Short note [Nota corta]

NYMPH CONTROL OF *Mahanarva andigena* WITH *Metarhizium anisopliae* IN GREENHOUSE AND FIELD CONDITIONS IN PASTAZA, ECUADOR[1]

[CONTROL DE NINFAS DE *Mahanarva andigena* CON *Metarhizium anisopliae* EN CONDICIONES DE INVERNADERO Y CAMPO EN PASTAZA, ECUADOR]

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SUMMARY

The efficacy of four native isolations of *Metarhizium anisopliae* in the control of *Mahanarva andigena* nymphs in greenhouse and field was evaluated. In both conditions the treatments were: four native T1 isolations: MT-51 (01), T2: P-50 (01), T3: T-63 (01) and T4: F55 (01), a commercial strain T5: Metazeb and T6 control treatment. In a greenhouse, 30 days old sugar cane seedlings were used, each one was infested with 10 fourth instar nymphs and subsequently sprinkled with 15 mL of a suspension of $1x10^8$ conidia/mL^{-1} for each isolation, the commercial strain at a dose of $2x10^{12}$ conidia/ha^{-1} (300 g of commercial product/ha^{-1}) and the control treatment (sterile distilled water + agral 90). In greenhouse mortality was recorded for seven days and the corrected and confirmed mortality was determined. In the field, the treatments were sprayed at the same concentrations. The effectiveness of the treatments was determined 7 and 14 days after the application, with the Henderson-Tilton formula. In the greenhouse treatments, T1, T2 and T3 caused high percentages of confirmed mortality, with values of 71.11, 67.5 and 54.45%, respectively, higher than the commercial strain, and in the field at 14 days, the application of treatments T1 and T2, reached an efficiency of 73.08 and 69.68%, higher than other treatments. These results demonstrate that the T1 isolations: MT-51 (01) and T2: P-50 (01) in greenhouse and field induced a high mortality of *M. andigena* nymphs, so they are the most promising for use as control biological agents of this insect pest in the cultivation of sugar cane.

Keywords: nymphs, native isolations, sugar cane, confirmed mortality.

RESUMEN

Se evaluó la eficacia de cuatro aislamientos nativos de *Metarhizium anisopliae* en el control de ninfas de *Mahanarva andigena* en invernadero y campo. En ambas condiciones los tratamientos fueron: cuatro aislamientos nativos T1: MT-51(01), T2: P-50(01), T3: T-63(01) y T4: F55(01), una cepa comercial T5: Metazeb y un tratamiento control T6. En invernadero se emplearon plántulas de caña de azúcar de 30 días de edad, cada una fue infestada con 10 ninfas de cuarto instar y posteriormente fueron asperjadas con 15 mL de una suspensión de $1x10^8$ conidios/mL^{-1} por cada aislamiento, la cepa comercial a una dosis de $2x10^{12}$ conidios/ha^{-1} (300 g de producto comercial/ha^{-1}) y el tratamiento control (agua destilada estéril + agral 90). En invernadero la mortalidad se registró durante siete días y se determinó la mortalidad corregida y confirmada. En campo se asperjaron los tratamientos a las mismas concentraciones. La eficacia de los tratamientos se determinó a los 7 y 14 días después de la aplicación, con la fórmula de Henderson-Tilton. En invernadero los tratamientos T1, T2 y T3, provocaron altos porcentajes de mortalidad confirmada, con valores de 71.11, 67.5 y 54.45%, respectivamente, superiores a la cepa comercial, y en campo a los 14 días, de la aplicación los tratamientos T1 y T2, alcanzaron una eficacia de 73.08 y 69.68%, superiores a los demás tratamientos. Estos resultados demuestran que los aislamientos T1: MT-51(01) y T2: P-50(01) en invernadero y campo indujeron

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una alta mortalidad de ninfas *M. andigena*, por lo que constituyen los más promisorios para uso como agentes de control biológico de este insecto plaga en el cultivo de caña de azúcar.

Palabras clave: ninfas, aislamientos nativos, caña de azúcar, mortalidad confirmada

INTRODUCTION

The sugar cane cultivation is affected by the high incidence of spittlebug (*Mahanarva andigena* Jacobi: Hemiptera: Cercopidae) in the province of Pastaza, which has caused large losses in agricultural yield (Valle, 2015). In the case of this pest, the most significant damage is done by the nymphs in their aerial habits, these are located in the buds and leaf sheaths (Rodríguez and Peck, 2007), directly attacking the leaf area, which directly affects photosynthesis and other metabolic processes of the plant, damaging the quality of raw materials for industrial processing, as well as its commercial value (Korndörfer *et al*., 2011). On average, they reach population levels of up to 52 nymphs per stem at times of greatest infestation, which corresponds to the months of March and April, a value that exceeds the economic damage threshold of 3 nymphs per stem (Valle *et al*., 2015). The high infestation cane lots show a yellowing of the foliage, similar to the effect of the application of a herbicide, which with the course of time dry (Obando *et al.*, 2013).

The chemical control of this pest is widely used in sugar cane, through the use of Thiamethoxam, Aldicarb, Carbofuran and Imidalcloprid, significantly reducing infestations of the pest in the sugarcane crop (Barbosa *et al.*, 2015). However, chemical control has some disadvantages compared to biological control such as the possibility of resurgence of the pest, generation of resistant insects, human and environmental contamination, which has motivated the search for ecological strategies to control these insects, with isolated of native *Metarhizium*, due to the fact that in other countries such as Brazil, they have demonstrated that the mycoinsecticides based on *Metarhizium anisopliae* have been effective in the control of this pest (Rezende *et al.*, 2015).

The insects colonized by *M. anisopliae* remain in the field, these infect healthy individuals, which reduces the possibility of resurgence of these pests in sugarcane plantations (Kassab *et al*., 2015), but does not affect the enemies of this plague such as the wasp *Salpingogaster nigra* (Schiner) and the predatory ant *Pheidole genalis* (Borgmeier) (Tiago *et al*., 2011). Therefore, the objective of this study was to determine the efficacy of native *Metarhizium anisopliae* isolations in the control of *Mahanarva andigena* nymphs under greenhouse and field conditions in the province of Pastaza.

MATERIALS AND METHODS

Location

The greenhouse experiment was conducted in the city of Puyo, Province of Pastaza, Ecuador, under conditions of average temperature of 23.72 ± 2.09 oC and relative humidity of 80.95 ± 5.34. In the field, the experiment was carried out in a farm with clay loam soil, located at Km 9 via Puyo - Napo (Coordinates UTM X: 166276, Y: 9843842, altitude 1 025 m)

Obtaining nymphs of *Mahanarva andigena* and isolations of *Metarhizium anisopliae*

In the study, fourth instar nymphs were used, collected on a farm with sugar cane cultivation POJ 93, located in the parish of Lieutenant Hugo Ortiz, province of Pastaza, with no history of application of chemical products.

Six treatments were used; four native T1 isolations: MT-51 (01), T2: P-50 (01), T3: T-63 (01), T4: F55 (01) previously validated and conserved in the Microbiology laboratory of the Amazon State University , a commercial strain T5: Metazeb and a T6 control treatment: sterile distilled water + agral 90 in a proportion of 0.5 mL/L^{-1}.

The inoculum production of the four native isolations was carried out on solid substrate (whole rice), according to the methodologies of Fanti and Alves (2013).

Application and evaluation

In the greenhouse, each seedling, once infested with 10 nymphs collected in the field, was sprinkled with 15 mL of a suspension containing $1x10^8$ conidia/mL^{-1} for each isolation (Torres *et al.*, 2013), the commercial strain at the recommended dose and the control treatment (sterile distilled water + agral 90 in proportion of 0.5 mL/L^{-1}), later it was covered with a transparent plastic cylindrical chamber (70 cm high and 45 cm in diameter), with two opposite lateral windows of 12 x 10 cm coated with antiaphid mesh. The application of the suspensions was done with manually calibrated sprayers, according to the methodology of Obando *et al.* (2013). Mortality was recorded daily, for seven days, with these data the corrected mortality was determined by the Schneider-

Orelli formula (Ciba-Geigy, 1981) and confirmed mortality. To determine the latter, each dead individual was placed in a humid chamber in order to verify the growth of the fungus on them.

For the application in the field, the preparation of the conidial suspensions of the isolations was carried out according to the methodology of Loureiro *et al.* (2012). The substrate colonized by the fungus was washed in water by the use of 20 L transparent plastic containers. The resulting broth was filtered in the backpack pump tank (20 L Jacto®) calibrated for each isolation. Once the suspensions of each treatment were prepared at the concentrations of the previous experiment, the application directed to the foliage and bud of the stems of sugar cane was carried out.

Prior to the application of the treatments, a population assessment of nymphs and adults of the insect was carried out. This same evaluation was performed 7 and 14 days after the treatments were applied, accounting for nymphs and adults, living and dead (Matabanchoy *et al.*, 2012), in 10 stems of the center of each experimental plot. The effectiveness of the treatments was calculated using the Henderson-Tilton formula (Ciba-Geigy, 1981).

Design and statistical analysis

The greenhouse test was organized according to a completely randomized design, with six treatments and five replications. Each replication was made up of a pot, with a 30 days old sugar cane seedling infested with 10 fourth instar nymphs for a total of 50 nymphs per treatment.

In the field, the design and treatments were similar to those applied under greenhouse conditions. Each experimental plot was made up of 4 grooves of 10.50 m in length, spaced at 2 m between rows, representing a total area of 84 m^2; plot size used by Barbosa *et al.* (2011). 3 m separation between plots was left. The work and general management of cultivation are carried out according to the traditional methods used by the producers of sugarcane in the area.

To compare the efficacy of the treatments, a simple classification variance analysis was performed and the means were compared using the Tukey test ($p \leq 0.05$), after transformation of the data through the expression arcose$\sqrt{x}$ / 100, with the INFOSTAT 2015 Version program.

RESULTS

At six days the highest corrected mortality of *M. andigena* nymphs under greenhouse conditions was observed in the T1 treatments: MT-51 (01), T2: P50 (01) and T3: T-63 (01), with values which ranged from 86.14, 72.60 and 68.48 respectively ($P < 0.05$) (Table 1). Regarding the confirmed mortality, from the sixth day it was possible to show that the nymphs that had infection by the fungus stopped producing the protective foam and showed a cream to white appearance. And the next day, the white mycelium was observed on its body. The T1 treatments: MT-51 (01), T2: P-50 (01) and T3: T-63 (01) caused the highest ($P < 0.05$) percentages of confirmed mortality with values of 71.11, 67.50 and 54.45%, respectively (Table 1). When evaluating the effectiveness of the treatments in the field, it was determined that the T1 treatments: MT-51 (01) and T2: P-50 (01) showed higher ($P < 0.05$) efficiency in the control of nymphs (55.4 and 51.5% and 73.0 and 69.6% at seven and fourteen days respectively) with respect to the other treatments (Table 1).

DISCUSSION

Regarding efficacy in the control of nymphs, Kassab *et al.* (2012) observed that isolations of *M. anisopliae* (BIO 08 and IBCB 425) showed an efficacy between 73.33 and 70.6% in the control of *M. fimbriolata*, 15 days after application, results similar to those obtained in this study with the T1 isolations: MT-51 (01) and T2: P-50 (01), this tendency may be influenced by the high capacity of infection of *M. anisopliae* (Freitas *et al*., 2012). This was shown previously by Barbosa *et al*. (2011) when evaluating the efficacy of *M. anisopliae* isolations, found an efficiency of 83% in the control of nymphs of *M. fimbriolata*. On the other hand, Kassab *et al.* (2014), when applying a commercial strain (Meitê®) of *M. anisopliae* at a dose of $3x10^{12}$ conidia/ha^{-1}, they obtained an efficacy of 50% on the control of nymphs of *M. fimbriolata*, 15 days after application. With the use of native isolations, greater efficiency is obtained, probably due to the adaptability and resistance to the adafoclimatic conditions of each zone (Loureiro *et al.*, 2012).

When evaluating the efficacy in the control of *Aeneolamia nymphs*, Matabanchoy *et al.* (2012) and Torres *et al.* (2013) showed that the native isolations of *M. anisopliae* exert better control in relation to commercial strains. It is possible that the favorable microclimatic conditions, existing in the study area of high precipitation and relative humidity, have contributed to a greater efficiency of the T1 isolations: MT-51 (01) and T2: P-50 (01). In this regard, Kassab *et al.* (2015) observed a higher parasitism of nymphs in the field when precipitation, temperature and relative humidity increased. Likewise, Loureiro *et al.* (2012) noted that temperatures between 25 and 28 ºC, and high relative humidity are optimal for the mycelial growth of *M. anisopliae*. Barbosa *et al.* (2011) pointed out that the

fungus *M. anisopliae* should be used for the specific control of nymphs, taking into account the favorable climatic factors, for an efficient control. Moreover, Carvalho *et al.* (2011) pointed out that *M. anisopliae* acts mainly on the nymphs, whereas in the adult state, there are restrictions due to the difficulty of penetration into the tegument of the insect. Likewise, the foam produced by the nymphs provides favorable moisture conditions for the growth of the fungus (Freitas *et al*., 2014).

Table 1. Mortality and efficacy (%) of *Metarhizium anisopliae* on *M. andigena nymphs* in greenhouse and field

	Corrected mortality	Confirmed mortality	Efficacy in field after application	
			7 days	14 days
T1	86.14 a	71.10 a	55.43 a	73.08 a
T2	73.08 ab	67.50 a	51.56 a	69.68 a
T3	68.48 abc	54.45 ab	40.20 b	57.07 b
T4	49.03 bc	26.90 bc	34.50 b	45.38 b
T5	31.03 c	16.60 cd	21.65 c	26.12 c
T6	0.00 d	0.00 d	0.00 d	0.00 d
EEM	0.07	0.08	0.06	0.08
Valor P	P<0.0023	P<0.0001	P<0.0001	P<0.0001

Means with different letters between columns indicate significant differences p≤0.05. T1: MT-51 (01), T2: P-50 (01), T3: T-63 (01), T4: F-55 (01), T5: Metazeb, T6: Control

CONCLUSIONS

The native isolations of *Metarhizium anisopliae* T1: MT-51 (01) and T2: P-50 (01) in greenhouse and field induced a high mortality of *M. andigena* nymphs, so they are the most promising for use as control agents of this insect pest in the sugarcane crop in climatic conditions similar to those of Pastaza, Ecuador.

REFERENCES

Barbosa, R., Kassab, S., Fonseca, P.R., Rossoni, C., Silva, A. 2011. Associação de *Metarhizium anisopliae* (Hyp.: Clavicipitaceae) e Thiamethoxam para o controle da cigarrinha-dasraízes em cana-de-açúcar. Ensaios e Ciência: Ciências Biológicas, Agrárias e da Saúde. 15: 41-51.

Barbosa, R., Kassab, S., Pereira, F., Rossoni, C. 2015. Controle químico e biológico de *Mahanarva fimbriolata* Stål, 1854 (Hemiptera: Cercopidae) para regiões produtoras de cana-de-açúcar de Mato Grosso do Sul. Ambiência.11: 247 – 255.

Carvalho, L.W., Micheletti, S.M., Carvalho, L.H., Dias, N.S., Pérez, K.G. 2011. Incidencia de *Mahanarva fimbriolata* después de aplicaciones de *Metarhizium anisopliae* e Imidacloprid en caña de azúcar. Revista Caatinga. 24: 20-26.

Ciba Geigy. 1981. Manual de ensayos de campo en producción vegetal, 2.a ed. Basilea, Suiza. pp 211.

Fanti, A.L., Alves, L.F. 2013. Isolados de fungos entomopatogênicos visando ao controle da broca da erva-mate (*Hedypathes betulinus*) Klug (Coleoptera; Cerambycidae). Semina: Ciencias Agrarias. 34: 1467-1478. DOI: 10.5433/1679-0359.2013v34n4p1467

Freitas, A.F., Loureiro, E.S., Almeida, M.E., Pessoa, L.G. 2012. Seleção de isolados de *Metarhizium anisopliae* (Metsch.) Sorok. (Deuteromycotina: Hyphomycetes) para o controle de *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera:Cercopidae) em cana-de-açúcar. Arq. Inst. Biol. 79: 247-254.

Freitas, A.F., Loureiro, E., Barbosa, M.E., Pessoa, L.G. 2014. Rendimento de conídios e germinação de diferentes isolados de *Metarhizium anisopliae* (Metsch.) Sorok. (Ascomycota: Clavicipitaceae) cultivados em arroz. Arq. Inst. Biol. 81: 75-78.

Kassab, S.O., Loureiro, E.S., Barbosa, R.H., Da Fonseca, R.B., Mota, T.A., Rossoni, C. 2012. Alteração no método de amostragem de *Mahanarva fimbriolata* (Stål, 1854) (Hem.: Cercopidae) e avaliação da eficiência de *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883 (Hyp.: Clavicipitaceae). Arq. Inst. Biol. 79: 621-625.

Kassab, S.O., Loureiro, E.S., Rossoni, C., Pereira, F. F., Barbosa, R.H., Costa, D.P., Zanuncio, J.C. 2014. Combinations of *Metarhizium anisopliae* with chemical insecticides and their effectiveness in *Mahanarva fimbriolata* (Hemiptera: Cercopidae) control on sugarcane. Florida Entomologist. 97: 146-154. DOI: http://dx.doi.org/10.1653/024.097.0120

Kassab, S.O., Loureiro, E., Rossoni, C., Pereira, F.F., Mota, T.A., Barbosa, R.H., Costa D.P. 2015. Control de *Mahanarva fimbriolata* (Stal)

(Hemiptera: Cercopidae) with entomopathogenic fungus and insecticides using two sampling methods on sugarcane fields. African Journal of Agricultural Reseach. 10: 803-810.

Korndörfer, A.P., Grisoto, E., Vendramim, J.D. 2011. Induction of Insect Plant Resistance to the Spittlebug *Mahanarva fimbriolata* Stål (Hemiptera: Cercopidae) in Sugarcane by Silicon Application. Neotropical Entomology.40: 387-392.

Loureiro, E.S., Filho, A.B., Almeida, J.E., Mendes, J. M., Pessoa, L. G. 2012. Eficiência de isolados de *Metarhizium anisopliae* (metsch.) Sorok. No controle da cigarrinha-da-raiz da cana-de-açúcar, *Mahanarva fimbriolata* (stal, 1854) (Hemiptera: Cercopidae), em condições de campo. Revista Arq. Inst. Biol. 79: 47-53.

Matabanchoy, J.A., Bustillo, A.E., Castro, U., Mesa, N.C., Moreno, C.A. 2012. Eficacia de *Metarhizium anisopliae* para controlar *Aeneolamia varia* (Hemiptera Cercopidae), en caña de azúcar. Revista Colombiana de Entomología. 38: 177-181.

Obando, J.A., Bustillo, A.E., Castro, U., Mesa, N.C. 2013. Selección de cepas de *Metarhizium anisopliae* para el control de *Aeneolamia varia* (Hemiptera: Cercopidae). Revista Colombiana de Entomología. 39: 26-33.

Rezende, J.A, Riguetti, A.B., Lopes, M., Delalibera, I., Rehner, S.A. 2015. Phylogenetic diversity of Brazilian *Metarhizium* associated with sugarcane agricultura. BioControl. 60: 495–505. DOI:10.1007/s10526-015-9656-5

Rodríguez, J.C., Peck, D.C. 2007. Biología y hábitos de *Mahanarva andigena* (Hemiptera: Cercopidae) en condiciones de casa de malla. Revista Colombiana de Entomología. 33: 31-35.

Tiago, P.V., Souza, H.M., Moysés, J.B., Oliveira, N. T., Lima, E. A. 2011. Differential pathogenicity of *Metarhizium anisopliae* and the Control of the Sugarcane Root Spittlebug *Mahanarva fimbriolata*. Brazilian Archives of Biology and Technology. 45: 435-440.

Torres, M., Cortez, H.M., Ortiz, C.F., Capello, S., De la Cruz, A. 2013. Caracterización de aislamientos nativos de *Metarhizium anisopliae* y su patogenicidad hacia *Aeneolamia postica*, en Tabasco, México. Revista Colombiana de Entomología. 39: 40-46.

Valle, S., Iparrguirre, M., Puertas, A., Rodríguez, S., Fiallos, A., Hdalgo, L., Miranda, I. 2015. Evaluación de dos métodos de monitoreo de *Mahanarva andigena* Jacobi en el cultivo de la caña de azúcar (*Saccharum* spp., híbrido) en la provincia de Pastaza, Ecuador. Revista Protección Vegetal. 30: 185-192.

Valle, S. 2015. Particularidades bioecológicas de *Mahanarva andigena* (Jacobi) como base para el manejo con *Metarhizium anisopliae* (Metschnikoff) Sorokin en la caña de azúcar *Saccharum* spp. híbrido en Pastaza, Ecuador (Tesis doctoral). Universidad de Granma, Cuba. pp 118.