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Efficacy of native isolates of *Metarhizium* spp. in the control of the weevil (*Metamasius hemipterus*) of sugarcane in laboratory conditions

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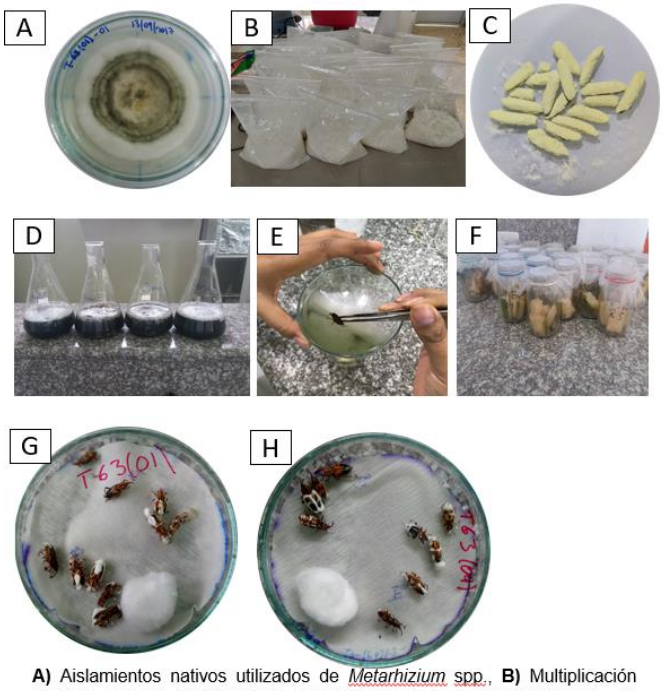
Graphical Abstract *(mandatory)*	**Abstract.** *(mandatory)*
	The objective of this work was to evaluate the efficacy of four native isolates of *Metarhizium* spp., in the control of adults of *Metamasius hemipterus* in laboratory conditions. A completely randomized design with seven treatments and five repetitions was used. The treatments were the four native isolates (TI6301, TS6304, PS5002, PS5003; at a concentration of 1 x10^8 conidia.mL^{-1}), Chemical (Engeo at a dose of 1mL.L-1 water), a commercial biological (Micosplag at a dose of 0.5 g.L^{-1} of water) and a control treatment with sterile distilled water. The treatments were applied by immersion. Mortality assessment was recorded at 5, 10, and 15 days after treatment application. Abbott's formula was used for the correction of mortality. To determine the



A) Aislamientos nativos utilizados de *Metarhizium* spp., **B)** Multiplicación masiva en arroz, **C)** Granos de arroz colonizados por *Metarhizium* spp., **D)** Preparación diluciones de cada aislamiento, **E)** Inmersión de adultos de *Metamasius hemipterus* en la suspensión de cada aislamiento nativo, **F)** Adultos de *M. hemipterus* en envases de vidrio de 250 mL con trozos de caña de azúcar y tapados con tela tul, **G y H)** Adultos muertos cubiertos con micelio de *Metarhizium* spp.

confirmed mortality, dead adults were conditioned in humid chambers to verify the presence of mycelium on dead insects. The data was processed by ANOVA, the comparison of means was made with the Tukey test (P <0.05). The chemical treatment showed an immediate response in adults of *M. hemipterus* with a 100% mortality efficiency before the first 24 hours, while the native isolates began to reflect mortalities from the second day. The native isolates of *Metarhizium* spp. TI6301, TS6304 and PS5002 caused accumulated and corrected mortalities above 70%, and Micosplag treatment reached an efficiency of 4.17%. Confirmed mortalities were between 72.4 and 76% with native isolates TS6304 and TI6301, respectively on adults of *M. hemipterus.*

Introduction *(optional), no page limit*

Metamasius hemipterus (Coleoptera: Curculionidae) is one of the main sugarcane pests. The most important damage occurs in the replanting stage when short stalks of sugarcane are planted. During the rooting process, the fermentative decomposition of internodes tissues attracts *M. hemipterus* to feed, mate and oviposit. Fermentative decomposition of the planted sugarcane stem occurs only in high humidity environments (Alpizar et al., 2012).

M. hemipterus is a pest of first importance for sugarcane in Ecuador where the insect limits the tonnage, the quality of the juices and the percentage of sucrose as a direct effect of the damage caused by the larvae inside the stems. In addition, broken stalks are seen in the field due to the effect of the perforations and galleries made by the larvae, usually very close to the ground, in the region near the roots. The wind, the very weight of the stems and in many cases the rain facilitates the lying of the affected stems. Generally at the bases of the stems, they present their internodes turned into a rotting mass of fermented and smelly bagasse. The rest of the plant, being deprived of the circulation of sap dries and dies (Risco, 1967).

In severe attacks, when the affected stems exceed 10%, the cane tonnage can be decreased by 10 or 15%. Under these conditions the losses in sucrose reached 20 and 30% as seen by Risco (1967). Several techniques have been used to manage bill populations. The application of synthetic insecticides has been ineffective, because most of their life goes inside the stems unless they are applied repeatedly or the application time coincides with the flight time of these beetles (Kushiyev et al., 2018). It has been reported that the spread of entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* infects larvae and adult beetles (Alpizar et al., 2012). *M. anisopliae* is often used worldwide due to its ability to infect a large number of insect species and its easy mass reproduction (Thaochan & Sausa, 2019).

Taking into account the environmental conditions of Pastaza province, the use of these fungi against *M. hemipterus* could be an ecologically friendly alternative to manage the populations of this pest insect in the sugarcane crop. Erper et al. (2016) point out that frequent rains and high humidity

throughout the year are ideal environmental conditions for the development of entomopathogenic fungi.

There are no studies in Pastaza province where the effect of native isolates of *Metarhizium* spp. in the control of the sugarcane weevil. Therefore, in order to evaluate the viability of the use of entomopathogenic fungi, which allows managing weevil populations in sugarcane crops in Pastaza province, the present work aimed to determine the efficacy of native isolates of *Metarhizium* spp. in the control of the weevil (*Metamasius hemipterus*) of sugarcane in laboratory conditions.

Materials and Methods

Study Sites

The study was conducted in the Microbiology laboratory of the Amazon State University. Inside the laboratory it was maintained at a temperature of 23 ± 1 oC and relative humidity of 80 ± 1%.

Collection of M. hemipterus adults

The methodology of Mendoza, Gómez and Gualle (2013) was used for the collection of *M. hemipterus* adults. To this end, traps were used, consisting of a plastic container of 4 L capacity, with a lid and two lateral openings of 8 x 9 cm. Inside, chopped cane was placed and each trap was distributed in the sugarcane crop at a distance between ten meter traps. From 5 to 8 days, the adults captured in the traps were collected and placed in glass containers for transfer to the laboratory.

Fungal isolates

Four native isolates of *Metarhizium* spp. were used in the study. The native isolates used were: TI6301 (obtained from nymph of Mahanarva andigena), TS6304, PS5002 and PS5003 (obtained from soil samples), which were multiplied in grains of precooked rice. Once colonized, a suspension of conidia with sterile distilled water + 0.1% Tween 80 was prepared and adjusted to a concentration of 1 x 10^8 conidia. mL^{-1} of each of the isolates.

Inoculation of fungal isolate

In the laboratory, the experimental unit consisted of 250 mL glass bottles containing filter paper, cotton moistened with sterile distilled water, a slice of sugarcane and 5 adults of *M. hemipterus*. 25 adults were used per treatment, distributed in a completely randomized design. For the conformation of the treatments, four native isolates of *Metarhizium* spp. described above, a chemical treatment composed of water solution + Engeo (1mL.L^{-1} water), a commercial biological treatment Micos plag (0.5 gL^{-1} water) + 0.1% Tween 80 and a control treatment with sterile distilled water + Tween 80 at 0.1%.

The adult weevils previously collected were superficially disinfected in 70% ethanol for 10 s and placed on autoclaved filter paper for drying (Castrillo et al., 2013). Subsequently the inoculation of each of the isolates of *Metarhizium* spp. It was carried out by the method of immersion of insects for 30 seconds in a Petri dish used by Mendoza, Gómez and Gualle (2013). After inoculation, the adult insects were confined in the glass jars with pieces of cane. Mortality records were made daily up to 15 days after inoculation.

The dead adults were superficially disinfected by soaking them in 0.1% sodium hypochlorite (NaOCl) for one minute, washed twice with sterile distilled water and placed in a humid chamber (Petri dish containing filter paper and cotton moistened with water sterile distilled) at a temperature of 27 ± 1 ^oC in the dark until fungal sporulation in order to determine the cause of mortality, with which the confirmed mortality was determined.

Statistical analysis

Adjustment was made to the data obtained, calculating the percentage of corrected mortality according to Abbott's formula (1925):

$$\% \text{ Corrected mortality} = \left(\frac{\% mt - \% mta}{100 - mta}\right) x100$$

Where: % mt = Percentage of treatment mortality; % mta = Percentage of mortality in the absolute control.

Variance Analysis (ANOVA) was performed to detect differences between treatments. In the comparison of means, the Tukey test, P <0.05, was used. The data was analyzed with the Insfostat version 2018 program.

Results and Discussion

The results showed that the chemical treatment gave an immediate response in adults of *M. hemipterus* with a 100% mortality efficiency before the first 24 hours, while the native isolates began to reflect mortalities from day two. Statistically treatments with native isolates of *Metarhizium* spp. did not reflect significant differences, only the native isolation TI6301 in numerical value obtained greater control action providing a mortality of 84% at five days, followed by the TS6304 treatment with 68% efficiency in the same time interval. 15 days after the end of the evaluation, treatment TI6301 increased its action on adults of *M. hemipterus* by 4%, ending with 88% mortality at the end of the trial (Table 1). The commercial biological treatment Micos Plag, in the trial had no entomopathogenic actions towards the pest, resulting in 8% mortality at 15 days.

Table 1. Percentage of adult mortality of *M. hemipterus* caused by the treatments of the native isolates of *Metarhizium* spp., Chemical and biological commercial at 5, 10 and 15 days of application.

TREATMENT	MORTALITY (%) DAY 5	MORTALITY (%) DAY 10	MORTALITY (%) DAY 15
CHEMICAL	100 a	100 a	100 a
TI6301	84 a	84 ab	88 ab
TS6304	68 ab	80 ab	80 ab
PS5002	28 bc	64 ab	76 ab
PS5003	32 bc	56 b	64 b
COMMERCIAL BIOLOGICAL (Micos plag)	0 c	4 c	4b c
CONTROL	4 c	4 c	8 c

Different letters in the same column indicate significant differences for Tukey (P< 0.05%) multiple range test.

In a study conducted by Ak (2019), it was determined that the TR-78-3 isolation of *M. anisopliae* caused a mortality of 90.35% on the weevil Sitophilus granarius (L.) at a concentration of $1x10^8$conidia.ml^{-1} at seven days of application. In the biossay, mortality began on the second day with constant deaths, reaching a maximum mortality rate of 88% 15 days after inoculation, values close to those referenced. These variations in insect mortality time and their effect on the insects under control may be related to the pathogenicity of each native isolation.

In another study Mendoza et al. (2013), showed that the most pathogenic *M. anisopliae* strains were those isolated from *Mahanarva andigena* and *Perkinsiella saccharicida*, which caused mortality of

91.7 and 90.0%, values slightly higher than those obtained in the study with the isolates TI6301 and TS6304.

On the other hand, Alvarado, Montes, Gomes, Bustillo and Mesa (2013), mention that the period of time to reach mortalities greater than 80% is 24 days, and the study obtained mortalities greater than 80% in the first 10 days with TI6301 and TS6304 native isolates. However, the commercial biological product did not show its maximum potential, which probably required more time to show its effectiveness.

From the mortality reflected in each of the treatments and by means of a correction with the mortality obtained in the absolute control by means of Abbott's formula, the efficacy of each of the isolates was determined. The treatments TS6304, TI6301, PS5002 and PS5003 showed no statistical differences between them, indicating in this analysis that the TI6301 isolation stands out numerically with 87.50% mortality, followed by TS6304 with 79.17% in terms of the treatment with the commercial biological Micos Plag obtained lower figures with 4.17% mortality, which suggests different biological activity possibly because it is a product imported into the country (Figure 1).

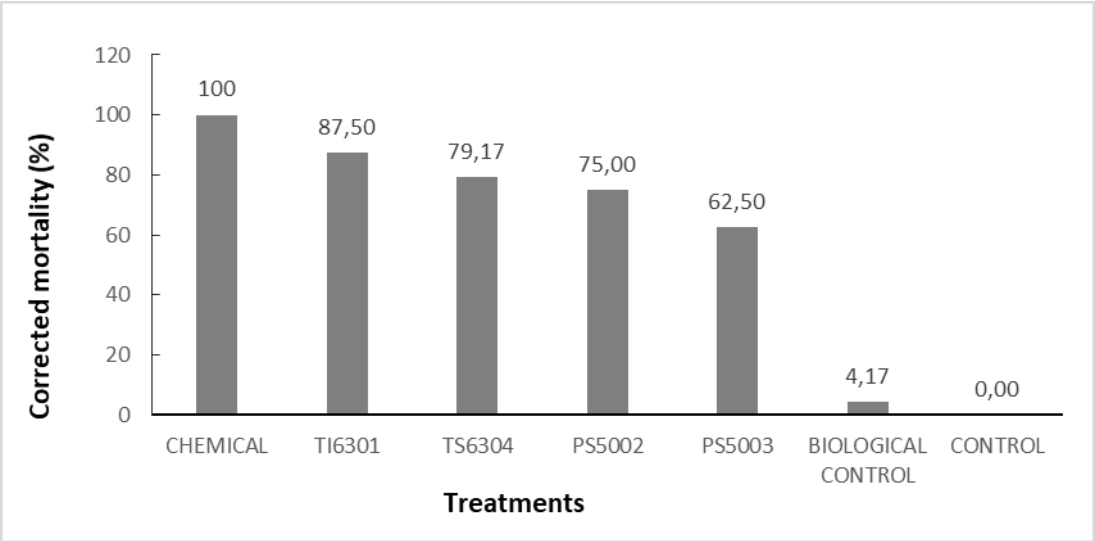


Figure 1. Corrected mortality *of M. hemipterus* adult caused by the treatments applied.

With respect to corrected mortality Barrios, Vieco, Barragán, Bustillo and Méndez (2016), indicate that three of the entomopathogenic fungi selected, caused a mortality greater than 90%, which reflect higher values with the results obtained in the investigation, since that three native isolates obtained mortality values of 75% to 87.50%.

The results of confirmed mortality in the treatments of *Metarhizium* spp., of the native isolates TI6301, TS6304, PS5002 and PS5003 statistically presented differences when contrasting with the control treatment, among which the TI6301 stands out numerically with 76% of mortality confirmed by the presence of dark green conidia on dead adults of *M. hemipterus*, followed by treatment TS6304 with 72.4% confirmed mortality.

Table 2. Confirmed mortality in adults of *M. hemipterus* caused by the native isolates of *Metarhizium* spp. fifteen days after application.

TREATMENT	CONFIRMED MORTALITY (%)
TI6301	76,0 a
TS6304	72,4 a
PS5002	64,0 a
PS5003	60,0 a
CONTROL	0,0 b
E.E.	11,56

In a study conducted by Kushiyev et al. (2018) showed that the isolation of *M. anisopliae* TR-106 against the weevil *Sitophilus granarius* (L.) caused 100% mortality and approximately 95% mycosis on dead insects after 8 days of application, which they are superior to those obtained in our study where 76% of confirmed mortality was determined by identifying the sporulation of fungi when manifesting dark green conidia characteristic of *Metarhizium*.

In another study conducted by Tuncer et al. (2019) showed that all applications of the isolation of *M. anisopliae* TR-106 caused approximately 100% mycosis rate in adults killed by *Xylosandrus germanus* beetles (Blandfort) eight days after application.

On the other hand, Thaochan & Sausa (2017) point out that the entomopathogenic fungus *M. anisopliae* is the most notable that inhabits the soil with high capacity for controlling a large number of species of insect pests of plants and animals, which are covered by mycelium, which is initially white, but that turns green once the fungus sporulates. Tuncer et al. (2019) mention that the use of this fungus can be effective when applied directly to insect pests or when they are later acquired by insects from the treated surface; therefore, billflies, which spend most of their life cycles within the host, are ideal targets for this fungus.

Conclusions

Under laboratory conditions the native isolates of *Metarhizium* spp. TI6301, TS6304 and PS5002 caused accumulated and corrected mortalities above 70%, and confirmed mortalities of 72.4 and 76% with native isolates TS6304 and TI6301, respectively on *M. hemipterus* adults. Which indicates that these two isolates are promising agents of biological control of this pest insect in terms of practical application according to the results obtained from similar studies.

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