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Coffee borer control
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Biological control of the coffee berry borer with entomopathogenous fungi in Nayarit, Mexico

Control biológico de la broca del café con hongos entomopatógenos en Nayarit, México

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ABSTRACT. The berry borer is the most damaging insect pest of coffee worldwide, affecting both yield and quality. Due to its economic importance, the borer has been the subject of considerably research around the world, both to determine its biology, as well as to develop economically and environmentally viable control technologies. Much of the work has focused on biological control with parasitoids and entomopathogens. The objective of this study was to isolate, identify and evaluate strains of *Beauveria bassiana* native to the coffee growing areas of Nayarit against the coffee berry borer under field conditions. The strains were obtained from soil and coffee fruit samples from 15 coffee orchards and were evaluated in an organic coffee production orchard. In general, the strains of *B. bassiana* showed good performance against the coffee berry borer with effectiveness higher than 76%. In the region of study, if control measures are not applied, the percentages of infestation could reach up to 56%. Regional *B. bassiana* strains are considered an option for biological control of the coffee berry borer.
Keywords: Biological effectiveness, Incidence, Severity, *B. bassiana.*

Resumen. La broca es la plaga de insectos más dañina del café en todo el mundo y afecta tanto el rendimiento como la calidad. Debido a su importancia económica, el barrenador ha sido objeto de considerables investigaciones en todo el mundo, tanto para determinar su biología, como para desarrollar tecnologías de control económica y ambientalmente viables. Gran parte del trabajo se ha centrado en el control biológico con parasitoides y entomopatógenos. El objetivo de este estudio fue aislar, identificar y evaluar cepas de *Beauveria bassiana* nativas de las zonas cafetaleras de Nayarit contra la broca del café en condiciones de campo. Las cepas se obtuvieron de muestras de suelo y fruto de café de 15 huertas y fueron evaluadas en un huerto de producción de café orgánico. En general, las cepas de *B. bassiana* mostraron un buen comportamiento contra la broca del café con efectividad superior al 76%. En la región de estudio, si no se aplican medidas de control, los porcentajes de infestación podrían llegar hasta el 56%. Las cepas regionales de *B. bassiana* se consideran una opción para el control biológico de la broca del café.
Palabras Clave: Efectividad biológica, Incidencia, Severidad, *B. bassiana.*

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INTRODUCTION

Mexico is a pioneer in the export of organic coffee and is the main producer of organic coffee in the world (Sosa *et al*. 2004). In the 2019-2020 cycle, according to the Agri-Food and Fisheries Information Service, cherry coffee production was 958 thousand tons, which represented an increase of 6.5% compared to the previous cycle (SIAP 2024). This crop is distributed over an area of 664 794 hectares with a record of 500 coffee growers, which include 58 producing regions in 15 states, in a total of 480 municipalities and 4 572 communities in the country. Nearly 80% of national coffee production is obtained in the following six Mexican states: Chiapas, Veracruz, Oaxaca, Puebla, Guerrero and Hidalgo (FIRA 2003, Santoyo *et al*. 1995).

Nayarit has a cultivated coffee area of more than 16 300 hectares distributed in six geographic regions. Production is largely due to the application of techniques that benefit the crop more than the increase in cultivated area. There are 5 310 coffee producers from 11 municipalities that on average generate 1 923 direct jobs and resources exceeding 150 million pesos (De La Peña-González 2018). Production at national level shows a slow recovery after the diminution observed in recent years due to the large production losses that occurred during 2012, caused by early and highly aggressive outbreaks of both the coffee borer and coffee rust (Torres-Castillo *et al*. 2020).

The coffee borer, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae), is the most damaging insect pest of coffee worldwide, affecting both yield and quality (Dufour *et al*. 1999). It is estimated that causes losses of more than $500 million dollars annually (Vega *et al*. 2009). Currently, in México the coffee borer has been reported in 244 municipalities at 11 states, generating economic damages equivalent to 100 million pesos (SENASICA 2019). The damage occurs when the female pierces the cherry and establishes itself in the seed, where it builds galleries for the development of the larvae, which feed on the endosperm of the seed, reducing the viability and quality of the coffee bean (Barrera 2002).

Due to its economic importance, the Coffee berry borer has been the subject of considerably research around the world, both to determine its biology and to develop control technologies that are currently considered to be economically and environmentally viable. In the last 15 years, research has focused on biological control with parasitoids and entomopathogens (Dufour *et al*. 1999). The use of biological control agents is an important alternative in the production of organic coffee. The application of virulent strains of entomopathogenic fungi, such as *Beauveria bassiana*, have been shown to be an effective tool for the management of the coffee borer in different parts of the world. In Colombia it was determined that a mixture of genetically different strains of *B. bassiana* were more virulent than individual strains. In the laboratory, the strains and mixtures (*Bb*9001,

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*Bb*9024, *Bb*9119-*M. anisopliae* Ma9236) caused mortality on the borer between 91% and 94% and the mixture affected the oviposition capacity of the insect by up to 87%. In the field, all treatments reduced infestation in trees between 18% and 47% compared to the control, reducing the population in infested fruits by 40% (Jaramillo *et al.* 2015).

In the case of *B. bassiana*, it was determined that a single dead body of coffee borer can produce up to 10 million spores, which favors its establishment and dispersal (Narváez *et al*. 1997). It was also observed that its effect is dependent on the density of the infestation. On the other hand, a higher degree of infection occurs in general at the lower parts of the coffee plant due to the effect of shading where there is greater relative humidity and less solar radiation (Bustillo-Pardey 2005). Its effect is observed with greater intensity in the first days when it reaches up to 70% with applications of the fungus of 5×10^9 spores per mL (Bustillo-Pardey 2005). It is considered that in the coffee plantations of Nayarit there is the presence of entomopathogenic fungi affecting the populations of *Hypothenemus hampei* and these native strains are better adapted and more virulent than those imported from other agroecosystems. The objective of the present study was to isolate, identify and reproduce native strains of the entomopathogenic fungus *Beauveria bassiana* and evaluate under field conditions its effect on the control of the berry borer in coffee orchards in the producing area of Compostela, Nayarit, México.

MATERIAL AND METHODS

Location

The evaluation of *Beauveria bassiana* isolates was carried out in the locality "La casa" in the Cumbres de Huicicila ejido, in Compostela, Nayarit (21° 01´ 48´´ Latitude N and 105° 0´45´´ Longitude W and 860 masl). The coffee orchard is considered a traditional rustic or mountain production system (Moguel and Toledo 1999), with a tendency towards organic, under rainfall and Arabica type coffee of three years old with Catimor (Caturra X Híbrido Timor) and Típica. Harvest is during the months of December to February, with reports of high incidence of coffee borer in previous cycles. The vegetation used as shade includes guamo (*Inga* spp.), Indian leather (*Bursera simaruba* (L.) Sarg. 1890), tepehuacate (*Lysiloma acapulcense* (Kunth) Benth.), tepezapote (*Ternstroemia tepezapote* Schltdl. and Cham.). It is considered to be part of the physiographic province of the Trans-Mexican Volcanic Belt, with a warm subhumid climate with vegetation considered as low deciduous forest and evergreen forest (Bravo-Bolaños *et al*. 2020).

Collection of samples for isolation of *Beauveria bassiana*

Soil collections samples (45) were carried out in October and November 2021 from a total of 15 orchards (three samples per orchard) representatives of the coffee-producing areas of

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the state of Nayarit (Figure 1). Selection of the orchards, was based on the suggestions of the technicians of the Nayarit State Plant Health Committee, as well as the technical advisors of the coffee processing company "Terruño Nayarita", mainly considering that chemically synthesized products will not be applied and that the orchards were representative of the production systems of the area of study. It is important to mention that in the producing area of Nayarit, only Arabica (Catimor) and Tipica type coffee is produced. Coffee orchards presented abundant vegetation that have not been disturbed by agricultural activities.

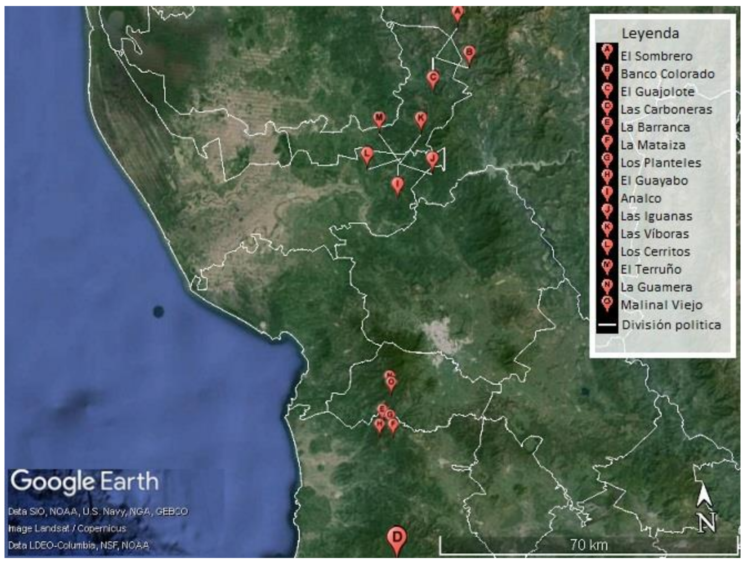

Figure 1. Location of the study area of the coffee-growing areas at the state of Nayarit, Mexico.

Soil samples were taken from three random coffee plants per orchard, where the superficial leaf litter from the base of the plants was removed and 1.0 kg was obtained from the first 30 cm of soil depth. Ten fruits per plant at physiological maturity were also collected at random from a total ten plants per orchard. The soil and fruit samples were placed in a "ziploc bags" with their identification data and placed in coolers with frozen gel for transport to the Entomology Laboratory of the Pabellón Experimental Field, belonging to the National Institute of Forestry, Agricultural and Livestock Research (CEPAB-INIFAP) for storage in a refrigerator at a temperature of 8 °C until processing (Table 1).

Sample processing.

The soil samples were sieved with a 2.0 mm mesh sieve, and a 300 g sample was placed in a 1.0 L capacity plastic jar. Then, the soil was moistened to field capacity and five *Tenebrio molitor* larvae were placed per jar and incubated at a temperature of 25 °C in a bioclimatic chamber. Jars were checked daily from the fifth day and the infected larvae were placed in individual containers to later scrape the mycelium with a bacteriological loop and place it in Petri dishes with PDA (Potato Dextrose Agar).

Table 1. Localities sampled for the isolation of entomopathogenic fungi in coffee orchards in Cumbres de Huicicila, Compostela Nayarit, México.

Locality	Municipality	Latitude	Longitude	Altitude	EPF*	Sample
El Sombrero	Ruiz	22°04′52′′	104°52′07′′	930	Bb	Soil
Banco Colorado	Ruiz	22°04′50′′	104°52′19′′	840	Bb	Fruit
El Guajolote	Ruiz	22°04′51′′	104°52′33′′	810	Bb	Soil
Las Carboneras	Compostela	21°01′48′′	105°00′45′′	860	---	Soil
La Barranca	Compostela	21°19′33′′	105°01′45′′	920	Bb	Fruit
La Mataiza	Compostela	21°17′38′′	105°00′24′′	930	Bb	Soil
Los Planteles	Compostela	21°18′47′′	105°00′45′′	870	---	Fruit
El Guayabo	Compostela	21°17′43′′	105°02′12′′	940	Bb	Soil
Analco	Santiago Ixcuintla	21°52′05′′	104°58′02′′	475	---	Soil
Las Iguanas	Santiago Ixcuintla	21°52′08′′	104°57′59′′	468	Bb	Fruit
Las Víboras	Santiago Ixcuintla	21°52′03′′	104°57′46′′	491	Bb	Soil
Los Cerritos	Santiago Ixcuintla	21°52′42′′	104°57′28′′	483	---	Fruit
El Terruño	Santiago Ixcuintla	21°52′06′′	104°57′38′′	466	Bb	Fruit
La Guamera	Xalisco	21°23′43′′	105°00′34′′	1020	Bb	Soil
Malinal Viejo	Xalisco	21°22′56′′	105°00′22′′	1020	---	Fruit

*EPF = entomopathogenic fungi; Bb = *Beauveria bassiana*

The dead larvae without the presence of mycelium were superficially disinfected with 2.0% sodium hypochlorite for three minutes and rinsed with sterile water three times to remove the remains of the disinfectant (Hatting *et al*. 1999). After that, the larvae were placed in a 9.0 cm plastic Petri dish with a layer of filter paper moistened with sterile water, the dishes were sealed with plastic tape (Plastipak) and incubated at 25 °C. Every 24 h, the Petri dishes were checked and the development of mycelium was determined.

In positive cases, mycelium was collected with a dissecting forceps and subsequently sown in a Petri dish containing PDA culture medium. The fungi were successively reseeded until pure cultures were obtained and were identified by the reproductive structures according to Bidochka *et al*. (1998).

For the morphological identification of *B. bassiana*, the taxonomic keys for the genus of Barnett and Hunter (1998) were used. For molecular identification, the procedure was as follows: from pure isolates of the *Beauveria bassiana* strains, DNA extraction was carried out with the CTAB method (Doyle and Doyle 1990). The quality and quantity of the extracted DNA was checked in Nanodrop (Model) for subsequent sequencing. The samples were sent for sequencing to the LANBAMA-IPICYT laboratory using the Oligos

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ITS1 and ITS4 and sequencing with the labeled dideoxynucleotide method on the model 3500 and 3130 Genetic Analyzer sequencers (Applied Biosystems). The obtained sequences were compared with the Genbank database using the BLASTN program. From the isolates previously identified, both from the soil and fruits, those with the fastest growth and sporulation were selected for evaluation in the field.

Field phase tests for coffee berry borer control.

In the field, 13 treatments were evaluated, including nine regional strains of *Beauveria bassiana* (Table 2), one strain from the guava agroecosystem (BbC20) that has been reported to be effective against guava weevil (*Conotrachelus dimidiatus*) in Malpaso, Calvillo, Aguascalientes (Cerna-Chavez *et al*. 2021), a homemade organic repellent commonly used in the Compostela producing area (made with 2.5 kg of mountain microorganisms, 1.0 kg of garlic, 1.0 kg of onion, 1.0 kg of hot chili , 1.0 kg of ginger, 0.5 kg of leaves of aromatic plants, 1.0 L of molasses, 1.0 L of vinegar and 1.0 L of 70% alcohol), an insecticide of biological origin Spinosad and a control with only water.

The study began on September 27th, 2022, in which the treatments were established and the first application of the treatments was carried out. The Petri dishes with active growth of *Beauveria bassiana* were scraped and placed in 10% Tween 20 oil, the spore suspensions were adjusted to a dose of $1x10^7$ spores per milliliter. At the time of application in the field, 1.0 mL of adherent (Bionex ®) per liter of water was applied to all the treatments with the exception of the control. While for the insecticide, 3.0 cm^3 L^{-1} of water was applied and in the case of the repellent, 5.0 mL L^{-1} of water was applied.

Table 2. Treatments for the biological control of the coffee berry borer (*Hypothenemus hampei* L.) in the Cumbres de Huicicila, Nayarit. México.

Treatment	EPF´s	Locality	Sample
1	*B. bassiana* (AG2) [a]	El Sombrero	Soil
2	*B. bassiana* (22G)[a]	Banco Colorado	Fruit
3	*B. bassiana* (M14) [a]	El Guajolote	Soil
4	*B.bassiana*(1-6 (2) T) [a]	La Barranca	Fruit
5	*B. bassiana*(3-1-T (2) [a]	La Mataiza	Fruit
6	*B. bassiana* (D (3) T)[a]	El Guayabo	Soil
7	*B. bassiana* (5-3 (1) [a]	Las Iguanas	Fruit
8	*B. bassiana* (Bb C20)[b]	Malpaso	Soil
9	*B. bassiana*(4-1(2) T) [a]	El Terruño	Fruit
10	*B. bassiana* (3-1-T(2) [a]	La Guamera	Soil
11	Repellent[c]		
12	Spinosad[d]		
13	Control[e]		

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[a] = regional strains; [b] = strains from the guava agroecosystem; [c] = homemade organic repellent: [d] = insecticide of biological origin; [e] = only water

Spraying was carried out on September 27, October 4, 12, 19 and 26. The treatments were applied with 4.0 L capacity spray pumps, with hollow cone nozzles and a different pump was used for each treatment.

A completely randomized design was used, with five replications (trees) per treatment, and five branches were marked per tree, where the experimental unit was a fruit. The variables measured were the number of fruits affected by berry borers and the number of borers with the presence of entomopathogenic fungi.

The data obtained were subjected to an analysis of variance and a Tukey mean comparison test (P = 0.05), with the JMP statistical package of SAS (2011). In the case of data in percentages, before submitting them to the ANOVA and Tukey test, a square root transformation ($\sqrt{x} + 0.5$)was performed.

RESULTS

According to the sequencing results, the recovered fungi correspond to *Beauveria bassiana* in a coincidence range from 94.56 to 99.43% with the values deposited in Genbank (Accessions: KC121560.1 and KC121558.1). In the coffee-growing areas of Nayarit, the entomopathogenic fungus *Beauveria bassiana* was detected in five soil samples (localities: El Sombrero, El Guajolote, El Guayabo, Las Víboras and La Guamera) and in five fruit samples (localities: Banco Colorado, La Barranca , La Mataiza, Las Iguanas and El Terruño); which indicates the presence of *B. bassiana* in the coffee-growing areas of the state, probably attributed to the tendency of not applying chemically synthesized insecticides in these production systems.

The statistical analysis showed that only treatments 2 (22G), 5 (3-1-T (2), 7 (5-3 (1) and 12 (Spinosad) are statistically different from the rest (Figure 2) and presented the lowest percentages of damaged fruits. The strains of these treatments come from the Banco Colorado (22G), La Mataiza (3-1-T (2) and Las Iguanas (5-3 (1) orchards, which are geographically distant from each other. This fact reveals, the existence of native strains with entomopathogenic potential for the control of the coffee borer in coffee-growing areas from the state of Nayarit.

In the field evaluation (Table 3) most of the strains presented percentages of healthy fruits greater than 60% with the exception of the strain D (3)T that showed percentages below this value like the control, with 52 and 44 % respectively. The highest percentage of healthy fruits occurred in the treatment with Spinosad and strains 3-1-T (2) and 5-3(1) with 85, 76 and 74 % respectively. The presence of fruits with entomopathogenic fungi was greater in strains 4-1(2)T, 3-1T(2) and 22G with 86.8, 79.2 and 78.6 % respectively compared to only 12.5 % in the control.

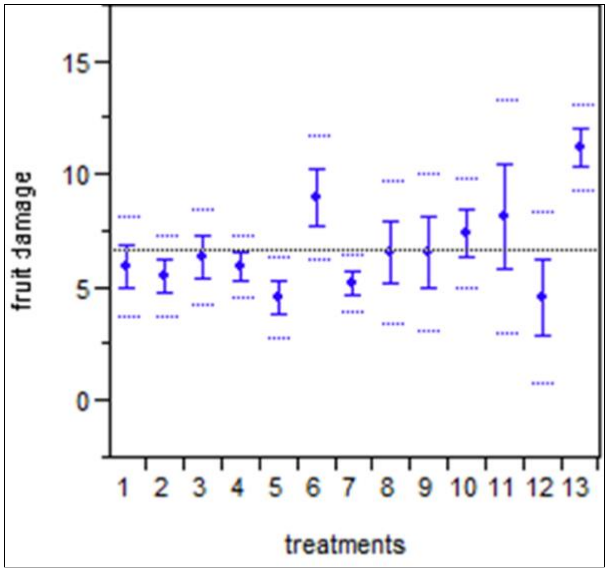

Figure 2. Comparison of means of biological control treatments against the coffee berry borer (*Hypothenemus hampei* L.) in Cumbres de Huicicila, Compostela, Nayarit. México. There is a statistical difference when the standard deviation lines do not overlap.

Table 3. Effect of the biological control treatments on the coffee berry borer (*Hypothenemus hampei* L.) in the area of Cumbres de Huicicila, Compostela, Nayarit. México.

	Treatments	Healthy Fruits (%)	Damaged Fruits (%)	Fruits with fungus *B. bassiana* (%)
1	*B. bassiana* (AG2)[a]	70	30	43.3
2	*B. bassiana* (22G) [a]	72	28	78.6
3	*B. bassiana* (M14) [a]	68	32	34.4
4	*B. bassiana* (1-6 (2)T) [a]	68	32	71.9
5	*B. bassiana* (3-1-T(2) [a]	76	24	79.2
6	*B. bassiana* (D(3)T)[b]	52	48	43.8
7	*B. bassiana* (5-3(1) [a]	74	26	65.4
8	*B. bassiana* (BbC20)[b]	66	34	35.3
9	*B. bassiana* (4-1(2)T) [a]	62	38	86.8
10	*B. bassiana* (3-1-T(3)[a]	63	37	43.2
11	Repellent[c]	60	40	40.0
12	Spinosad[d]	85	15	46.7

13	Control[e]	44	56	12.5

[a]= regional strains; [b]= strains from the guava agroecosystem; [c]= homemade organic repellent; [d]= insecticide of biological origin; [e] = only water

Likewise, a polynomic response of second grade (P≤0.05; R^2 = 0.59) was observed in the relationship between the percentage of the presence of *B. bassiana* with the number of healthy fruits (Figure 3), observing that the higher number of healthy fruits was found in the range of 60 to 70 of the percentage of presence of *B. bassiana*. Then, healthy fruits showed a slightly decrease at greater values of the presence of the fungus, although this decrease was not statistically significant (P≥0.05).

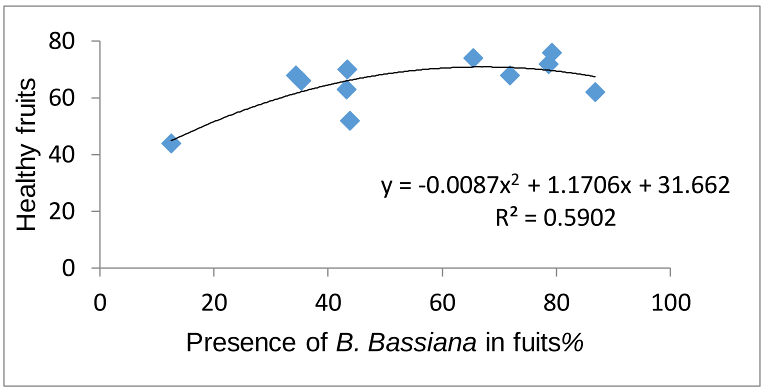

Figure 3. Relationship between the percentage of presence of the fungus *B. bassiana* vs percentage of healthy fruits.

DISCUSSION

Kalyabina *et al.* (2021) mentioned that the use of chemically synthesized insecticides causes habitat loss, affects the interaction between organisms and the conversion of environments within an ecosystem. The values of healthy fruits shown in this study by the control were high, because entomopathogens naturally exist in the region. Sánchez-Peña (2011) reported that the entomopathogenic fungus *Beauveria bassiana* is commonly found in undisturbed habitats. El Husseini (2019) pointed out that *B. bassiana* is naturally located in the soil. Suu *et al.* (2022) reported values of 93.67% of coffee cherries without damage using applications of *B. bassiana*.

However, efficiency values may vary due to environmental and management factors. Villalba (1995) mentioned that high control percentages were achieved with the appropriate use of spray technology. Pérez-González *et al.* (2014) argue that the dispersal of *B. bassiana* will depend on the strain, temperature, and the ability to establish. The fungus *Beauveria* sp. is capable of adapting to a wide range of environments around the world (Pérez-González *et al.* 2014). Ibrahim *et al.* (2020) mentioned that the infection is random, through factors that favor the activation of *B. bassiana*. Humidity, temperature, precipitation, solar radiation, the age of the host and the pathogenicity of the entomopathogen influence the effectiveness of control (Fernandes *et al.* 2015). The entomopathogenic fungus *B. bassiana* is present throughout the world, parasitizing several species of insects, including the coffee berry borer (Vuelta-Lorenzo *et al*. 2017). Fargues and Remaudiere (2020) pointed out that in the field the number of conidia that reach the insect is generally reduced, causing a reduction in control efficiency. This indicates that the fungus is already naturally established, although its incidence is low, however, it was increased with the strains 4-1(2)T, 3-1T (2) and the BbC20 strain, which presented an average of 68% of healthy fruits with an average of 69 % with presence of fungi, being lower in the last strain.

This slight decrease in healthy fruits observed in strains (4-1(2)T) and (1-6 (2)T) could probably be due to a lower concentration of secondary metabolites in these strains. *B. bassiana* has been reported to produce several toxic metabolites, such as beauvericin and bassianolide, which exhibit insecticidal activity. Additionally, the importance of other metabolites such as bassianin, beauverolides, bassiatin, and oosporein in the biological control potential of *B. bassiana* has been highlighted. Nevertheless, it has been determined that the virulence of *B. bassiana* strains lies more in the concentration of these metabolites, rather than in a single metabolite (Chaithra *et al*. 2022, Stuart *et al*. 2022).

Several studies have revealed that not all strains of *B. bassiana* are equally effective; it is possible that they are different, because the enzymes (lipases, chitinases, proteases) involved in the control of the coffee berry borer have different efficiency of action, which leads to having greater or lesser control of the insect (Cruz *et al*. 2006). Wraight and Ramos (2002) mentioned that *B. bassiana* produces hydrolytic enzymes such as proteases, lipases, chitinases and acid oxalic. The *B. bassiana* strain applied in treatment 5 was more efficient in the infection of the berry borer, probably due to a greater activity of those enzymes present in the strain (3-1-T(2). This agrees with Dhawan and Joshi (2017) who reported that the virulence of *B. bassiana* is related to a greater activity of the enzymes lipases, chitinases and proteases.

CONCLUSIONS

The presence of *Beauveria bassiana* with entomopathogenic potential is present in the coffee-growing areas of the state of Nayarit. A wide distribution was found since it occurred in 10 of the 15 monitored orchards with the greatest presence in the soil. The strain with the greatest control over the coffee berry borer was (3-1-T (2) with 76% of healthy fruits. The greatest presence of fruits with berry borer infected with *B. bassiana* was observed in the strains 4-1(2) T, 3-1 -T(2) and 22G with 86.8, 79.2 and 78.6% respectively. The isolated strains increased the percentage of fruits with berry borers infected with *B. bassiana* from

34.4 to 86.8%. The use of native strains of *B. bassiana* constitutes a sustainable strategy in the control of the coffee berry borer. In this type of studies, it is important to isolate and evaluate the greatest number of strains possible, since the results showed that not all strains have the same potential for entomopathogenic activity.

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INTEREST CONFLICT

All authors agree and express that there is not conflict of interest in the present study. Likewise, all the participants in the manuscript carried out significant contributions.

LITERATURE CITED

Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi. 4th ed. APS Press. Virginia, EE.UU 218p.

Barrera JF (2002) La broca del café: Una plaga que llegó para quedarse. En: Barrera JF (ed) Tres plagas del café en Chiapas. El Colegio de la Frontera Sur, México. pp: 17-20.

Bidochka MJ, Kasperski JE, Wild GAM (1998) Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near northern habitats. Canadian Journal of Botany-Revue Canadienne De Botanique 76: 1198-1204.

Bravo-Bolaños O, López-García J, Sánchez-González A (2020) Estructura y composición florística de los bosques de *Quercus* del volcán Sanganguey, Nayarit, México. Botanical Sciences 98(3): 441-452. https://doi: 10.17129/botsci.2490

Bustillo-Pardey AE (2005) El papel del control biológico en el manejo integrado de la broca del café, *Hypothenemus hampei* (Ferrari) (Coleóptera: Curculionidae: Scolytinae) Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 29 (110): 55-68.

Cerna-Chávez E, Lira-Ramos KV, Ochoa-Fuentes Y, Delgado-Ortiz JC, Cepeda-Siller M, González-Gaona E (2021) Actividad de extracto de plantas y hongos entomopatógenos para el control del picudo de la guayaba (*Conotrachelus dimidiatus* Champion) (Coleoptera: Curculionidae). Biotecnia 23(1): 70-76. https://doi.org/10.18633/biotecnia.v23i1.1336

Chaithra M, Prameeladevi T, Prasad L, Kundu A, Bhagyasree SN, Subramanian S, Kamil D (2022) Metabolomic diversity of local strains of *Beauveria bassiana* (Balsamo) Vuillemin and their efficacy against the cassava mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae). Plos One 17(11): e0277124. https://10.1371/journal.pone.0277124.

Chapman EG, Messing RH, Harwood JD (2015) Determining the origin of the coffee berry borer invasion of Hawaii. Annals of the Entomological Society of America, 108(4): 585–592. https://doi.org/10.1093/aesa/sav041

Cruz LP, Gaitán AL, Góngora CE (2006) Exploiting the genetic diversity of *Beauveria bassiana* for improving the biological control of the coffee berry borer through the use of strain mixtures. Applied Microbiology and Biotechnology 71(6): 918-926. https://doi.org/10.1007/s00253-005-0228-3

Dhawan M, Joshi N (2017) Enzymatic comparison and mortality of *Beauveria bassiana* against cabbage caterpillar *Pieris brassicae* LINN. Brazilian Journal of Microbiology 48(3): 522-529. https://10.1016/j.bjm.2016.08.004

De La Peña-González MI (2018) La exportación de café nayarita a España. EDUCATECONCIENCIA 20(21): 22-34. https://doi.org/10.58299/edu.v20i21.58

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Dufour B, Barrera JF, Decazy B (1999) La broca de los frutos del cafeto: ¿La lucha biológica como solución? En: Bertrand B, Rapidel B (eds) Desafíos de la caficultura en Centroamérica. CIRAD, IICA. San José, Costa Rica. pp: 293-325.

El-Husseini MM (2019) Effect of the fungus *Beauveria bassiana* (Balsamo) Vuillemin, on the beet armyworm, *Spodoptera exigua* (Hübner) larvae (Lepidoptera: Noctuidae), under laboratory and open field conditions. Egyptian Journal of Biological Pest Control 29: Article 52. https://doi.org/10.1186/s41938-019-0149-y

Fargues J, Remaudière G (2020) The production and uses of *Beauveria bassiana* as a microbial insecticide. Biocontrol Science and Technology 10(5): 731-752. https://doi.org/10.1007/s11274-016-2131-3

Fernandes ÉKK, Rangel DEN, Braga GUL, Roberts DW (2015) Tolerance of entomopathogenic fungi to ultraviolet radiation: A review on screening of strains and their formulation. Current Genetics 61(3): 427-440. https://doi.org/10.1007/s00294-015-0492-z

FIRA (2003) Situación de la Red Café, oportunidades de desarrollo en México. FIRA-Banco de México. México. Boletín informativo 519(34):105.

Hatting JL, Humber RA, Poprawski TJ, Miller RM (1999) A survey of fungal pathogens of aphids from South Africa, with special reference to cereal aphids. Biological Control 16(1): 1-12. https://doi.org/10.1006/bcon.1999.0725

Ibrahim L, Fergani A, Yehia R (2020) Characterization and pathogenicity of *Beauveria bassiana* strains isolated from *Galleria mellonella* L. (Lepidoptera: Pyralidae) in Turkey. Egyptian Journal of Biological Pest Control 30(1): 1 https://doi.org/10.1186/s41938-020-00253-4

Jaramillo JL, Montoya EC, Benavides P, Góngora CEB (2015) *Beauveria bassiana* y *Metarhizium anisopliae* para el control de broca de café en frutos del suelo. Revista Colombiana de Entomología 41(1): 95-104.

Kalyabina VP, Prusov AN, Ugarova IA, Krasnov GS, Kudryavtseva AV, Fesenko EE (2021) Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Frontiers in Environmental Science 9. https://doi.org/10.3389/fenvs.2021.735471

Moguel P, Toledo VM (1999) Biodiversity conservation in traditional coffee systems of Mexico. Conservation Biology 13(1): 11-21. https://doi.org/10.1046/j.1523-1739.1999.97153.x

Narváez M, González MT, Bustillo AE, Cháves B, Montoya EC (1997) Producción de esporas de aislamientos de *Beauveria bassiana* y *Metarhizium anisopliae* en diferentes sustratos. Revista Colombiana de Entomología 23(3-4): 125-132. https://doi.org/10.25100/socolen.v23i3-4.122

Pérez-González VH, Guzmán-Franco AW, Alatorre-Rosas R, Hernández-López J, Hernández-López A, Carillo-Benítez MG, Baverstok J (2014) Specific diversity of the entomopathogenic fungi *Beauveria* and *Metarhizium* in Mexican agricultural soils. Journal of Invertebrate Pathology 119(5): 54-61. https://doi.org/10.1016/j.jip.2014.04.009

Sánchez-Peña SR, San-Juan LJ, Medina RF (2011) Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, Mexico, and their virulence towards thrips and whiteflies. Journal of Insect Science 11(1). https://doi.org/10.1673/031.011.0101

Santoyo CH, Díaz CS, Rodríguez PB (1995) Sistema Agroindustrial café en México. Diagnóstico, problemática y alternativas. México, Universidad Autónoma de Chapingo. 157p.

SAS (2011) Statistical Analysis System User's Guide. SAS Institute, Inc. Cary, NC. USA.

SENASICA (2019) Mapa dinámico fitosanitario-broca de café (*Hypothenemus hampei*) en México. Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria.

Secretaría de Desarrollo Rural (SADER). http://sinavef.senasica.gob.mx/MDF/. Data consulted: 07 April, 2024.

SIAP (2024) Cultivo de café en México. Secretaría de Agricultura y Desarrollo Rural. Servicio de Información Agroalimentaria y Pesquera. https://www.gob.mx/agricultura/articulos/cultivo-de-cafe-en-mexico. Data consulted: 07 April, 2024.

Sosa ML, Escamilla PE, Díaz CS (2004) Organic coffee. In: Wintgens JE (ed) Coffee: growing, processing, sustainable production. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany. p. 339-354.

Suu TB, Lan DD, Dinh TNH (2022) Infestation and evaluation efficacy of monitoring by alcohol trap and application of local *Beauveria bassiana* for control of coffee Berry borer in the North-western Region of Vietnam. Annals of Biology 38(1): 96-101. https://doi.org/10.1016/j.aob.2022.01.011

Stuart AKDC, Furuie JL, Cataldi TR, Stuart RM, Zawadneak MAC, Labate CA, Pimentel IC (2022) Fungal consortium of two *Beauveria bassiana* strains increases their virulence, growth, and resistance to stress: A metabolomic approach. PLoS One. 17(7): e0271460. https://doi.org/10.1371/journal.pone.0271460

Torres-Castillo NE, Melchor-Martínez EM, Ochoa-Sierra JS, Ramírez-Mendoza RA, Parra-Saldívar R, Iqbal HMN (2020) Impact of climate change and early development of coffee rust – An overview of control strategies to preserve organic cultivars in Mexico. Science of The Total Environment 738: 140225. https://doi.org/10.1016/j.scitotenv.2020.140225

Vega FE, Infante F, Castillo A, Jaramillo J (2009) The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): A short review, with recent findings and future research directions. Terrestrial Arthropod Reviews 2: 129-147. https://doi.org/10.1163/187498309X434183

Villalba GD (1995) Calibración de aspersoras manuales de espalda. Federación Nacional de Cafeteros de Colombia. Gerencia de Producción y Desarrollo. Boletín de extensión No. 75.16p.

Vuelta-Lorenzo DR, Rizo-Mustelier M, Basile-Rodriguez R (2017) Evaluación del efecto del hongo *Beauveria bassiana*, Trampas y el nematodo *Heterorhabditis bacteriophora* en el control de la broca del café (*Hypothenemus hampei*) en Las Yaguas. Ciencia en su PC 4: 38-52.

Wraight S, Ramos ME (2002) Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato *beetle Leptinotarsa decemlineata*. BioControl 23(2): 164-178. https://doi.org/10.1023/A:1015575220028