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Antifungal effect from *Zingiber officinale, Aloe vera* and *Trichoderma* sp. for control of *Moniliophthora roreri* in *Theobroma cacao* in Huánuco, Peru

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto antifúngico de *Zingiber officinale* jengibre, *Áloe vera* y *Trichoderma* sp. para controlar *Moniliophthora roreri* en *Theobroma cacao* en Huánuco, Perú.

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ABSTRACT

Keywords:
Biological control
Moniliophthora roreri
Peru
Theobroma cacao
Fungicides

Theobroma cacao is the main raw material to produce chocolate, as well as for use in the food, cosmetic, and pharmaceutical industries. However, *Moniliophthora roreri* is one of the most destructive fungal diseases and the main limiting of cacao production worldwide. Thus, this work aimed to assess the inhibitory effect of extracts of *Zingiber officinale* (T1) and *Aloe vera* (T2), and *Trichoderma harzianum* + *Bacillus subtillis*. (T3) on *Moniliophthora roreri* infection in *Theobroma cacao*; in addition, a control (T4) was also evaluated. Each treatment was applied to six plants of cacao. Incidence of monilia infection and fruit weight were monitored every 15 days (in total four periods) after the application of the treatment by spray. Significant differences (P<0.05) were found among treatments for incidence. It was observed that spraying entire cacao trees after two times (approximately 30 days) showed a reduction of monilia infection. After all periods, T1, T2, and T3 showed an incidence of monilia infection by 20.5, 17.7, and 14.9% respectively, compared to cultural control of 41.1%. This reduction of moniliasis infection translates into an increase in fruit weight average for T3 (8.4 kg), T2 (7.3 kg), and T1 (6.9 kg). In contrast, in the control (T3), the fruit weight average decreased by 5.3 kg. Biological control showed efficient management of pathogens as *M. roreri.* It is recommended to use such antifungal (*Aloe vera*) spray over at least 120 days which would decrease infection incidence even more.

RESUMEN

Palabras clave:
Control biológico
Monilia
Perú
Cacao
Fungicidas

El cacao es la principal materia prima para producir chocolate, así como para su uso en las industrias alimentaria, cosmética y farmacéutica. Sin embargo, *Moniliophthora roreri* es una de las enfermedades fúngicas más destructivas y la principal limitante de la producción de cacao en todo el mundo. Así, este trabajo evaluó el efecto inhibidor de extractos obtenidos de *Zingiber officiale* (T1) y *Aloe vera* (T2), y *Trichoderma harzianum* + *Bacillus subtillis* (T3) en la infección producida por *Moniliophthora roreri* en *Theobroma cacao*; además, un cultivo control (T4) también fue evaluado. Cada tratamiento se aplicó a seis plantas de cacao. La incidencia de la infección por monilia y el peso de la fruta se monitorearon cada 15 días (en total cuatro períodos) después del tratamiento por pulverización. Se encontraron diferencias significativas (P<0.05) entre los tratamientos por incidencia. Se observó que la pulverización de árboles enteros de cacao después de dos veces (aproximadamente 30 días) mostró una reducción de la infección por monilia. Después de todos los períodos, T1, T2 y T3 mostraron una reducción en la infección por monilia de 20,5, 17,7 y 14,9% respectivamente, en comparación con el control cultural de 41,1%. Esta reducción de la infección por moniliasis se tradujo en un aumento del peso promedio de los frutos en T3 (8,4 kg), T2 (7,3 kg) y T1 (6,9 kg). En cambio, el control cultural (T3), el peso promedio de la fruta disminuyó a 5,3 kg. El control biológico mostró un manejo eficiente de patógenos como *M. roreri*. Recomendamos el uso de este tipo de antifúngicos (en especial *Aloe vera*) aplicados durante al menos 120 días lo que disminuiría más la incidencia de la infección.

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Theobroma cacao (L.) or cacao tree is cultivated mainly in tropical areas of Latin America due to its economic and ecosystem importance (Toala *et al*., 2019). Traditionally, cacao seeds have been exploited for the manufacture of mainly chocolate and candies but based on their organoleptic and nutritional characteristics are used also in food, pharmaceutical, and cosmetic industries (Delgado *et al*., 2018; López *et al*., 2020).

According to the Ministry of Agriculture of Perú (MINAGRI), in Latin America, Brazil, Ecuador, Perú, and Colombia are the countries with the highest cacao production, while Costa de Marfil, Ghana, and Nigeria are the largest producers in the African continent (about 50% of world production) (MINAGRI, 2019). Peru owns 60% of the world´s cacao varieties and its production has been growing at an average annual rate of 15.6% for 10 years consecutively (MINAGRI, 2019). However, in Peru, the moniliasis is the main limiting of cacao production by reducing their production up to 40% (López *et al*., 2020). As consequence, many cacao fields are abandoned or replaced by other more profitable activities (Berget *et al*., 2021).

Cacao production worldwide is limited by fungal diseases, and it is estimated that they cause about 30% of loss and generate an economic imbalance for exporting countries (Delgado-Ospina *et al*., 2021). The most destructive pathogens for cacao are of the genus *Moniliophthora sp*., especially *M. perniciosa* (Dos Santos *et al*., 2020) and *M. roreri* (Bailey *et al*., 2018), which cause moniliasis and the witches' broom disease, both endemic and highly invasive in cacao. Moniliasis caused by *M. roreri* exclusively affects the cacao fruits at any stage of development and may cause losses of up 90% of production (Bailey *et al*., 2018). These fungi reproduce on the cobs and are dispersed by spores that come into contact with other cobs (Tirado-Gallego *et al*., 2016). Symptoms include bumps, premature yellowing or maturation, and oily and necrotic spots, which cause total loss of seeds or a decrease in their organoleptic quality (Joya-Dávila *et al*., 2015).

To control and reduce these diseases, many times farmers have preferred the application of chemicals (Tirado-Gallego *et al*., 2016); nonetheless, its use can generate high costs and cause serious damage to the environment, soil, and human health (Anzules-Toala *et al*., 2021; Torres-de-la-Cruz *et al*., 2019). Other types of control are cultural control (*phytosanitaryness*), agronomic practices, the use of biological agents (especially *Trichoderma* sp. *Bacillus* sp.) of fungus and bacteria since these are friendly to the environment and easy application (Toala *et al*., 2019; Villamil *et al*., 2016; Villamizar-Gallardo *et al*., 2017). For instance, Seng *et al*. (2014) applied *Trichoderma sp*. to control *M. roreri* in Costa Rica and reported a monilia reduction of 11% in only 35 days.

In addition, Joya-Dávila *et al*. (2015) used an extract of *Zingiber officinale* and reported from 88 to 100% control over this pathogen formation and germination. De Rodríguez *et al*. (2005) evaluated the inhibitory effect of *Aloe vera* pulp and liquid fraction on three phytopathogenic fungi (*Rhyzoctonia solani, Fusarium oxysporum,* and *Colletotrichum coccodes*) isolated from a potato crop. Results showed an inhibitory effect on *F. oxysporum* and a reduction in the rate of colony growth. Similarly, Rosca-Casian *et al*. (2007) evaluated the antifungal activity of *Aloe vera* against four pathogenic species *Alternativa* viz., *A. alternat, A. citri,* and *A. tenuissima*, founding significant inhibition on growth and biomass production.

According to the Ministry of Agriculture of Peru (MINAGRI), cacao cultivation is of the largest economic importance in the province of Leoncio Prado. Nevertheless, its production in this province is affected by moniliasis between 12 to 24% (MINAGRI, 2008). Biological control is considered a promising alternative to cope with agrochemicals and plant diseases because is less costly and gives protection to the crop or fruit throughout the crop period. Likewise, their application does not cause toxicity to the plants, is safer for the environment and for the people who apply them. Thus, this work aimed to assess the inhibitory effect of extracts of *Zingiber officinale*, *Aloe vera,* and *Trichoderma harzianum* + *Bacillus subtillis*, in the incidence of *Moniliophthora roreri* on *Theobroma cacao*.

MATERIALS AND METHODS

Study site

The study was carried out at the 14-year-old commercial cacao plantation (CCN-51) during January – February from 2021 in the Jose Crespo and Castillo district, Leoncio Prado Province, Huánuco-Peru (08°56′00"S; 76°02′30"O). The plantation was located at 540 masl, showing a tropical climate, with annual precipitation of 3179 mm, an average temperature, and relative humidity of 23.8 °C and 86%, respectively (SENAMHI, 2021). During the study period (3 months) the maximum temperature was 34.4 °C and the average was 30.4±2.4 °C. Likewise, the minimum temperature was 19 °C and the average of 21.5±0.7 °C, average precipitation was13.4±22.3 mm with the highest values among January-February (97.7 mm day^{-1}) (Figure 1).

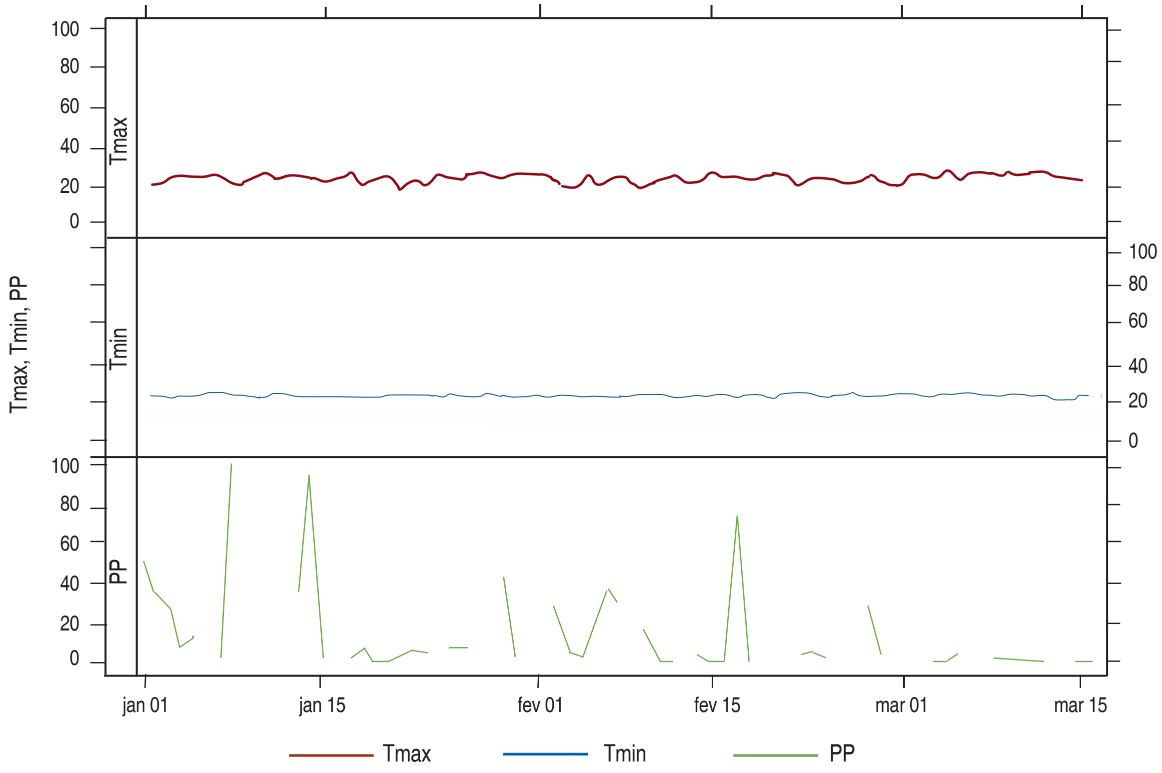

Figure 1. Maximum temperature (Tmax), minimum temperature (Tmin), and precipitation (PP) during the development of the study.

Experimental design

The experiment was conducted employing a random block design (DBCA) with 4 treatments and 3 replications. The experimental area was 630 m^2 with 96 plants of cacao divided into two plots. Each plot had 48 plants (6 columns with 8 plants) at 3x2 m^2. Data were taken from three central plants to avoid the edge effect.

Treatments

In total, four treatments were applied; three biological treatments as fungicides: i) T1: *Zingiber officinale* (it was macerated, and 333 mL of juice obtained was diluted in 20 L of water), ii) T2: *Aloe vera* (it was carried out a mixture of 200 g of *Aloe vera*, 200g of paico leaves (*Dysphania ambrosioides*), 200 g of dried horsetail (*Equisetum arvense*), and 200 g of soap glycerin solid blue, all diluted in 2 L water), iii) T3: a combination (20 L total) of *Trichoderma harzianum* T-22 (w/v, 14 g 10 L^{-1} water) and a biofungicide Serenade® (*Bacillus subtillis* QST173 (v/v, 15 mL 10 L^{-1} water), and iv) T4: cultural control (nothing applied). The cultural control management was applied (sanitary pruning + removal of sick cobs) every 15 days. Treatments were applied throughout four periods in 2021: P1 (11 January), P2 (27 January), P3 (11 February), and P4 (26 February), using a nutrifield PH-B20 manually operated backpack sprayer. Sprays were delivered at a rate of 10 L min^{-1}, wetting the entire trunk, leaves, fruits, and branches from the ground up to 3 m.

Monitoring process

Fruits were monitored for monilia infection on 6 plants;

each plant may contain a number determined of cobs in the four periods: 11 (first evaluation after treatment application) and 27 in the second period in January, and 11 (third period) and 26 (fourth period) in February 2021. At each period, the fruits greater than 15 cm were assessed, considering: i) the number of healthy cobs; ii) the number of cobs affected with *M. roreri*; iii) the number of seeds in healthy cobs, and iv) total weight of the seeds. Before applying any treatment, it was observed that most cobs of cacao were infected by *M. roreri*. Thus, fruits infected totally with monilia were cut and removed, from their trees, burned, and buried to avoid their proliferation (Figure 2). Incidence (%) of cobs harvested with moniliasis were quantified using the following equation: I(%)=(CI/CH)x100. Where I(%): Percentage of fruits infected with the disease; CI: Number of infected cobs; and CH: Number total cobs harvested) (Toala *et al*., 2019). The total weight was calculated by weighing (kg plant^{-1}) the grains extracted from ripe fruits harvested during the period of evaluation.

Figure 2. Cacao fruits: A) infected with monilia, B) Burned, and C) Buried.

Data analysis

Data were submitted to one-way analysis of variance (ANOVA) and subsequent Tukey´s test (value of P<0.05 was significant) to compare the mean among different treatments applied. Graphs and statistical analysis were performed using R software, version 3.3.6 (R Team Core, 2019).

RESULTS AND DISCUSSION

This study shows the inhibitory effect of three biological agents (*Zingiber officinale*, *Aloe vera*, and *Trichoderma harzianum* + *Bacillus subtillis*) for the management of moniliasis on cacao fruit in the study area. This work was carried out in the field for 3 months, where biological agents were sprayed (at each start of monitoring) on trees and fruits, and the incidence of moniliasis was monitored every 15 days.

Figure 3 shows the percentage of healthy and infected cobs by *Moniliophthora roreri*, evaluated at each period (P1, P2, P3, and P4) and using three biological treatments: *Zingiber officinale* (T1) *Aloe vera* (T2) and *Trichoderma harzianum* + *Bacillus subtillis* (T3), and control (T4). It was observed among the period an increase of healthy cobs and a reduction of infected cobs. For instance, T1 showed a decrease of infected cobs among periods: 51.9% (P1) to 10% (P2), 20% (P3), and 0% (P4). Likewise, in the P1 (after 15 days of evaluation and the first application of treatments) the T1, T2, T3, T4 were reported that 51.9, 38.9, 45.5, and 55.6% were still infected, respectively. In contrast, in P2 (after 30 days of evaluation and second application of treatments) for T1, T2, T3, and T4 were found that 10.0, 16.6, 0.0, and 44.4% were still infected, but showing an infectious reduction. For P3 (after 45 days of evaluation and third application of treatments) T1, T2, T3, and T4 showed cobs infected of 20, 33.3, 25, and 44.4%, respectively. Finally, P4 (after 45 days of evaluation and third application of treatments) presented cobs infected of 0.0, 6.2, 0.0, and 20% for T1, T2, T3, and T4, respectively. T1 and T3 after four periods of analysis number of infected cobs was eliminated. Nonetheless, T2 showed a minimal presence (6.2%) of *M. roreri,* and T4 (cultural control) kept almost the same number of cobs infected for all periods.

These results showed that the use of biological treatments for moniliasis control can reduce (T2) and eliminate

(T1 and T3) the incidence of this fungal disease present in fruit cacao. Joya-Dávila *et al*. (2015) produced hydro-distilled *Z. officinale* to inhibit the moniliasis infection and reported from 88 to 100% control over their formation and incidence. Besides, Tamayo *et al*. (2016) also reported the effectiveness of *Z. officinale* on moniliasis with a reduction ranging from 40 to 50%. The antifungal or antimicrobial effect of *Z. officinale* corresponds to the gingerol, zingerone, and paradol (bioactive compounds) who contains high flavonoid, phytochemical, and pharmacological contents (Nortaa and Kankam, 2020). These results found in other studies are according to those carried out in this research.

Aloe vera (T2, Figure 3) as fungicide showed a decrease in moniliasis on *Theobroma cacao*. In the scientific literature, there is no reported information on the application of *Aloe vera* and its antifungal and antibacterial effect on moniliasis. However, this bio-fungicide showed successful resulted to control the inhibition of *Mycosphaerella fijiensis* (Jaramillo *et al.,* 2017). Mendy *et al*. (2019) evaluated two types of *Aloe vera* extract against mycelium growth of four pathogenic fungi of papaya fruit: *Fusarium* sp., *Aspergillus niger*, *Colletotrichum gloeosporioides*, and *Lasiodiplodia theobromae*, and was observed a reduction of incidence on papaya fruit after 72 h inoculation. Sitara *et al*. (2011) reported a complete inhibition of *Drechslera hawaiensis* and *Atternaria alternata* and partial inhibition of *Penicilim digitatum* when was applied *Alove vera* gel (0.35%). Likewise, Castillo *et al.* (2010) found efficacy in inhibiting mycelium growth of two common fungi: *Penicillium digitatum* and *Botrytis cinerea* when added *Aloe vera* gel at several concentrations.

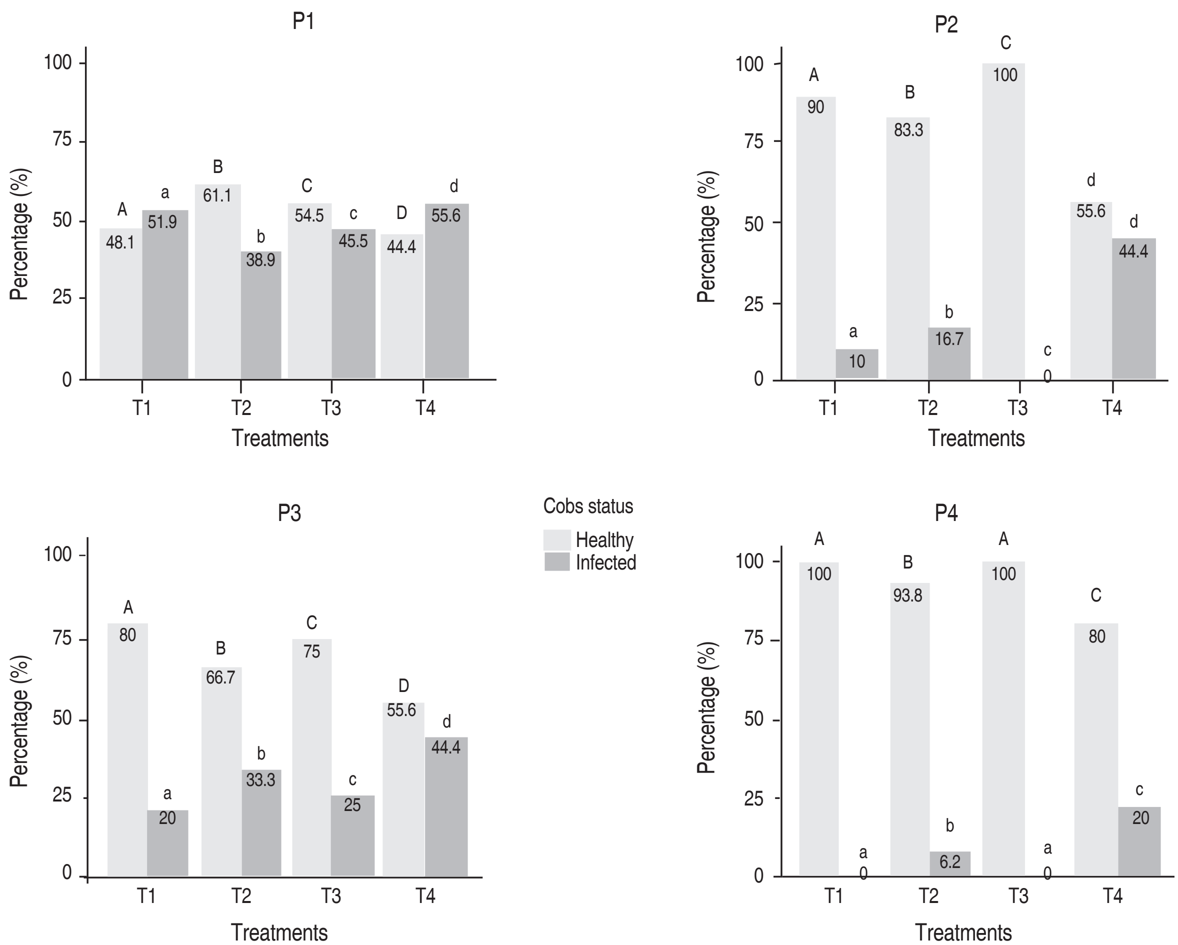

Figure 3. Percentage of healthy and infected cobs by *Moniliophthora roreri*, at each period (P1, P2, P3 and P4) and treatment (T1, T2, T3 and T4).

On the other hand, Seng *et al*. (2014) reported monilia infection reduction by 11% in only 35 days when *Trichoderma sp.* (T3) was used as a spray on the entire cacao tree. This result was similar to the present findings (after 30 days). Likewise, Carvajal *et al*. (2015) assessed the antagonistic activity of two isolated species of *Trichoderma sp.* over *M. roreri* under field conditions and found damage reduction of 19.5 and 11.2% in only 28 days, respectively. However, significant differences among treatments were not reported.

Figure 4 shows the healthy, infected, and total of cobs studied by treatment during all periods. It is noted that the healthy cobs followed this order: T3 (*Trichoderma sp.,* 87.3%) > T2 (80.4%1) > T1 (70.2%) > T4 (65.4%). Significant differences (P<0.05) were found among treatments. The better effect of *Trichoderma sp.,* may be explained because this fungus is a natural antagonist of *M. roreri* and their species produce over 40 different metabolites capable of inhibiting several phytopathogenic microorganisms (Leiva *et al*., 2020).

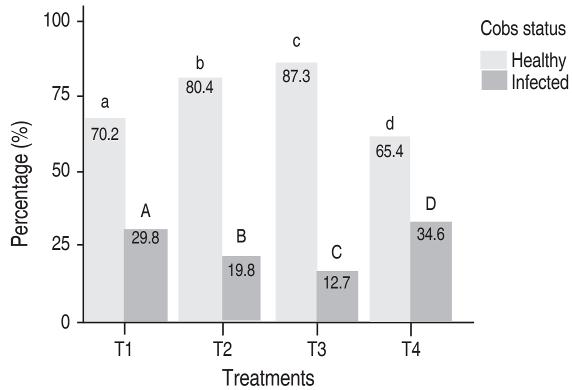

Figure 4. The percentage of healthy and infected by *M. roreri*, monitored for each treatment.

Table 1. Moniliasis incidence (%) and fruit weight (kg plant^{-1}) per period and treatment to control the disease of *M. roreri*.

Treatment	Period	Incidence per treatment (%)	Fruit weight (kg) per treatment	Incidence average (%)	Total fruit weight (kg)
T1	P1	51.9	2.4	20.6 a	6.9 a
	P2	20.5	1.2		
	P3	10.0	1.3		
	P4	0.0	2.0		
T2	P1	38.9	2.3	17.7 b	7.3 a
	P2	16.7	1.0		
	P3	9.1	1.6		
	P4	6.3	2.4		
T3	P1	45.5	0.9	14.9 c	8.4 b
	P2	14.3	0.6		
	P3	0.0	2.1		
	P4	0.0	4.8		
T4	P1	55.6	0.6	41.1 d	5.4 c
	P2	44.4	0.8		
	P3	44.4	1.1		
	P4	20.0	2.9		
Total				24.2	27.9

Values on each column followed by the same letter do not differ significantly (P<0.05).

The infected cobs are ordered of the following manner: T4 (34.6%) > T1 (29.8%) > T2 (19.6%) > T3 (12.7%). Significant differences (P<0.05) were found among all treatments. The control sample was less effective compared to other treatments. Toala *et al*. (2019) reported no control (even was observed an increase of 2% when compared to the initial and final incidence) of this disease when only a control treatment was applied.

Table 1 presents the moniliasis incidence (%) and fruit weight (kg) for each period and treatment and their average on control of *M. roreri.* Incidence of moniliasis average is ordered as follows: T4 (41.1%) > T1 (20.5%) > T2 (17.7%) > T3 (14.9%). Likewise, fruit weight average was: T3 (8.4 kg) > T2 (7.3 kg) > T1 (6.9 kg) > T4 (5.4%). T3 (*Trichoderma* sp. + *Bacillus subtillis*) showed better performance reducing the incidence of moniliasis and obtaining the higher fruit weight. De Sousa *et al*. (2021) evaluated five isolated *Trichoderma* spp., on seed treatment and seedling production of *Theobroma cacao* and found fungi incidence of 26.5% only in the control treatment, while incidence fungi was 0% for all *Trichoderma* isolates. Likewise, Seng *et al*. (2014) reported a significant reduction of incidence of monilia infection by 11%. As well, significant differences (P<0.05) were reported among incidence and fruit weight (except T1 and T2). Increasing fruit weight is probably related to better bio-fungicide control. A similar finding was reported by Trocoli *et al*. (2017) who reported an increase in fruit weight among 30 to 56.5% of pineapple after applying *Trichoderma* sp. on *Fusarium guttiforme*. Likewise, Siswanto *et al*. (2020) revealed better quality and weight of cacao beans after fruit be sprayed by biological and botanical pesticides.

CONCLUSIONS

The results indicated that the use of biological fungicides decreases the incidence of moniliasis and increased fruit weight. T3 (*T. harzianum + bacillus*) showed better performance in decreasing moniliasis infection than T1 (*Zingiber officinale*) and T2 (*Aloe vera*). Likewise, T3 showed a higher increase in fruit weight compared to other treatments. However, *Aloe vera* plant should be studied to apply more different extraction methods with different concentrations to find better antifungal effects.

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