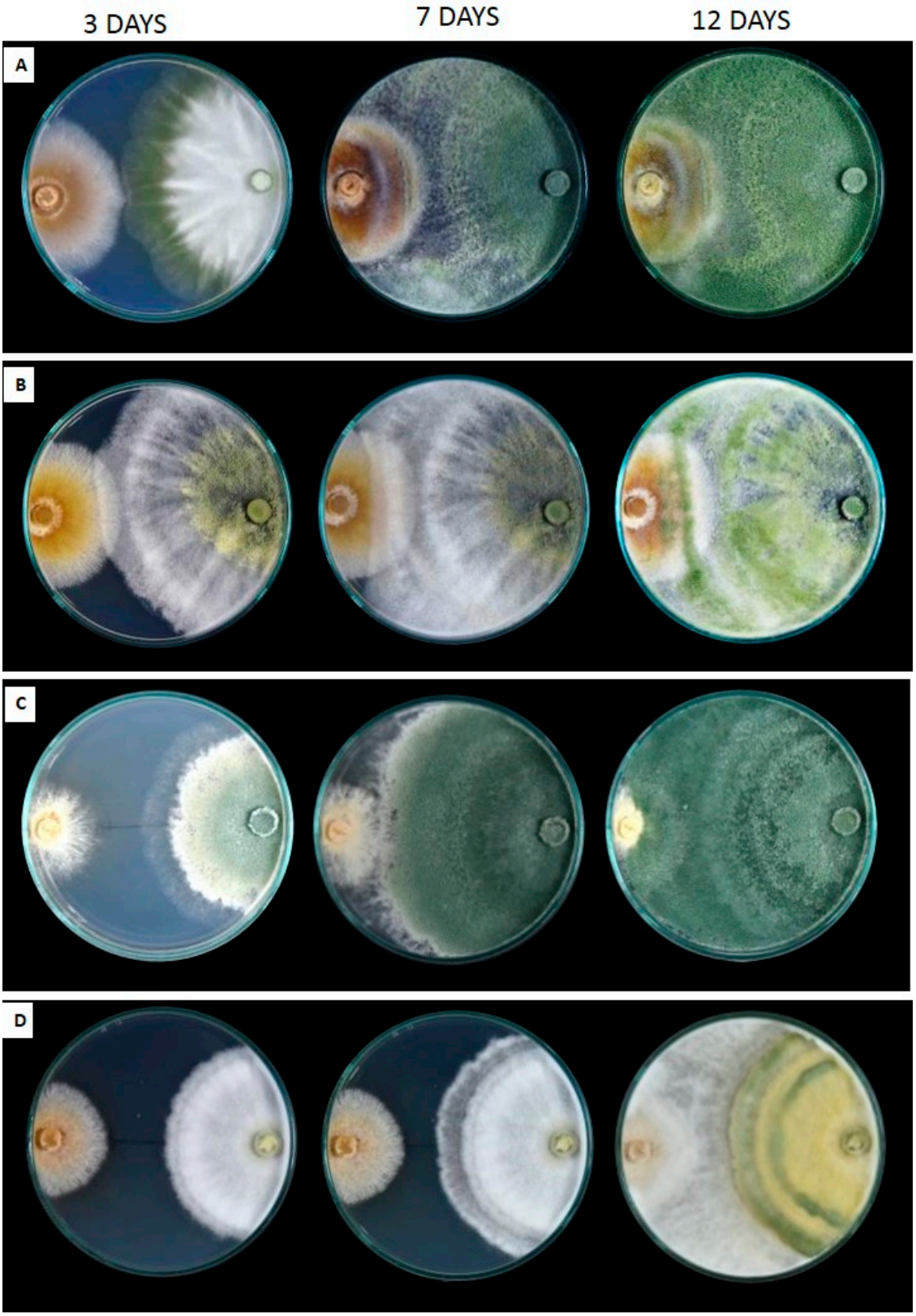

Figure A3. In vitro antagonism tests for: (**A**) CP 10-3; (**B**) CP53-2; (**C**) CP24-6; (**D**) CP38-2. From left to right 3, 7, and 12-day-old cultures. In each dish, on the left FPR strain, and on the right *Trichoderma* strain.

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