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Isolation, Identification, and Morphometric Characterization of Native Isolates of *Beauveria* spp. from Banana Crops

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Abstract **Original Research Article**

A survey of entomopathogenic fungi was carried out from specimens of *Cosmopolites sordidus* infected with mycosis in commercial plantations and from banana crop soil samples. From a total of 72 samples, 15 native isolates of *Beauveria* spp. were obtained, of which three came from adult *Cosmopolites sordidus* and twelve from soil samples, where their main macroscopic physiological and microscopic morphometric characteristics were recorded. Colonies on PDA, SDA and EMA culture media are initially white to felted and eventually become creamy powdery with concentric rings. Reverse light yellow, Conidia hyaline, globose to subglobose1.0-2.5 (1.9 µm) x 1.0-2.0 (1.7 µm) Conidiogenous cells, with globose bases. 2.0-3.0 (2.3 µm) x 2.0-2.5(2.1 µm) and conidiophores 25 to 50 µm long with tubular septate mycelium. Radial growth rate was measured in three different media, the results obtained show that all the isolates present a similar radial growth in the Sabouraud Dextrose Agar and malt extract culture media, the highest radial growth was obtained by isolate BBT324 with a radial growth of 12.75 mm in 10 days and the lowest radial growth with 6.05 mm was obtained in the Papa Detroxa Agar medium with isolate BBT23, the concentration of the *Beauveria* spp. conidia, isolate BBT34 was the highest in the Papa Detroxa Agar medium with isolate BBT23, BBT34 isolate was the one that presented the highest percentage of concentration 5.7 million spores per milliliter of water, followed by BBT41 with 3.95 million spores per milliliter of water and finally BBT31 with 1.01 million spores per milliliter of water.

Keywords: Entomopathogenic fungi, mitosporic fungi, biological control, pests.

INTRODUCTION

Mitosporic fungi are a globally recognized group of microbial agents to be used in both biological pest control and integrated pest management (IPM) programs. This is due to their mode of action, which is by direct contact with the host cuticle, and the availability of technologies to mass-produce them. Some genera can also be formulated in oils and thus applied with ultra-low volume techniques (Bateman *et al.*, 1993; Bateman *et al.*, 1996., Castillo-Arévalo, 2022).

These are widely distributed in nature in diverse ecosystems, where they play an important role in regulating pest insect populations; they cause lethal infections in their host, including soil-dwelling ones through epizootics (Gürlek *et al.*, 2018). Entomopathogenic fungi are distributed in approximately more than 100 genera and 750 species, some of which are of agronomic interest as microbial control agents (Garcia *et al.*, 2019). These fungi include *Beauveria bassiana*, *Metarhizium* spp, *Isaria fumosorosea* and *Lecanicillium* spp, among others, which are used for the control of dozens of pests in a wide variety of crops (Lacey *et al.*, 2015).

It has been shown that to arrive at a highly effective product to control a pest species, one must start from a wide range of isolates, selected in principle in terms of their virulence. Isolates that cause epiphytotic pests, such as some of the imperfect entomopathogenic fungal genera, are promising candidates for use in pest control, provided they achieve good tolerance to adverse environmental factors, ease of production, adequate storage behavior and high safety in mammals (Bateman *et al.*, 1996).

Classically, there are three ways to enrich the number of fungal isolates to be used as candidates for biological pest control. The first is by isolation of the fungus from mycorrhizal arthropods and nematodes, the second from soil samples and finally directly from crop collections (Rhodes and Smith, 1992).

The success of a biological pest control program based on entomopathogenic fungi depends on the production of inoculum for field applications, the selection of strains or isolates with high virulence, good growth, sporulation, and resistance to adverse environmental conditions (Garcia *et al.*, 2015). In the search for biological control agents, one of the basic strategies is the initial exploration of native natural enemies, before introducing exotic agents, because native strains are adapted to the environmental conditions of the area; unlike strains of transient microorganisms, which due to their lack of adaptability may be ineffective in different agroecosystems (García *et al.*, 2011).

The black boll weevil is considered one of the most important pests of banana and other musaceae in most tropical and subtropical countries (Castrillón, 2004).

In Nicaragua, the incidence of weevils has reduced the yields and income that this crop provides to the country's producers; on the other hand, the food security that this crop provides has been threatened by diseases that affect the plant and its fruits (Castillo-Arévalo, 2022).

Under local conditions, losses of 40 to 50% have been detected (Castillo-Arévalo, 2022). Currently, the only cost-effective alternative is the application of chemical insecticides on the soil surface (Vargas *et al.*, 2015), however, their adverse effects on the environment and workers are known if used inappropriately. The black weevil and the striped banana weevil have shown the ability to develop resistance to most insecticides (Gold and Messiaen, 2000). furthermore, the use of chemical pesticides is costly and has harmful effects on human health, animals, and the environment, as well as generating insect resistance (Bautista *et al.*, 2018), (Rezende *et al.*, 2015).

The objective of the work was to isolate and characterize fungal species to select promising isolates as biological control agents. For the characterization of the strains, it was necessary to consider the macroscopic characteristics of the colony and microscopic (conidiophore, conidium, phialides).

MATERIAL AND METHODS

Location of the study area

The study was carried out in the Department of Rivas, in the municipalities of Belén, Buenos Aires and Potosí, a total of 72 farms.

Soil samples and adult samples of *Cosmopolites sordidus* with signs of mycosis were collected in municipalities with the largest banana production area (Belén, Buenos Aires and Potosí). With a total of 72 farms, 24 farms with a minimum size of 10 hectares in production of SENSA ¾ plantain were selected in each municipality. For soil sampling, the methodology of Sevim et al. (2010) and Hernández Domínguez was used with some modifications. In each farm, one hectare with an established crop was selected, where five zigzag points were delimited; at each point an independent 600 g soil subsample was taken at a depth of 15 to 20 cm near the roots of each plant. Soil samples were taken with a small shovel and placed in airtight ziploc® type refrigerated bags for transfer to the bioplagicide laboratory of the Universidad Nacional Agraria. Ten subsamples were taken from each farm, which made up the composite sample per farm.

Figure 1: Geographical location of the farms under study

Methodological design

Sample collection

The sample was selected using the finite population sample calculation formula used by (Castillo-Arévalo, 2022), which is used when the universe or total number of units under study is known.

$$n = \frac{n\,N*z2\;p*q}{(N-1)\;e2+z2*p*q} \quad \text{..... Equation 1}$$

Meaning of the letters:

n = Sample size.
N = Universe population.
Z = 95% confidence level
P = Proportional estimate of the population
Q = (1-P)
E = Standard error of the sample, 5% (0.05)

Isolation, obtaining of monosporic cultures of *Beauveria* spp and macroscopic physiological and microscopic morphometric characterization.

Isolation was performed in the biopesticide laboratory of the Universidad Nacional Agraria, from soil samples, for which the serial dilution methodology was used (Hernández-Domínguez and Schapovaloff et al., 2015). From the composite soil sample, 90 g were taken and placed in a 250 mL Erlenmeyer flask containing 50 µL of 0.01% Tween® 80 solution (Panreac Química S.L.U., Barcelona, Spain). The mixture was homogenized in an orbital shaker at 120 rpm for 24 h at a temperature of 28 0C. Serial dilutions were made from each soil suspension. 1mL of the 10-1 dilution was taken and evenly distributed at 10-1 10-2 10-3 10-4, then an aliquot of 20 µL was taken in a Petri dish containing Potato Dextrose Agar (PDA) (DifcoTM Becton, Dickinson and Company, USA) and with 1% of the volume to be prepared of 25 % lactic acid (J.T.Baker®, USA). The characterization of the isolates was carried out in three culture media: Malt Extract, Potato Dextrose and Agar and Sabourad Dextrose Agar. After eight days of inoculation of the Petri dishes, visual observations were made and mounting was performed under the light microscope with a 40x lens for purification, the colonies that presented characteristics compatible with the genus of entomopathogenic fungi were isolated (Bischoff et al., 2009; Humber, 1997, 2012). According to Driver et al., (2000), molecular analyses for the identification of *Beauveria bassiana*, based on rDNA techniques are one of the most efficient methods for the classification of species; however, the characterization of the native isolates found in this study was performed based on the dicotypic keys described at the species level, with the help of the species descriptions of entomopathogenic fungi of the CMI, according to the macroscopic physiological and microscopic morphometric characteristics (CMI, 1979), and (Claro et al., 2006); the characteristics taken into account for identification were the microscopic morphological characters of the conidia, phialides and conidiophores (width and length), as well as the macroscopic physiological characteristics of the colonies, such as: colony color, type of growth, appearance of the colony and the presence of exudate. For isolation from mycotic insects, the methodology proposed by Garcia et al., (2012) was used. Infected weevils collected in the field were placed in a 2 mL microtube (Eppendorf, Germany) with Whatman 42 filter paper (Sigma Aldrich, USA) and taken to the laboratory where they were superficially disinfected with 1% sodium hypochlorite for 3 min. Excess hypochlorite was removed by triple washing in sterile distilled water; to avoid excess humidity, the insects were placed on sterile paper. Each insect was placed in a humid chamber composed of a 90 mm (Ø) Petri dish (100 × 15 mm) containing Whatman 42 filter paper and two slides were placed on it in the form of a cross, and the insect was placed on it (90 % RH) at 27±1 °C for eight days. When fungal growth was observed on the insect body, the insect was isolated and seeded on acidified PDA medium. The isolation was incubated for eight days at 27±1 °C, which allowed its development until sporulation and identification of the microorganism obtained. From the isolates obtained from soil samples and mycotic insects, pure monosporic cultures were made, according to the procedure described by Estrada et al., (1999). Each isolate was assigned a code, composed of the initial letter of the genus and species of the fungus, the initial letter of the investigator and the treatment number. All isolates were kept refrigerated at 4 °C for use in subsequent assays. For macroscopic characterization, the microculture technique was applied. Filter paper was placed inside each 90 mm (Ø) Petri dish (100 × 15 mm) and two slides were placed on top of it in the form of a cross. After sterilization, 1.5% water-agar was added to the top slide, on which an aliquot of each isolate obtained from a serial dilution with a concentration of 1x10-4 conidia per microliter was seeded. The filter paper was then moistened with sterile distilled water, sealed with parafilm® M (Sigma Aldrich, USA) and the plates were incubated at 27±1 °C. From the third day to five days, microscopic observations were made with a 40x lens (BB.1153-PLi, Euromex, Euromex Microscopen Spain, S.L.) to observe reproductive structures. At least 300 structures of each isolate were observed with the aid of a digital camera (CMEX-3 Digital camera with 3 Mp CMOS sensor. DC.3000c, The Netherlands), incorporated in the BB.1153-PLi microscope (Euromex), with 40x magnification.

Growth Rate

To measure the growth rate, we used the radial growth methodology described by French and Hebert (1982) from purified in vitro cultures of *Beauveria* spp of each isolate found, transferred a circle of 5 mm in diameter from the edge of the growing colony, placed in the center of a Petri dish of 90 mm (Ø) in PDA, SDA, and EMA culture media (Difco™, Becton, Dickinson and Company, USA). (Difco™, Becton, Dickinson and Company, USA), a growth margin of 24 hours was left after which readings were taken every 12 hours for ten

days and placed in an incubation room at 27 ± 1 °C, with eight hours of light. From each isolate (15) 15 replicates were prepared, five for each culture medium, for a total of 225 experimental units. The radial growth rate was recorded using a 6", millimeter, standard digital Vernier caliper with five-digit digital display (Truper®, Mexico) every two days until day 10 after transfer according to Torres de la C et al., (2013) and Cárdenas et al., (2016). The data obtained were submitted for statistical analysis. The 5 mm corresponding to each initial mycelial circle was subtracted from the values obtained in the measurement. The growth rate was calculated at the end of the trial with the formula proposed by Guigón-López et al., (2010).

$$\text{Growth rate} = \frac{\text{Final growth} - \text{Initial growth}}{\text{Incubation time}}$$

…….. **Equation 2**

Conidial production

To determine the concentration of conidia for each isolate, the methodology proposed by Torres de la C *et al.*, (2013) was followed. The conidia were obtained from the culture surface of the Petri dishes after 20 days of incubation under aseptic conditions with a thirteen-centimeter metal spatula. Once the conidia were harvested, the Petri dish was washed with 50 mL of sterile distilled water (SDA) + Tween 80 (0.01%). The conidial solution was shaken for 10 seconds at 3000 rpm in a Vortex. A Neubauer chamber (Improved Brigh Line) was used to count the conidial yield of the different isolates in the different culture media. With a micropipette, 20 µL of the 10-5 dilution were taken and deposited in the chamber, allowing five minutes for the conidia to drop to the bottom of the chamber for better reading accuracy. For the reading, the five secondary squares were used, with the aid of the 40x objective of the light microscope. The concentration of conidia per mL was estimated by the formula:

$$\text{Conidia concentration/mL} = \frac{\text{Total spores counted x 250.000}}{\text{Number of frames x Dilution}}$$

…….. **Equation 3**

Statistical analysis

The data were processed using the statistical package InfoStat. 2020. The normality of the data and homogeneity of variances were analyzed. Comparison of means was performed by analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

RESULTS

Table 1: Main macroscopic physiological and microscopic morphometric characteristics of native isolates of *Beauveria* spp.

Isolation code	Sample origin	Identification	Physiological macroscopic characteristics	Microscopic morphometric characteristics
BBT324	*Cosmopolites sordidus* (coleóptero), Buenos Aires	*Beauveria bassiana*	Colonies on PDA, SDA and EMA culture media are initially white to felted and eventually become creamy powdery with concentric rings. Light yellow reverse.	Conidia hyaline, globose to subglobose1.0-2.5 (1.9 µm) x 1.0-2.0 (1.7 µm) conidiogenous cells, with globose bases. 2.0-3.0 (2.3 µm) x 2.0-2.5(2.1 µm) and conidiophores 25 to 50 µm long with tubular septate mycelium.
BBT31	*Cosmopolites sordidus* (coleóptero), Buenos Aires	*Beauveria bassiana*	In PDA, SDA and EMA, cottony and white colonies and white at the beginning cream and powdery with time. Yellow reverse light	Very abundant conidiophores with dense clusters of phialidic cells. Conidiogenous cells globose and others bottle-shaped with interme-1.5-3.0 (2.7 µm) x 2.0-3.0 (22 µm). (22 µm). Conidia hyaline, sometimes with a well-defined apex, globose to ellipsoidal, sometimes with a sharply defined apex.1.5-3.0 (2.3 µm) x 1.5-3.0 (21 µm), with tubular septate mycelium
BBT11	Soil, Potosí	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become light beige and slightly powdery as they sporulate. dusty, as they sporulate sporulating with time. Yellow reverse light	Conidiogenous cells very typical of the species, with globular bases. species-typical conidiogenous cells, with globular bases. 2.0-3.0 (2.3 µm) x 2.0-2.5 (2.2 µm). (2.2 µm). Conidia very homogeneous, hyaline, globose, some sub globose, 2.0-2.0 µm, with tubular septate mycelium.

Isolation code	Sample origin	Identification	Physiological macroscopic characteristics	Microscopic morphometric characteristics
BBT41	*Cosmopolites sordidus* (coleóptero), Buenos Aires	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become light creamy and slightly powdery, light-yellow backing	Conidiogenous cells globose one and the other bottle-shaped with intermediate forms, 2.0-3.5 (2.3 µm) x 1.0-2.5 (1.9 µm). Conidia hyaline, globose to sub-globose subglobose1.5-2.0 (1.9 µm) x1.0-2.0 (1.7µm), with tubular septate mycelium.
BBT52	Soil, Belén	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become light beige light beige and slightly powdery as they sporulate dusty as they sporulate sporulating over time. Some coremios Reverse light yellow	They present coremium, where the conidiogenous cells are found in the form of bottle. With intermediate shapes, 2.0-3.5 (2.3 µm) x 1.0-2.5 (1.9 µm). Conidia hyaline, globose to sub-globose subglobose1.5-2.0 (1.9 µm) x 1.0-2.0 (1.7µm), with tubular septate mycelium.
BBT323	Soil, Belén	*Beauveria bassiana*	In PDA, SDA and EMA, cottony and white colonies and white at the beginning beige and slightly dusty with time. dusty with time. Honey-colored reverse	Conidiogenous cells very typical of the species, with globose bases globose, 2.0-3.0 (2.4 µm) x 2.0-2.5 (2.3 µm). Conidia very homogeneous, hyaline, globose, some slightly sub globose, 2.0-2.0 µm. Conidiogenous cells very typical of the species, with globose bases
BBT23	Soil, Belén	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and turn beige and become beige and powdery as they sporulate. As they sporulate with time. Reverse side light yellow	Conidiogenous cells, predominantly bottle-shaped, 3.0-4.0 (3.3 µm) x 3.0-2.0 (2.3 µm). Conidia variable, although predominantly ellipsoidal, apiculate base, sometimes apiculate, hyaline, 2.0-3.0 (2.3 µm) x 2.0-3.0 (2.3 µm), with tubular septate mycelium.
BBT42	Soil, Potosí	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and turn beige and become beige and powdery as they sporulate. As they sporulate with time. Reverse side light yellow	Conidiogenous cells, typical of the species with globose bases, 2.0-3.0 (2.2 µm) x 2.0-2.5 (2.3 µm). Conidia globose to subglobose, hyaline, some apicuous, 2.0-2.5 (2.5) µm. 2.0-2.5(2.2 µm) x 1.5-2.0 (1.8 µm), conidia globose to subglobose, hyaline, some apiculate. (1.8 µm), with tubular septate mycelium Conidiogenous cells, typical of the species with globose bases,
BBT1429	Soil, Belén	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and turn beige and become beige and powdery as they sporulate. As they sporulate with time. Reverse side very light yellow	Conidiogenous cells, typical of the species with globose bases, 2.0-3.0 (2.3 µm) x 2.0-2.5 (2.4 µm). Conidia sub globose to slightly ellipsoidal, the latter predominating, hyaline, 2.0-3.0 (2.6 µm) x 2.0, with tubular septate mycelium.
BBT1426	Soil, Belén	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are	Conidiogenous cells predominantly bottle-shaped, 3.0-

Isolation code	Sample origin	Identification	Physiological macroscopic characteristics	Microscopic morphometric characteristics
			white and are white and cottony and become light beige and powdery as they sporulate as they sporulate with time. Pale yellow reverse	4.0 (3.2 x 3.0-2.0) bottle-shaped, 3.0-4.0 (3.2) x 3.0-2.0 (2.4 µm). Conidia, predominantly ellipsoidal forms, some globose to subglobose, hyaline, sometimes apiculate, 2.0-3.0 (2.5 µm) x 2.0-2.5 (2.2 µm)
BBT141	Soil, Potosí	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become light beige and powdery as they sporulate as they sporulate over time. Discrete tendency to form coremium. Reverse side yellow honey	Conidiogenous cells, predominantly bottle-shaped, 3.0-4.0 bottle-shaped, 3.0-4.0 (3.3) x 3.0-2.0 (2.5 µm). Conidia, predominantly ellipsoidal forms with defined apex, some subglobose, hyaline, 2.0-3.0 (2,6 µm) x 2,0
BBT322	Soil, Belén	*Beauveria bassiana*	Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become beige and powdery as they sporulate over time. As they sporulate over time. Tendency to form coremium. Yellow reverse Colonies in PDA, SDA, and EMA that at the beginning are white and are white and cottony and become	Conidiogenous cells, typical of the species with globose bases, 2.0-3.0 (2.3 µm) x 2.0-2.5 (2.4 µm). Conidia globose to subglobose, slightly hyaline, 2.0-3.0 slightly, hyaline, 2.0 x2.0 µm. Conidiogenous cells, typical of the species
BBT321	Soil, Belén	*Beauveria bassiana*	Colonies in MEA that are initially white and are white and cottony and become pale beige and powdery as they sporulate over time. Light yellow reverse Colonies in MEA that are initially white and are white and cottony and become pale.	Conidiogenous cells with globose bases, 2.0-3.0 globose, 2.0-3.0 (2.1 µm) x 2.0-2.5 (2.4 µm). Conidia subglobose to slightly elliptic, hyaline, 2.0-3.0 (2.3) x 1.5-2.5 (2.1) µm
BBT34	Soil, Potosí	*Beauveria bassiana*	Colonies on PDA, SDA and EMA that are initially white and cottony and become pale beige and slightly powdery as they sporulate over time. Tendency to form coremium. Reverse side light yellow	Conidiogenous cells predominantly bottle-shaped 3.0-4.0(3.3) x 3.0-2.0 (2.7 µm). Conidia sub globose to slightly elliptic to ellipsoidal, hyaline, 2.0-3.0 (2.3) x 2.0 µm.
BBT112	Soil, Potosí	*Beauveria bassiana*	Colonies on PDA, SDA and EMA that are initially white and cottony and become pale beige and slightly crusty as they sporulate over time. Reverse side light yellow	Conidiogenous cells predominantly bottle-shaped, 3.0-4.0 (3.4) x 3.0-2.0 (2.8 µm). Conidia slightly elliptic to ellipsoidal, apex inconspicuous, hyaline, 2.0-3.0 (2.5) x 1.0-2.0 (1.6) µm

Of the soil samples obtained, only 16.66% were found to contain isolates of *Beauveria* spp. Of a total of 72 samples processed, 12 were successful samples from the soil, and only three corresponded to samples obtained by sandwich trapping with rotting banana weevil adults (Figure 1).

Figure 1: Isolated of *Beauveria* spp. Obtained in sandwich trapping of rotting banana weevil adults

The macroscopic characterization of *Beauveria* spp. used in the study indicates that the colonies presented high vertical growth of white color with cottony appearance due to the abundance of conidia, then the colonies become slightly powdery, and pale yellow on the back of the plate (Figure 2). Similar results were obtained by Garcia *et al.*, (2011) and Castillo *et al.*, (2012) who report that the colony of B. bassiana is woolly and powdery in appearance, white at first and then yellowish in the center. The growth of these colonies was circular, regular and with defined borders, coinciding with the observations of Garcia *et al.*, (2006) and Castillo *et al.*, (2012). Microscopic morphometric characteristics Hyaline conidia, globose to subglobose1.0-2.5 (1.9 µm) x 1.0-2.0 (1.7 µm) Conidiogenous cells, with globose bases. 2.0-3.0 (2.3 µm) x 2.0-2.5(2.1 µm) and conidiophores 25 to 50 µm long with tubular septate mycelium (Figure 4 & 5).

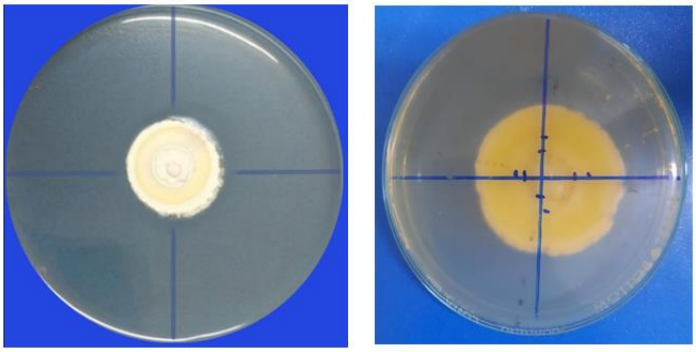
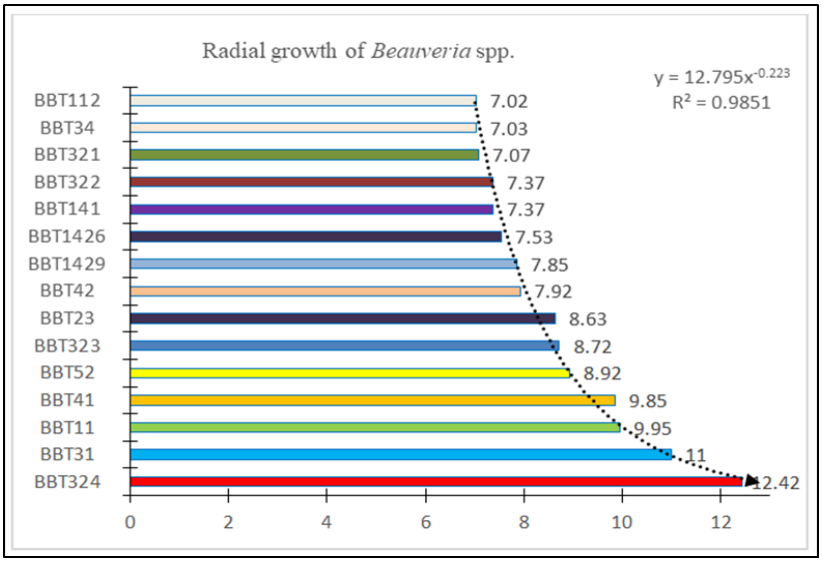


Figure 2: Macroscopic characterization of Beauveria spp.



Radial growth of conidia per native isolate of *Beauveria* spp. on PDA, SDA, and EMA culture medium at 10 days of development at 27 ± 1°C

Significant differences ($p < 0.0001$) were found in the radial growth of conidia by each native isolate of *Beauveria* spp. in PDA, SDA, and EMA culture medium. In general, the native isolate that had the best radial growth for each of them was BBT324, followed by BBT31 showing good potential in terms of conidia production, a basic aspect for the production of entomopathogenic fungi on a commercial scale, while the isolates BBT34, BBT112 had the least radial growth for each of them, and BBT324 and BBT31 showed good potential in terms of conidia production, a basic aspect for the production on a commercial scale of entomopathogenic fungi.

Figure 4: Microscopic morphometric characteristics Conidiophores of native isolates of *Beauveria* spp. in PDA, SDA, and EMA culture medium at 10 days of development at 27 ± 1°C

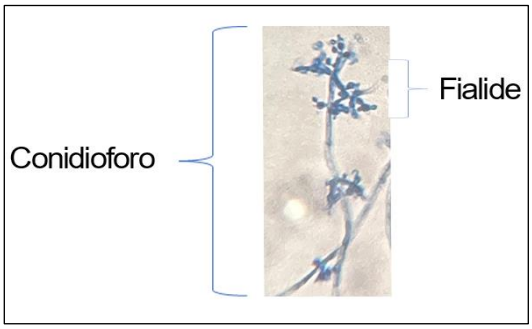


Figure 5: Microscopic characteristics of conidia conidiophores of native isolates of *Beauveria* spp. in PDA, SDA, and EMA culture medium at 10 days of development at 27 ± 1°C

Table 2: Radial growth of native isolates on EMA medium at 10 days of development at 27 ± 1°C

Isolated	E.E.									
BBT324	12.75	0.29	A							
BBT41	12.45	0.29	A							
BBT23	11.95	0.29	A	B						
BBT31	11.60	0.29	A	B						
BBT11	10.95	0.29		B	C					
BBT1429	9.70	0.29			C	D				
BBT323	9.50	0.29				D	E			
BBT42	9.35	0.29				D	E			
BBT52	8.65	0.29				D	E	F		
BBT1426	8.30	0.29					E	F	G	
BBT322	7.75	0.29						F	G	H
BBT34	7.10	0.29							G	H
BBT141	7.05	0.29							G	H
BBT321	6.90	0.29								H
BBT112	5.75	0.29								
C.V.				13.75						
p-valor				0.0001						
F; df; n				60.16; 299; 300						

Means with a common letter are not significantly different ($p > 0.05$).

SE=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom from error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

In the analysis of variance, a significant difference was found (p=0.0001). In general, it was observed that isolate BBT324 had better radial growth (12.75 cm) followed by isolate BBT41 (12.45 cm); the two that presented less radial growth were: BBT321 with (6.90 cm) followed by isolate BBT112 (5.75 cm), not presenting ideal characteristics for biocontrol candidates.

Table 3: Radial growth of native isolates in PDA medium at 10 days of development at 27 ± 1°C

Isolated	E.E.										
BBT23	6.05	0.23	A								
BBT321	6.50	0.23	A	B							
BBT1429	6.55	0.23		A	B						
BBT42	6.70	0.23		A	B	C					
BBT322	6.75	0.23			B	C					
BBT112	7.00	0.23		B	C	D					
BBT1426	7.25	0.23			C	D	E				
BBT141	7.30	0.23			C	D	E				
BBT34	7.50	0.23				D	E				
BBT52	7.90	0.23					E	F			
BBT41	8.35	0.23						F			
BBT323	8.45	0.23						F			
BBT11	9.15	0.23							G		
BBT31	11.00	0.23								H	
BBT324	11.85	0.23									I
C.V.				13.27							
p-valor				0.0001							
F; df; n				50.86; 299; 300							

Means with a common letter are not significantly different (p > 0.05).
SE=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom from error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

In the analysis of variance, a significant difference was found (p=0.0001). In general, it was observed that isolate BBT324 had better radial growth (11.85 cm) followed by isolate BBT31 (11.00 cm); the two that presented less radial growth were: BBT321 with (6.50 cm) followed by isolate BBT23 (6.05 cm), not presenting ideal characteristics for biocontrol candidates.

Table 4: Radial growth of native isolates in SDA medium at 10 days of development at 27 ± 1°C

Isolated	E.E.										
BBT34	6.50	0.22	A								
BBT1426	7.05	0.22	A	B							
BBT1429	7.30	0.22		B	C						
BBT322	7.60	0.22		B	C	D					
BBT42	7.70	0.22			C	D	E				
BBT141	7.75	0.22			C	D	E				
BBT321	7.80	0.22			C	D	E				
BBT23	7.90	0.22			C	D	E				
BBT323	8.20	0.22				D	E	F			
BBT112	8.30	0.22					E	F			
BBT41	8.75	0.22						F			
BBT11	9.75	0.22							G		
BBT52	10.20G	0.22							G	H	
BBT31	10.40	0.22								H	
BBT324	12.65	0.22									I
C.V.				11.36							
p-valor				0.0001							
F; df; n				54.63; 299; 300							

Means with a common letter are not significantly different (p > 0.05).
SE=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom from error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

In the analysis of variance, a significant difference was found (p=0.0001). In general, it was observed that isolate BBT324 had better radial growth (12.65 cm) followed by isolate BBT31 (10.40 cm); the two that presented less radial growth were: BBT1426 with (7.05 cm) followed by isolate BBT34 (6.50 cm), not presenting ideal characteristics for biocontrol candidates.

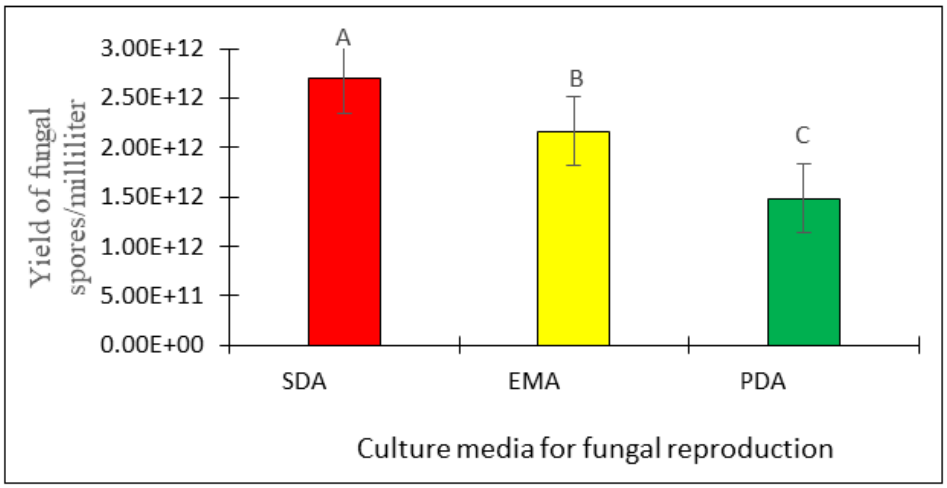


Figure 6: Conidial production of native isolate of *Beauveria* spp. On PDA, SDA, and EMA culture medium after 10 days of development at 27 ± 1°C.

The analysis of variance for the spore production variable, indicates a greater obtaining in the concentration of *Beauveria* spp. conidia, being SDA the one that presented the highest percentage of concentration 2.7 million spores per milliliter of water, followed by EMA with 2.16 million spores per milliliter of water and finally PDA with 1.48 million spores per milliliter of water.

The analysis of variance performed on the conidia concentration variable indicates that there are statistical differences between the media (p<0.0001); it also indicates statistical differences between the mean separations (Figure 6), likewise the interaction was significant which indicates that the evaluated media influence the concentration of conidia of the *Beauveria* spp. isolates (Figure 6), and also the interaction was significant which indicates that the evaluated media influence the concentration of conidia of the *Beauveria* spp. isolates (Figure 7).

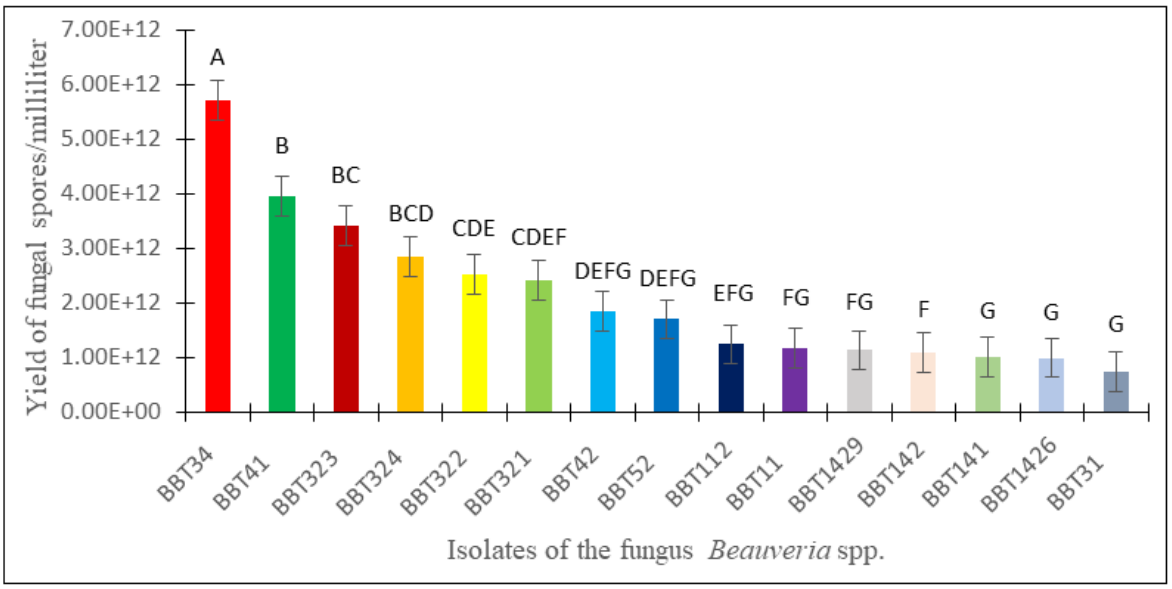


Figure 7: Concentration of conidia for each native isolate of *Beauveria* spp. in PDA, SDA, and EMA culture medium at 10 days of development at 27 ± 1°C

The analysis of variance for the spore yield variable, indicates a production in the concentration of *Beauveria* spp. conidia, being the isolate BBT34 the one that presented the highest percentage of concentration 5.7 million spores per milliliter of water, followed by BBT41 with 3.95 million spores per milliliter of water and finally BBT31 with 1.01 million spores per milliliter of water.

The analysis of variance performed on the conidia concentration variable indicates that there are statistical differences between the media ($p<0.0001$); it also indicates statistical differences between the mean separations (Figure 7), likewise the interaction was significant, which indicates that the evaluated media influence the concentration of conidia of the *Beauveria* spp. isolates (Figure 8), and also the interaction was significant, which indicates that the media evaluated influence the concentration of the conidia of the *Beauveria* spp. isolates (Figure 7).

DISCUSSION

With respect to the species of interest detected, it can be observed that there was a predominance of isolates belonging to the species *Beauveria bassiana* (Castro *et al.*, 2013) and (Castillo-Arévalo, 2023), in the same way as in this study.

On the other hand, Dhoj *et al.*, (2006) used 15 g of soil approximately for everyone of Galleria and put the trap insect in contact with the soil for up to 18 days; however, in this work, only one useful soil sample was obtained by trapping with Galleria, which represented 7% success. While in this research we took soil samples of 600 g to obtain the isolates with 16, 66 % of effectiveness, having double the effectiveness.

Lecuona (1996) he assures that the method by trapping with Galleria mentioned above is effective to obtain new fungal isolates is the isolation of single fungal colonies (cfu) from soil samples by serial dilutions, however, it is widely accepted that this method is very expensive and is preferred for the isolation and selection of bacterial species, such as Bacillus thuringiensis (Johnson and Bishop, 1996), in addition it involves a lot of field sampling time and experience, The reality is that among the most successful control agents with microorganisms are those isolates obtained directly from the host insects, as occurred in this research, which showed better radial growth behavior were the isolates from naturally parasitized insects and not from culture collections or indirect methods through selective culture media.

López (1995) in his research he indicates that this fungus is commonly found in coffee berry borer, relating it to this research, which was found in adults of *Cosmopolites sordidus*.

As can be seen in this study, the macro and microscopic characteristics for *Beauveria* isolates were highly similar, relating to the research developed by (Bridge *et al.*, 1993; Bridge and Arora, 1998; Bielikova *et al.*, 2002).

Castro *et al.*, (2013), and Garcia *et al.*, (2012), found similar results to this study in their research work where the radial growth of conidia per isolate is very similar to this one, they also pointed out that a high production of conidia makes them more effective in the field.

The growth rate of a fungus is largely determined by the organism's ability to take advantage of nutrient sources.

The organism's ability to take advantage of available nutrient sources (carbohydrates and proteins, among others), degrade proteins, among others), to degrade the substances present in the growth substrate, and by the ability of the fungus' metabolism to and by the ability of that organism's metabolism to function within the range of temperature temperature range offered by the medium (Iskandarov *et al.*, 2006; Fargues *et al.*, 1992). In this regard, Shah *et al.*, (2005) Safavi *et al.*, (2007) and (Castillo-Arévalo, 2021) state that the radial growth rate is a variable that depends on several factors, including variable that depends on several factors, for example, the type of strain used, the nature of the substrate on which the fungus grows nature of the substrate where the fungus grows, as well as the ratio of the substrate used, among other factors. In the same way, in this research it was observed that the promptness of growth of fungal isolates depends on the medium that has a high source of carbohydrates and glucose.

The results found in this study are like those reported by Zare and Gams (2001), who indicate that 20 days after inoculation, radial growth was 18 mm in PDA, 15 mm in EMA and 16 mm in SDA. They also describe that this fungus presents a growth of colonies that reach 15 to 25 mm in diameter in 10 days at 24°C in PDA culture medium.

Elósegui *et al.*, (2003) in their research, no significant differences were obtained between the media, with colony diameter fluctuating between 31 and 37 mm; however, interaction was observed due to a maximum growth observed in the SDA transfer culture.

The results obtained show that all the isolates present a similar radial growth in the Sabouraud Dextrose Agar and malt extract culture media; however, the highest radial growth was obtained by isolate BBT324 with a radial growth of 12.75 mm in 10 days; it is important to emphasize that in these culture media quite uniform growth was observed. Regarding this finding (Retamal, 2008) indicates that the culture

medium in which the entomopathogenic fungus grows and sporulates better is Sabouraud Dextrose Agar, being in this study malt extract the medium that most developed the isolate with the highest development in the three media under study.

According to Monzón (2001) and Alcántara (2019), they show that when the source of sugars is higher in the culture medium, it favors the production of spores increasing its production in the concentration of the conidia of entomopathogenic fungi.

CONCLUSION

Fifteen native species of *Beauveria* spp. were isolated and characterized with taxonomic keys for the main macroscopic, physiological, and microscopic morphometric characteristics. These were subjected to different tests where the variables radial growth, morphology and conidia production were evaluated in different artificial culture media.

All isolates showed similar radial growth on Sabouraud Dextrose Agar and malt extract media, the highest radial growth was obtained by isolate BBT324 with a radial growth of 12.75 mm in 10 days and the lowest radial growth with 6.05 mm was obtained on Papa Detroxa Agar medium with isolate BBT23, the concentration of *Beauveria* spp. conidia was the highest on Papa Detroxa Agar medium with isolate BBT34. isolate BBT34 was the highest on Papa Detroxa Agar medium with isolate BBT34, isolate BBT34 had the highest percentage concentration of 5.7 million spores per milliliter of water, followed by BBT41 with 3.95 million spores per milliliter of water and finally BBT31 with 1.01 million spores per milliliter of water.

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