El Colegio de la Frontera Sur

Toxicidad de una mezcla de *Beauveria bassiana* y Spinosad contra la broca del café en laboratorio.

Tesis
presentada como requisito parcial para optar al grado de
Maestría en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en Entomología Tropical

Por

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DEDICATORIA

A Dios

Por darme salud como tiempo para poder realizar y cumplir mis metas

A mi madre

Cándida de León Pérez por darme la vida, ya que sin ella no tendría el gusto de vivir y placer de conocer y saber todo lo bueno que tiene a vida

A mi hermosa hija

Ambar Darinne Morales González por llegar a mi vida y darme el mayor y mejor placer en la vida, ser su padre, ya que día a día aprendo nuevas cosas, el sentido de la vida y darme a conocer lo que es el verdadero amor. Te amo.

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INDICE

I. INTRODUCCION

La región del Soconusco se caracteriza por tener una diversidad de cultivos, donde el café ha sido el cultivo por excelencia, del cual depende gran parte de la economía de esta región (Montoya *et al*., 2010). Este aromático grano responde a los impulsos externos de la economía, como incrementos y decrementos del mercado internacional, que rige el comportamiento de los precios internacionales (Neumann, 2009 y McCall, 2010). Los decrementos ha traído como consecuencia, el abandono de los cafetales en los últimos años por parte de diversos grupos poblacionales, principalmente los jóvenes (Montoya *et al*., 2010), propiciado en parte, por la falta de apoyo y asistencia técnica del sector gobierno encargado de ver por el bienestar de la población, en apoyo a la sanidad de este cultivo que da sustento a tantas familias.

Los insectos son un grupo de organismos que impactan en la agricultura y en la salud humana. Han sido uno de los principales problemas en la agricultura, ya que ocasionan daños en los cultivos, tanto en su rendimiento como en su producción, provocando fuertes pérdidas económicas (Toro *et al.,* 2003; Rogg, 2000). Se invierten muchos recursos por parte de los agricultores tanto humanos como económicos y por parte del gobierno para su control e investigación. El cultivo del café no se escapa a esta problemática, ya que se ha visto afectado por diversos problemas fitosanitarios, entre ellas, la principal plaga de este cultivo, la broca del café (*Hypothenemus hampei* Ferrari), insecto del orden Coleóptera de la familia Curculionidae. Esta plaga, que ingresó a México en 1978 a través de la frontera sur de Chiapas, no solo es la más importante en la zona, sino que también a nivel mundial. Actualmente este insecto se encuentra en la mayor parte de los estados productores de café del país (Barrera, 2008).

En el Soconusco, Chiapas, se mencionan niveles de infestación del 1 al 42% de los frutos que son perforados por este insecto (Pereira, 2011). Desde su introducción en los cafetales chiapanecos, las instituciones de gobierno como SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria) realizaron campañas para combatir esta plaga, implementando el uso de Endosulfan, insecticida que demostró ser eficiente contra la broca, pero con el tiempo presentó algunos problemas ambientales así como resistencia de la broca al insecticida en otros países (Brun *et al*., 1989). Esto provocó que en México se restringiera su uso y dejó de usarse en los cafetales. Con el paso del tiempo, *H. hampei* se desplazó e invadió a otras entidades del país (SENASICA, 2013).

La utilización de productos químicos para el control de plagas de manera indiscriminada, ha provocado daños ecológicos y ambientales, desde la contaminación de los suelos, el agua, el aire, hasta la muerte de organismos no blancos (USDA, 2000). Por ello, se han buscado alternativas más sustentables para combatirlas, entre ellas el control biológico. Para el caso de *H. hampei*, uno de los organismos que se ha utilizado dentro de esta estrategia de control ha sido el hongo *Beauveria bassiana* (Pereira, 2011), patógeno. Provoca una enfermedad en los adultos de la broca. Este hongo, además de su uso contra la broca del café, se ha utilizado con otros insectos plaga, como la palomilla dorso de diamante (*Plutella xilostella*) y el picudo del plátano (Monzón, 2001; Amutha *et al*., 2010). Los insectos muertos por este hongo presentan una cubierta blanca algodonosa sobre el cuerpo, la cual está formada por el micelio y esporas del hongo (Monzón, 2001). Los resultados obtenidos con *B. bassiana* no han sido consistentes. Por lo que se ha continuado con las investigaciones para mejorar la eficiencia del hongo, desde la protección de las esporas de los rayos solares (Velez *et al*., 1997; Benavides *et al*., 2012), la realización de formulados para una mayor efectividad (Benavides *et al*., 2012; Morales *et al*., 2014), hasta la combinación con otras técnicas de manejo integrado de plagas (SAGARPA, 2007), entre ellas, la mezcla con insecticidas químicos (SENASICA, 2013).

Por otro lado, se han buscado alternativas a los insecticidas convencionales, que sean menos agresivas al medio ambiente y organismos no blancos. Una de ellas ha sido la utilización de bioinsecticidas como el Spinosad (espinosinas A y D), producto de la fermentación aeróbica del actinomiceto *Saccharopolyspora spinosa*, que provoca parálisis, inapetencia y finalmente la muerte de los insectos (Pineda *et al*., 2007). Este producto comercial, ha sido evaluado en diversos organismos plaga, entre ellos, *Heliothis virescens*, *Helicoverpa zea*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Spodoptera littoralis*, *Pseudoplusia includens*, *Trichoplusia ni*, *Plutella xyllostella*, *Corcyra cephalonica*, *Choristoneura fumiferana*, *Lymantria dispar*, *Plodia interpunctella*, *Aedes aegypti*, *Anopheles albimanus*, *Musca domestica*, *Ceratitis capitata*, *Liriomyza trifollii*, *Bactrocera dorsalis*, *Cryptolestes ferrugineus*, *Tribollium castaneum*, *Rhyzoperta dominica*, *Sitophilus oryzae*, *Oryzaephilus surinamensis* y *Chrysomela scripta* (Moulton *et al*., 2000; Bond *et al*., 2004; Huang *et al*., 2004; Ju-chun y Hai-tung, 2006; Pineda *et al*., 2006). Aunque el mayor efecto de este bioinsecticida es a través de la ingestión por lo insectos, se ha sugerido que también tiene cierto efecto por contacto (Dow Agrosciences, 2015). El uso de

Spinosad es una buena opción para incluirse en programas de manejo integrado de plagas en cultivos hortícolas, ornamentales y frutícolas contra grupos de insectos como Lepidóptera, Díptera y Coleóptera (Pineda *et al*., 2007). Ha sido avalado por importantes instituciones como el Departamento de Agricultura de los Estados Unidos, quien ha certificado su uso en cultivos orgánicos, debido a su baja residualidad en el ambiente (USDA, 2000).

Las evaluaciones de Spinosad contra coleópteros han incluido a *Tribolium castaneum*, *Callosubruchus maculatus*, *Sitophilus oryzae*, entre otros, pero para *H. hampei*, solo existe un reporte. Para el caso de *Tribolium castaneum* se han evaluado concentraciones de Spinosad de 100, 200 y 300 ppm, en formulaciones con aceites, determinándose que la mejor concentración para este insecto fue de 300 ppm, con el 100% de mortalidad a los 21 días (Khashaveh *et al*., 2009). En el caso de *Callosubruchus maculatus* se mezclaron concentraciones de Spinosad de 100, 200, 300 y 400 ppm con 1Kg de cereal, los cuales se colocaron en cilindros de 2 Kg durante 35 días, se reportó que a los 10 días las concentraciones de 200 y 300 ppm habían alcanzado casi el 100% de mortalidad (Khashaveh *et al*., 2011). En relación a *Sitophilus oryzae* a una concentración de 1 ppm, Spinosad mezclado con trigo de diferentes clases, provocó una mortalidad de 69 al 100% a los 7 días (Fang *et al*., 2002). Con respecto a *Hypothenemus hampei*, el único reporte sobre Spinosad es el de Chávez *et al*. (2006) quienes evaluaron su efecto en una dieta donde alimentaron a los adultos de la broca y su efecto por contacto en papel filtro a 10 y 400 ppm. Ellos reportan que a 10 ppm en la dieta provocó el 90% de mortalidad a los adultos a los 8 días, mientras que por contacto a la misma concentración provocó el 50% de mortalidad a los 3 días. Hasta donde sabemos, no hay otro estudio similar, y menos aún, donde se haya evaluado una combinación de Spinosad y el hongo *B. bassiana* contra este coleóptero,

Este trabajo de investigación abre una nueva alternativa, al utilizar el bioinsecticida Spinosad en combinación con otro agente ya conocido para el control de la plaga, el hongo *Beauveria bassiana*.

A lo largo de la historia, en algunos trabajos donde se ha evaluado la compatibilidad de insecticidas con entomopatógenos, se ha encontrado generalmente que existe una sinergia entre ellos, esto significa que el efecto de total de la mezcla es mayor que la suma de los efectos de los componentes individuales (Hewlett, 1960; Gisi, 1996). También se ha reportado sinergia en algunas mezclas de Spinosad mezclado con hongos patógenos. Por ejemplo, Spinosad mezclado con *Metarhizium anisopliae*, rebajó la tasa de alimentación del gusano de alambre (*Agriotes lineatus* y *Agriotes obscurus*) produciendo una elevada mortalidad sobre estos (Ericsson *et al*., 2007). Sin embargo, en otro trabajo parecido, donde se mezcló Spinosad con *B. bassiana* para determinar su toxicidad sobre el gusano cortador negro (*Agrotis ípsilon*), se reportó que no hubo diferencias significativas entre los individuos tratados respecto al control de pupas y adultos. En ese mismo trabajo se determinó que *B. bassiana* fue más efectivo que Spinosad solo sobre larvas del tercer estadio del gusano (Gosselin *et al*., 2009). Algo importante que se menciona en ese artículo fue la compatibilidad entre ambos productos, por lo que dejan la puerta abierta para evaluar la mezcla de ellos con otros insectos plaga.

Por lo anterior, el objetivo de este trabajo fue evaluar la toxicidad de Spinosad solo y en una mezcla con el hongo *Beauveria bassiana* contra la broca del café *Hypothenemus hampei*.

II. CAPITULO DEL ARTÍCULO Additive effect the mixture of *Beauveria bassiana* and Spinosad on the mortality the coffee berry borer in the laboratory

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ARTICULO

Additive effect the mixture of *Beauveria bassiana* and Spinosad on the mortality the coffee berry borer in the laboratory

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I.- ABSTRACT

Laboratory experiments were conducted to evaluate the effectiveness of the bioinsecticide Spinosad and its compatibility with the fungus *Beauveria bassiana* against *Hypothenemus hampei* adults. Different concentrations of both insecticides were evaluated separately for 12 days to determine the median lethal time (LT_{50}) and the median lethal concentration (LC_{50}). Spinosad at concentrations of 200, 300 and 400 ppm caused 61, 76 and 79% mortality in *H. hampei* females, with a LT_{50} of 8.5, 3.5 and 2.5 days, respectively, and LC_{50} of 60.4 ppm. The native *B. bassiana* strain Bb-Hy caused 28, 50, 71 and 86 % mortality at concentrations of $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ spores/mL with LT_{50} of 4 and 1.5 days for concentrations of $1x10^7$ and $1x10^8$, respectively, and LC_{50} of $2.47x10^6$. Toxicity of the mixture was evaluated using the concentration of 200 ppm Spinosad and $1x10^6$ *B. bassiana* for an observation period of five days, according to the analysis of CL50 and TL50. The mixture *B. bassiana* and Spinosad caused 94% mortality of adult female *H. hampei* at five days of observation, while in the same period the individual effect of Spinosad or *B. bassiana* alone caused 61 and 49% mortality, respectively. Our results show that the combination of the fungus *B. bassiana* with the insecticide Spinosad has an additive effect on mortality of adult coffee borers. No negative effect on the fungus was observed when mixed with the insecticide, indicating there is compatibility and the mixture can be used against adult *H. hampei*.

KEY WORDS

Hypothenemus hampei, *Beauveria bassiana*, Spinosad.

II.- INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei*, is the major pest of coffee worldwide. In 1978, it entered Mexico through the southern border of Chiapas to Guatemala. Currently, it is found in most of the coffee-producing states of the country (Barrera 2008). The Soconusco, Chiapas, infestations of up to 42% perforated fruits have been reported (Pereira 2011).

Control chemical have been used to control this pest. However the misuse of insecticide, may ecological and environmental damage or resistance of the target pest. For example, in New Caledonia *H. hampei* adults developed resistant to Endosulfan, the main insecticide used for its control (Brun et al. 1989; SENASICA 2016). For this reason safer, alternatives the manage the CBB have been investigated. Among these is the entomopathogenic fungus *Beauveria bassiana* (De la Rosa et al. 1997, 2000; Benavides et al. 2012). Results obtained with *B. bassiana* against *H. hampei* have been encouraging, achieving mortalities above 50% at a concentration of $1x10^6$ spores/mL in the laboratory and of 14.3% in field applications. However, research aimed to improve its effectivity has continued. For example, it has developed formulas to use the fungi in combinations the integrated with other strategies from pest management (SAGARPA 2007; Benavides et al. 2012; Morales et al. 2014). Combinations have included mixtures of entomopathogens and chemical insecticides (Ericsson et al. 2007; Gosselin et al. 2009; Sharififard et al. 2011).

In recent years, with the upsurge of organic coffee, more attention has been given to “green” insecticides, thus denominated because of their reduced or negligible environmental impact. Spinosad is one of these insecticides; besides being accepted in diverse organic crops, it has demonstrated insecticidal activity against a large number of agricultural pests (USDA 2000). Spinosad (spinosyns A and D) is a natural insecticide produced by aerobic fermentation of actinomycetes *Saccharopolyspora spinosa* (Kirst 2010), which produces insect paralysis and death. Although it exhibits effect by contact, its greatest activity is through ingestion (OMRI 2001). This insecticide has been regarded as a viable alternative to be included in integrated pest management programs in vegetable, ornamental and fruit crops (Pineda et al. 2007). Moreover, it is approved by important institutions such as the United States Department of Agriculture, which has certified it for use in organic crops because of its low persistence in the environment (USDA 2000).

Few formal assessments of Spinosad against *H. hampei* has been carried out. One of the first laboratory studies reported that this insecticide caused 90% mortality of the CBB 2 days after application at a concentration of 4800 ppm, while a concentration of 10 ppm caused the same percentage of mortality but after 8 days (Chávez et al. 2006). In Costa Rica, field applications were performed on adults released in confined sleeves at a dosage of 1 mL/L Spintor 12SC, which caused the death of 21% *H. hampei* adults. However, this percentage was lower than the mortality that occurred in the control, mostly caused naturally by *Beauveria bassiana* (ICAFE 2009).

A number of studies have shown that joint application or use of Spinosad with *B. bassiana* does not have significant effects on viability, growth or sporulation of the fungus. Thus the fungi can be used in combination with other IPM strategy, offering additional advantages by introducing multiple factors of mortality and physiological interaction in the insect that can derive in additive or synergetic effects (Rajanikanth 2010). This would open up the possibility of using it in a more efficient mixture against insect pests.

Because the entomopathogen *B. bassiana* and the insecticide Spinosad can be used independently as control methods against the CBB, during the first stages of infestation in the field, and since they have different modes of action, we hypothesized that in combination they can have greater impact on borer populations. For this reason, this study was conducted to evaluate the toxicity of Spinosad, as well as its compatibility and effectiveness when combined with *Beauveria bassiana* on CBB mortality in the laboratory.

III. MATERIALS AND METHODS

Site and general conditions of the experiments

The study was conducted in the Biological Control Laboratory of the research line "Arthropod ecology and pest management" of El Colegio de la Frontera Sur (ECOSUR), located in Tapachula, Chiapas. The experimental room was maintained at a temperature of 28 ±2 ºC, relative humidity 80 ±2 % and 12 ±2 h light-dark photoperiod.

Biological material and chemical product

Hypothenemus hampei.

The biological material used was CBB obtained from ripe Arabic coffee fruits collected in the field one week before the bioassays. Perforated fruits from the field were transported to the laboratory where they were placed on absorbent paper inside a recipient that had a screen bottom. One day before the bioassays, we selected the fruits from which adult borers were extracted. A dark-colored powder coming from the borer gallery entrance indicated that inside the fruits there were mainly *H. hampei* adults. The fruits were disinfected with 0.5% sodium hypochlorite (Bustillo and Marín 2002) and dried. The adult borers were then extracted and placed in glass tubes containing a meridic diet (Villacorta and Barrera 1993) where they remained until the bioassays.

Beauveria bassiana.

The strain of the fungus *B bassiana* used was the native strain Bb-Hy, isolated from CBB collected on a coffee farm in the Soconusco Region two years ago. The strain is part preserved in silica gel and belongs to the ECOSUR ceparium. It was reactivated two months before the bioassays on adult fruit flies, *Anastrepha* sp., provided by the rearing laboratory of the same institution. The fungus was later reproduced in solid SDA (Sabouraud Dextrose Agar) medium and its conidia were harvested 30 days later to prepare a stock solution in sterile distilled water with 0.05% Tween 80. This stock solution was used to prepare the concentrations used in the bioassay. Viability of the conidia was determined before the bioassays, observing their germination following the methodology described by Vélez and collaborators (1997). Germination was more than 95%.

Spinosad.

The commercial Spinosad product used for the bioassays was Spintor 125 SC™ (120 g a.i./L). The different dilutions were prepared with sterile distilled water.

Individual pathogenicity of Spinosad and *B. bassiana*

In this experiment, lethal concentrations of Spinosad and *B. bassiana* were determined separately. These determinations served as the basis for later mixtures. In the case of the insecticide, we used concentrations of 200, 300 and 400 ppm, which are frequently used in bioassays with coleopterans (Khashaveh et al. 2009 and 2011). The fungus was used in concentrations of $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$, which are used in bioassays against *H. hampei* (De La Rosa et al. 1997; Morales et al. 2014).

For the bioassays, 20 adult *H. hampei* females were used per replication, with five replicates per treatment. The solution of the concentrations of the different treatments (Spinosad and *B. bassiana*) were sprayed on using perfume-type atomizers with a capacity of 20mL. Two mL of the suspension was sprayed over the adult borers in each replication. Later, the *H. hampei* adults were placed in tubes containing meridic diet (Villacorta and Barrera 1993). The control treatment consisted of applications of sterile distilled water for the case of Spinosad and sterile distilled water with 0.05% Tween 80 for the case of *B. bassiana* on adult borers. The tubes were checked daily and dead borers were removed and placed in moist chambers to stimulate mycelial growth. The last observation was 12 days after application.

Pathogenicity of the mixture of Spinosad and *B. Bassiana*

Based on the above results, we decided to use the lowest lethal concentration of both Spinosad and *B. bassiana* that killed 50% of the *H. hampei* adults. We also decided to observe the treatments up to the fifth day, taking the LT_{50} of the previous bioassay into account. The bioassays were conducted following the same methodology described in the previous section. In this case, the treatments were mixtures of Spinosad and *B. bassiana* at the lethal concentration selected previously (Spinosad 200 ppm and *B. bassiana* $1x10^6$), a relative control of Spinosad at 200 ppm, a relative control of *B. bassiana* at $1x10^6$ and an absolute control consisting of sterile distilled water plus 0.05 % Tween 80. Dead borers were placed in moist chambers to verify the presence of mycelia.

Statistical analysis

Survival was analyzed with R software (R Core Team 2015) to determine LT_{50} and with the Probit analysis for CL_{50} of the separate Spinosad and *B. bassiana* treatments. Data from the experiments evaluating the mixtures of Spinosad and *B. bassiana* were analyzed with a generalized linear model with binomial response. An Analysis of variances (ANOVA) was performed and means were compared with Tukey.

IV. RESULTS

Individual pathogenicity of Spinosad and *B. bassiana*

The insecticide Spinosad caused 61, 76 and 79% mortality of *H. hampei* females at concentrations of 200, 300 and 400 ppm, respectively, at day 12 of observation (Figure 1). LT_{50} for the same concentrations was 8.5, 3.5 and 2.5 days, respectively (Table 2). The LC_{50}

obtained was 60.4 ppm with lower and upper fiducial limits of 29.9 and 75.8 ppm, respectively.

The native strain BbHy of *B. bassiana* caused mortalities of 28, 50, 71 and 86% at concentrations of $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ spores/mL respectively (Figure 2). LT_{50} for concentrations $1x10^7$ and $1x10^8$ was 4 and 1.5 days, while at concentrations of $1x10^5$ and $1x10^6$ it was not possible to determine because insect mortality did not surpass 50% (Table 1). LC_{50} for *B. bassiana* was $2.47x10^6$, with lower and upper fiducial limits of 4.38 $x10^5$ and 4.34 x 10^6 conidia/mL, respectively. The presence of mycelia on the dead borers treated with the fungus was 0, 23, 49, 67 and 83% for the control and the concentrations of concentraciones$1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$, respectively.

Pathogenicity of Spinosad and *B. bassiana* mixture

The mixture of the insecticide Spinosad and the fungus *B. bassiana* at the lowest concentrations used against *H. hampei* caused 94% mortality at five days. In contrast, the relative control concentrations of Spinosad and *B. bassiana* caused 61 and 49% mortality, respectively, while with the absolute control mortality was only 1% (Figure 3). The statistical analysis showed differences in the response to the mixture, relative to the separate concentrations. The concentrations of the insecticide and *B. bassiana* independently were statistically equal, but different from the mixture and the control (χ^2 = 225.34; df = 3; $P<0.001$).

V. DISCUSSION

In this study we found that both Spinosad as the fungus affects the survival of the drill, but the combination of both was better. Although some studies have proved the effect of Spinosad against Coleopteran insects (Chávez et al. 2006; Huang and Subramanyam 2007; Khashaveh et al. 2011; Leng and Reddy 2012, Reddy et al. 2014) and others have also evaluated the effect of the fungus *B. bassiana* mixed with Spinosad (Gosselin et al. 2009; Amutha et al. 2010; Gowrish et al. 2013; Reddy et al. 2014), this is the first study in which a native strain of the fungus combined with the bioinsecticde is tested against the CBB *H. hampei*. The results showed that the mixture of the fungus *B. bassiana* and the biological insecticide Spinosad achieved higher mortality of adult coffee borers *H. hampei* than their individual application in the laboratory, whereby a synergy occurs.

Ericsson and collaborators (2007) reported similar effects with the mixture of Spinosad with *Metharizium anisopliae*; they found that the mixture has an additive effect, causing higher mortality of the wireworm *Agriotes obscurus* and *A. lineatus* than individual applications. However, Gosselin and collaborators (2009) evaluated the effect of Spinosad and *B. bassiana* alone and combined against the lepidopteran *Agrotis ipsilon*; they observed different responses: antagonistic, synergetic and additive effects on third instar larvae.

In the case of the CBB, our results show that the combination of Spinosad and *B. bassiana* has an additive effect resulting in higher mortality of *H. hampei* adults when the two control agents were integrated than when each was applied separately, results similar to studies and definitions of the following authors (Rajanikanth 2010; Tammes 1964; Colby 1967). The mortality achieved by the mixture was 94% compared with the absolute treatments of Spinosad (61%) and the fungus *B. bassiana* (49%) five days after application.

Amutha and collaborators (2010) observed in the laboratory that Spinosad had a sligh toxic effect on *B. bassiana*. They suggest, however, that it can be used safely in a mixture. Spinosad may have a toxic effect on the growth of the fungus, since only 43% of the *H. hampei* adults used in the mixture treatment placed in moist chambers exhibited mycelia. For this reason, it is necessary to conduct more studies in this respect.

Our results suggest that Spinosad is an excellent candidate for controlling the coffee borer *H. hampei*, causing mortality rates of 50% to more than 80%, alone or combined with the fungus *Beauveria bassiana.* However, it is necessary to continue evaluating the interactions between Spinosad and *B. bassiana* in the laboratory and the field in order to consider their use in an integrated *H. hampei* management program.

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Tables

Table 1. Lethal time 50 (LT_{50}) of the treatments with Spinosad and *B. bassiana* separately on adult *H. hampei* females.

	TL_{50} Spinosad					TL_{50} *B. bassiana*		
Trata	TL50	Lower	Upper		Trata	TL50	Lower	Upper
dl400	2.5	1.5	3.5		d10_8	1.5	1.5	3.5
dl300	3.5	2.5	6.5		d10_7	4	3.5	8.5
dl200	8.5	6.5	10.5		d10_6	-------	4.5	-------
ctrl	-------	-------	-------		d10_5	-------	-------	-------
					ctrl	-------	-------	-------

Figure captions

Figure 1. Mortality of coffee berry borers subjected to different concentrations of a commercial Spinosad product (Spintor 125 SC™) to determine lethal concentration.

Figure 2. Mortality of coffee berry borers treated with different concentrations of the native *Beauveria bassiana* strain BbHy.

Figure 3. Mortality of adult *H. hampei* females caused by the commercial product Spinosad (200 ppm) and the fungus *B. bassiana* ($1x10^6$) mixed (Spin_Beauve) and separate. The same letters indicate that there is no statistical difference between treatments, according to Tukey at 0.05 %.

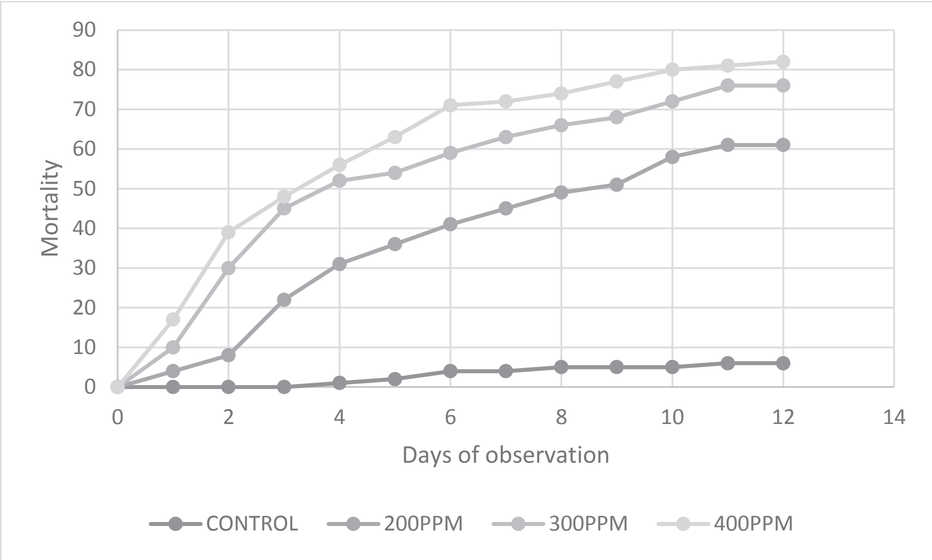
Mortality
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days of observation
CONTROL
200PPM
300PPM
400PPM

Fig 1.

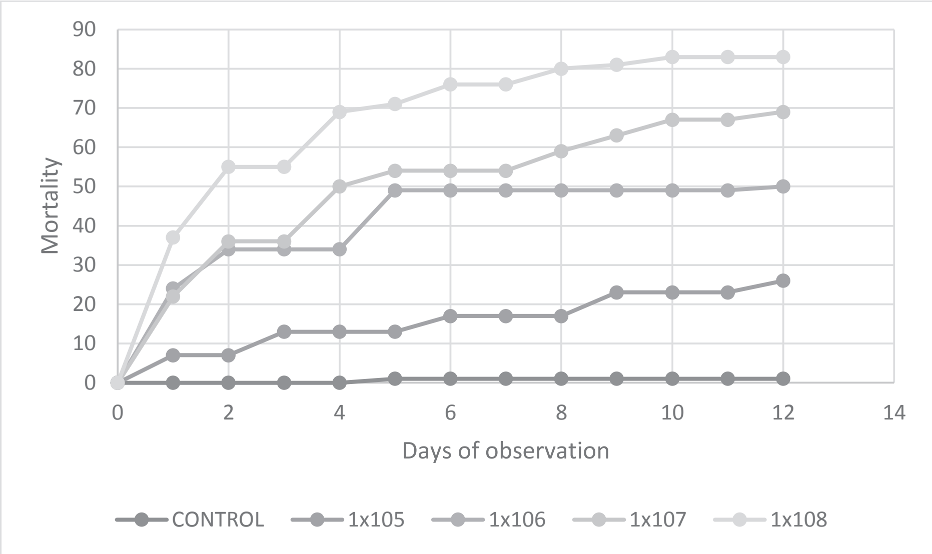
90
80
70
60
50
40
30
20
10
0
Mortality
0
2
4
6
8
10
12
14
Days of observation
CONTROL
1x105
1x106
1x107
1x108

Fig. 2

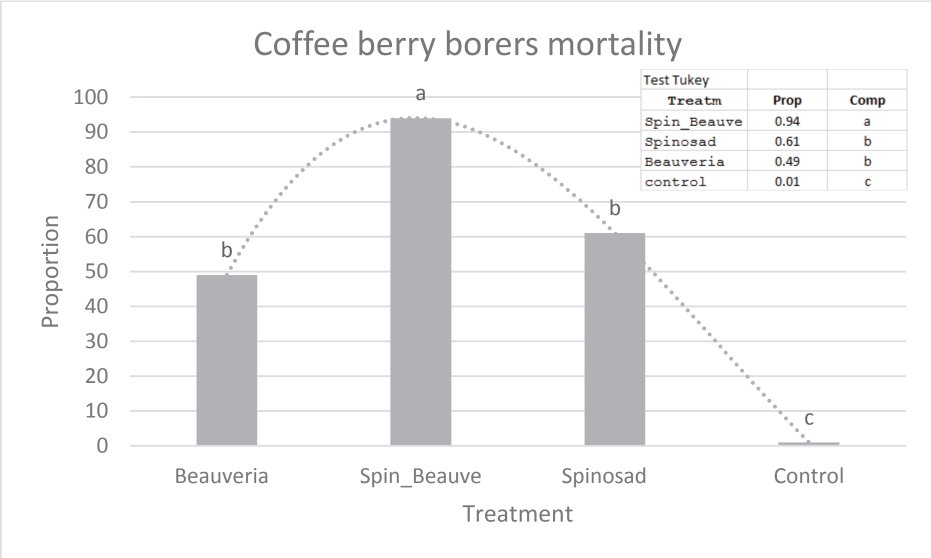
Coffee berry borers mortality
Test Tukey
Treatm Prop Comp
Spin_Beauve 0.94 a
Spinosad 0.61 b
Beauveria 0.49 b
control 0.01 c
Proportion
100
90
80
70
60
50
40
30
20
10
0
a
b
b
c
Beauveria
Spin_Beauve
Spinosad
Control
Treatment

Fig. 3

III. CONCLUSIONES GENERALES

Como estudio novedoso y por ser la primera vez que se reporta en la broca del café, el efecto de Spinosad solo y combinado con el hongo *B. bassiana*, además de causar la muerte sobre *H. hampei*, se pueden obtener las siguientes conclusiones.

- Nuestros resultados sugieren que Spinosad puede ser un excelente candidato para el control de la broca del café, *H. hampei*, ya que presenta porcentajes de mortalidad que van del 50 a más del 80% ya sea en forma independiente o mezclado con el hongo *B. bassiana*.

- La combinación de Spinosad y el hongo entomopatógeno *B. bassiana* causa una mayor mortalidad sobre los adultos de *H. hampei*, lo que sugiere un efecto de sinergia, ya que provocó el 94% de mortalidad al mezclar las concentraciones más bajas evaluadas en el presente estudio (200 ppm-$1x10^6$ conidias/mL: Spinosad – *B. bassiana*), que de manera independientes, donde provocaron mortalidades de 61% y 49% respectivamente.

- No se observó ningún efecto inhibitorio sobre el hongo *B. bassiana* cuando se mezcló con el bioinsecticida Spinosad.

- El efecto de Spinosad sobre la broca, así como de la mezcla con el hongo *B. bassiana*, representa un potencial para el control de esta plaga, pero también da pauta para seguir evaluando las diferentes interacciones que puedan ocurrir a concentraciones aun menores y en otros organismos plaga.

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