# Combinations of Metarhizium anisopliae with Chemical Insecticides and their Effectiveness in Mahanarva fimbriolata (Hemiptera: Cercopidae) Control on Sugarcane

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# COMBINATIONS OF *METARHIZIUM ANISOPLIAE* WITH CHEMICAL INSECTICIDES AND THEIR EFFECTIVENESS IN *MAHANARVA FIMBRIOLATA* (HEMIPTERA: CERCOPIDAE) CONTROL ON SUGARCANE

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Abstract

Some insecticides can be used jointly with entomopathogenic fungi, and therefore the combination of chemical and biological control measures can be a safe and effective method to control insect pests. The aim of this study was to evaluate the costs and efficacy of combinations of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) with thiamethoxam and imidacloprid on spittlebug (*Mahanarva fimbriolata* (Stål); Hemiptera: Cercopidae) control on sugarcane. The experiment was conducted as a randomized block design (RBD) with 10 treatments and 4 replications. The treatments included a control (untreated), thiamethoxam (250 g $ha^{-1}$), imidacloprid (700 g $ha^{-1}$), *M. anisopliae* (*M. a.*) ($3 \times 10^{12}$ conidia $ha^{-1}$), A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65 g $ha^{-1}$ of thiamethoxam), A2 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 125 g $ha^{-1}$ of thiamethoxam), A3 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 187.5 g $ha^{-1}$ of thiamethoxam), A4 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 175 g $ha^{-1}$ of imidacloprid), A5 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 350 g $ha^{-1}$ of imidacloprid), and A6 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 525g $ha^{-1}$ of imidacloprid). The reductions in the numbers of *M. fimbriolata* nymphs per treatment compared to the control were similar at 15 DAT (days after treatment) in all treatments except combination A5 (*M. anisopliae* and thiamethoxam). At 30 DAT, the numbers of nymphs were significantly reduced in all treatments except A3, and their effectiveness ranged from 14.28% to 92.85%. At 45 DAT the numbers of *M. fimbriolata* nymphs per treatment were significantly reduced in the following treatments: imidacloprid alone at 700g $ha^{-1}$, A1, A2, A3, A4 and A6; and the combinations A1 and A2 caused the lowest *M. fimbriolata* nymph infestations and effectiveness rates of 77.41 and 87.09 %, respectively. At 75 DAT the 2 best control efficacies occurred in treatments A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ of + 65g $ha^{-1}$ of thiamethoxam) (82.1%) and A5 (78.6%) ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 350 g $ha^{-1}$of imidacloprid). At 90 DAT the number of nymphs in the control had increased 2.8 fold over the number at 75 DAT. Very good control efficacies at 90 DAT occurred in all treatments with the combination of the fungus with an insecticide. At 105 DAT the numbers of nymphs had surged in all treatments, and no treatment provided effective control. The treatments with the highest earnings per hectare were A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65 g thiamethoxam) and *M. anisopliae* alone at the recommended dose of $3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$. Our findings demonstrate the effectiveness of using either thiamethoxam or imidacloprid in combination with *M. anisopliae* to control *M. fimbriolata* nymphs on sugarcane, but greater net earnings per hectare occurred with the lowest rate of the thiamethoxam combination than with any of the imidacloprid combinations.

Key Words: biological control, entomopathogenic fungi, imidacloprid, sugarcane, thiamethoxan.

Resumen

Algunos insecticidas se puede utilizar con hongos entomopatógenos y por lo tanto, la asociación de los controles químico y biológico puede ser una estrategia segura y eficaz para el control de insectos-plaga. El objetivo de este estudio fue evaluar los costos y eficacia de combinaciones de *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) con insecticidas thiamethoxam e imidacloprid para el control de la chicharrita (*Mahanarva fimbriolata* (Stål); Hemiptera: Cercopidae) en caña de azúcar . El experimento fue conducido en un delineamiento en bloques casualizados (DBC), con 10 tratamientos y 4 repeticiones. Los tratamientos que incluidos el control (sin tratamiento), thiamethoxam (250 g ha$^{-1}$), imidaclopride (700 g ha$^{-1}$), *M. anisopliae* (*M.a.*) (3×10$^{12}$ conidios ha$^{-1}$), A1 (3×10$^{12}$ conidios ha$^{-1}$de *M. a.* + 65 g ha$^{-1}$ de thiamethoxam), A2 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.* + 125g ha$^{-1}$ de thiamethoxam), A3 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.* + 187.5 g ha$^{-1}$ de thiamethoxam), A4 (3×10$^{12}$ conidios ha$^{-1}$ de *M.a* + 175 g ha$^{-1}$ de imidacloprido), A5 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.* + 350 g ha$^{-1}$de imidacloprido) y A6 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.* + 525g ha$^{-1}$ de imidacloprido). Las reducciones en el número de ninfas *M. fimbriolata* por tratamiento en comparación con el control fueron similares a los 15 DAT (días pos tratamiento) en todos los tratamientos excepto A5 combinación (*M. anisopliae* y thiamethoxam). A los 30 DAT, el número de ninfas se redujeron significativamente en todos los tratamientos, excepto A3, y su eficacia varió de 14,28% para 92,85%. A los 45 DAT, los números de ninfas *M. fimbriolata* por tratamiento se redujeron significativamente en los siguientes tratamientos: imidacloprido solo en 700 g ha$^{-1}$, A1, A2, A3, A4 y A6; y las combinaciones de A1 y A2 causaron la más bajo infestaciones de ninfas *M. fimbriolata* y sus tasas de eficacia fueron de 77,41 y 87,09%, respectivamente. A los 75 DAT, los 2 mejores eficacias de control se produjeron en tratamientos A1 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.* + 65 g ha$^{-1}$ de thiamethoxam) y A5 (78.6%) (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.*+ 350 g ha$^{-1}$ de imidacloprido). A los 90 DAT, el número de ninfas en el control había aumentado 2,8 veces más el número a 75 DAT. Muy buenas eficacias de control en 90 DAT, se produjo en todos los tratamientos con la combinación del hongo con un insecticida. A los 105 DAT, el número de ninfas habían aumentado en todos los tratamientos, y ningún tratamiento había proporcionado un control efectivo. Los tratamientos con los mayores rendimientos hectárea fueron A1 (3×10$^{12}$ conidios ha$^{-1}$ de *M. a.*+ 65 g de thiamethoxam) y *M. anisopliae* solo a la dosis recomendada de 3×10$^{12}$ conidios ha$^{-1}$ de *M. a*. Nuestros resultados demuestran la eficacia de thiamethoxam y imidacloprid en combinación con *M. anisopliae* para el control de ninfas *M. fimbriolata* en caña de azúcar, pero mayores beneficio neto por hectárea se produjeron con la tasa más baja de la combinación de thiamethoxam que con cualquiera de las combinaciones de imidacloprid.

Palabras clave: caña de azúcar, control biológico, hongos entomopatógenos, imicacloprido, thiamethoxam.

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Brazil produces the most sugarcane (Ravaneli et al. 2011; Vacari et al. 2012; Simões et al. 2012; Rossato et al. 2013), and *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is one of the major pests of this crop (Garcia et al. 2006; James et al. 2011; Garcia et al. 2011; Volpe et al. 2012). Nymphs and adults of *M. fimbriolata* can cause injuries and reduce of sugarcane productivity by 20 to 40 tonnes per hectare (Mendonça 2005). Furthermore, attacked sugarcane stalks lose quality, reducing sugar and alcohol production capacity (Dinardo-Miranda et al. 2002; Madaleno et al. 2008; Garcia et al. 2010; Carvalho et al. 2011; Korndörfer et al. 2011).

Chemical insecticides and the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae), are used to control *M. fimbriolata* nymphs and adults on sugarcane (Dinardo-Miranda et al. 2004 a, 2004 b; Loureiro et al. 2005; Li et al. 2010; Cuarán et al. 2012). Some chemical insecticides that are compatible with entomopathogenic fungi and other biological control agents may be used in various combinations to provide safe and efficient control of insect pests (Asi et al. 2010; Russell et al. 2010; Bitsadze et al. 2013).

The insecticides, imidacloprid and thiamethoxam, do not reduce the viability, vegetative growth, conidial production, and germination of *M. anisopliae* and this demonstrates the compatibility of these insecticides with this entomopathogen (Botelho & Monteiro 2011; Akbar et al. 2012; Silva et al. 2013).

Thus, the combination of chemical insecticides and *M. anisopliae* can used for the management of *M. fimbriolata*. The insecticide causes insect death in less time, and colonization of individuals killed by the entomopathogenic fungus increases the residual pest control effect (Dinardo-Miranda et al. 2008; Jin et al. 2011). This demonstrates the importance of understanding the combination of entomopathogenic fungi with insecticides in sugarcane fields.

The aim of this study was to evaluate the costs and efficacies of combinations of *M. anisopliae* with imidacloprid and thiamethoxam in *M. fimbriolata* control on sugarcane.

## Materials and Methods

The pest control materials used in this study were as follows: *Metarhizium anisopliae* (Meitê®) procured from Ballagro Agro Tecnologia, Bom Jesus dos Perdões city, São Paulo state, Brazil; thiamethoxam (Actara 250 WG®) obtained from

Syngenta Proteção de Cultivos Ltda, Paulínia city, São Paulo state, Brazil; and imidacloprid (Evidence 700 WG®) obtained from Bayer Crop Science, São Paulo city, São Paulo state, Brazil.

The experiment was conducted in a sugarcane field of the company "Energética Santa Helena Ltda" in Nova Andradina, Mato Grosso do Sul State, from Nov 2012 to Apr 2013. The experimental area (S 22° 16' 73" W 53° 18' 23", and 380 m) was planted with sugarcane (variety 'SP81-3250') with no flaws in the sprouting plants.

The plots included 10 rows of sugarcane spaced 1.4 m apart and 10 m long, totaling an areas of 140 $m^2$. The experiment was a randomized block design (RBD) with 10 treatments and 4 replications. The treatments included the control (untreated), thiamethoxam (250 g $ha^{-1}$), imidacloprid (700 g $ha^{-1}$), *M. anisopliae* (*M. a.*) ($3 \times 10^{12}$ conidia $ha^{-1}$), A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65 g $ha^{-1}$ of thiamethoxam), A2 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 125 g $ha^{-1}$ of thiamethoxam), A3 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 187.5 g $ha^{-1}$ of thiamethoxam), A4 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 175 g $ha^{-1}$ of imidacloprid), A5 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 350 g $ha^{-1}$ of imidacloprid), and A6 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 525 g $ha^{-1}$ of imidacloprid).

The experiment was started on 23 Nov 2012, when the level of infestation of *M. fimbriolata* in the experimental area reached on averaged 3.82 ± 0.23 nymphs (average ± standard error) per linear foot (30.4 cm) of furrow of sugarcane (Mendonça 2005; Dinardo-Miranda et al. 2008; Table 1). Since the application of mixtures of insecticides is prohibited in Brazil, each product was applied with a separate Jacto® sprayer (Pompéia city, São Paulo State, Brazil) that was calibrated to apply 150 L $ha^{-1}$ (Mendonça 2005). The spray was directed at the base of the stumps such that 30% of the spray volume reached the stems and 70% reached the sugarcane plant roots (Loureiro et al. 2005). The surfactant Tween® (0.01% polysorbate 80) was used in the treatments of fungal suspensions.

Nymphs of *M. fimbriolata* were sampled every 2 weeks, up to 105 days after treatment (DAT) (Mendonça 2005) in 2 linear feet (60.8 cm) of furrow planting of sugarcane in each plot. The nymphs of *M. fimbriolata* on the basal internodes of plants were counted after removal of the residual straw.

Climate data (average temperature, relative humidity, and rainfall) were obtained from Inmet (Instituto Nacional de Meteorologia) (Fig. 1) to establish the relationship between the infestation of *M. fimbriolata* and abiotic factors. The value of total recoverable sugar (TRS) (Landell et al. 1999) was obtained from 20 sugarcane stalks removed randomly per treatment on 15 Apr 2013.

UDOP (2013) provided the value of a tonne of sugar per ha, the estimated yield (total recoverable sugar, TRS) per ha of 68 tonnes, which is the average yield of Mato Grosso do Sul, State (Unica 2013) × value of a tonne per ha], maintenance cost of sugarcane (MCS) excluding the costs of pest control materials and their application for the control of *M. fimbriolata* (Udop 2013), costs of pest control materials and their application and earnings per ha [(the estimated value of the TRS produced per ha) – MCS – control cost)], were calculated in dollars (US$). The values of outsourced services and purchased products to manage the sugarcane field and to control *M. fimbriolata* were obtained from consulting firms and agricultural database of Udop (2013) (Table 2).

The population data for nymphs of *M. fimbriolata* were subjected to analysis of variance, and the significant means were compared by the Scott–Knott test at 5% probability. The efficacy of these treatments was calculated using Abbott's formula (Abbott 1925).

## Results

Table 3 shows that at 15 DAT the number of nymphs were significantly reduced compared to

Table 1. Pretreatment infestation by the spittle bug *Mahanarva fimbriolata* (Hemiptera: Cercopidae) on sugarcane in the experimental area.

| Treatments | Nymphs (average ± standard error) |
|---|---|
| Control (untreated) | 4.50 ± 0.88 |
| Thiamethoxam (250 g $ha^{-1}$) | 3.75 ± 1.19 |
| Imidacloprid (700 g $ha^{-1}$) | 3.00 ± 0.95 |
| *Metarhizium anisopliae* (*M.a*) ($3 \times 10^{12}$ con $ha^{-1}$) | 3.25 ± 0.18 |
| A1 (Rec. Dose of *M. a.* + 65 g $ha^{-1}$ of Thia.) | 4.75 ± 0.62 |
| A2 (Rec. Dose of *M. a.* + 125 g $ha^{-1}$ of Thia.) | 4.00 ± 1.28 |
| A3 (Rec. Dose of *M. a.* + 187.5 g $ha^{-1}$ of Thia.) | 3.50 ± 0.75 |
| A4 (Rec. Dose of *M. a.* + 175 g $ha^{-1}$ of Imida.) | 3.75 ± 1.12 |
| A5 (Rec. Dose of *M. a.* + 350 g $ha^{-1}$ of Imida.) | 3.50 ± 0.68 |
| A6 (Rec. Dose of *M. a.* + 525 g $ha^{-1}$ of Imida.) | 4.25 ± 0.94 |

Rec. Dose of *M. a.* - Recommended dose of *M. anisopliae* for the control of *M. fimbriolata* ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$); Thia. insecticide thiamethoxam; Imida. insecticide imidacloprid.

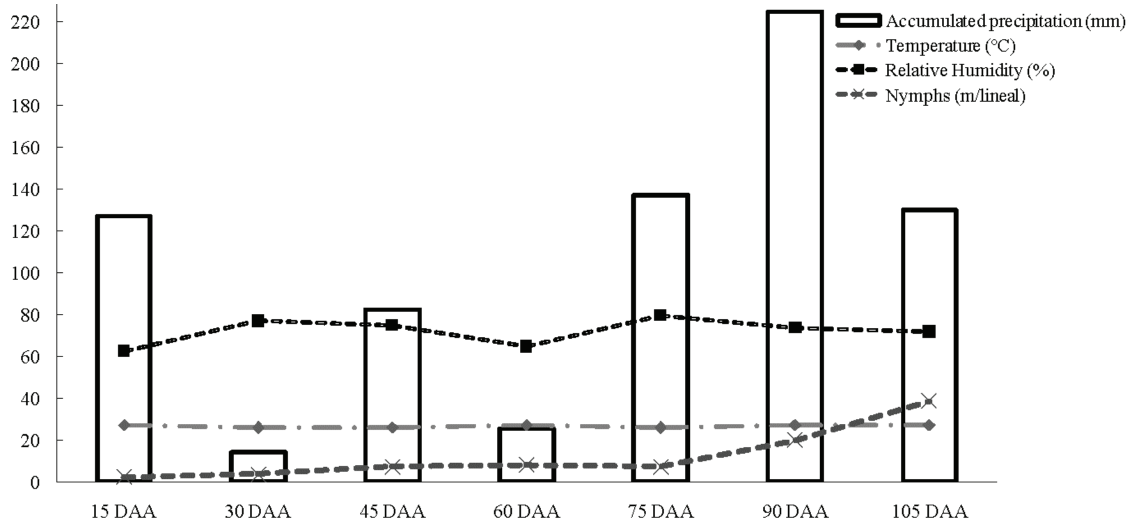


Fig. 1. Cumulative precipitation (mm), average temperature (°C), relative humidity (%) and number of *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs in the experimental area at 15 to 105 days after application (DAT) of insect control treatments.

the control in all treatments except A5 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ of + 350 g $ha^{-1}$ of imidacloprid).

At 30 DAT, the numbers of nymphs were significantly reduced compared to the control in all treatments except A3 (*M. anisopliae* plus the high rate of thiamethoxam) (Table 3). The efficacies of the treatments ranged from 14.28% to 92.85% (Table 4).

At 45 DAT the numbers of *M. fimbriolata* nymphs per treatment were significantly reduced compared to the control in the following treatments: imidacloprid alone at 700g $ha^{-1}$, A1, A2, A3, A4 and A6 (Table 3). Again the performance of A5 was anomalous.

At 60 DAT, the numbers of nymphs were significantly reduced compared to the control in all treatments. The best efficacies occurred in A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65g $ha^{-1}$ of thiamethoxam) and A2 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 125 g $ha^{-1}$ of thiamethoxam), which 77.4% and 87.1%, respectively (Table 4).

TABLE 2. MAINTENANCE COSTS PER HA OF SUGARCANE (MCS) AT NOVA ANDRADINA, MATO GROSSO DO SUL STATE IN 2012 AND 2013.

| MCS | Value (US$) |
|---|---|
| Agricultural inputs for cultivation | 548.50 |
| Mechanized operations (applications) | 59.78 |
| Manpower | 16.01 |
| Administrative expenses | 21.30 |
| Total | 645.59 |

At 75 DAT, the numbers of nymphs were significantly reduced to the greatest extent in the following 3 treatments: A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65g $ha^{-1}$ of thiamethoxam), A3 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 187.5 g $ha^{-1}$ of thiamethoxam), and A5 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 350 g $ha^{-1}$ of imidacloprid). Also the numbers of nymphs were significantly reduced compared to the control in all of the remaining treatments except A2 and A6, yet the numbers of nymphs in the latter 2 treatments were numerically less than in the control. The 2 best control efficacies relative to the control at 75 DAT occurred in treatments A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ of + 65g $ha^{-1}$ of thiamethoxam) (82.1%) and A5 (78.6%) ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 350 g $ha^{-1}$ of imidacloprid).

At 90 DAT the number of nymphs in the control had increased 2.8 fold over the number at 75 DAT. However, the numbers of nymphs were significantly reduced compared to the control in the following treatments: thiamethoxam (250 g $ha^{-1}$), A1, A2, A3, A4, and A5. The 2 best control efficacies a 90 DAT occurred in treatments A1 ($3 \times 10^{12}$ *M. a.* conidia $ha^{-1}$ + 65g $ha^{-1}$ of thiamethoxam) (85.9%), thiamethoxam (250 g $ha^{-1}$) (83.3%) and A2 ($3 \times 10^{12}$ conidia $ha^{-1}$ of *M. a.* + 125 g $ha^{-1}$ of thiamethoxam) (82.1%).

At 105 DAT the number of nymphs in the control had increased 2 fold over the number at 90 DAT. Also at 105 DAT, the number of nymphs were significantly reduced compared to the control in all treatments. However the numbers of nymphs in all treatments were equal or greater than the number in the control , which indicated that none of the treatments could still provide an economically useful level of control.

TABLE 3. INFESTATION LEVELS OF *MAHANARVA FIMBRIOLATA* (HEMIPTERA: CERCOPIDAE) NYMPHS PER LINEAR FOOT [30.4 CM] IN SUGARCANE PLOTS TREATED EITHER WITH THIAMETHOXAM, IMIDACLOPRID, OR *METARHIZIUM ANISOPLIAE*, OR WITH THE COMBINATION OF EITHER INSECTICIDE WITH THE FUNGUS.

| Treatments | 15 DAT | 30 DAT | 45 DAT | 60 DAT | 75 DAT | 90 DAT | 105 DAT |
|---|---|---|---|---|---|---|---|
| Control (untreated) | 2.00 ± 0.81 a | 3.50 ± 1.29 a | 7.00 ± 3.27 a | 7.75 ± 3.39 a | 7.00 ± 2.16 a | 19.50 ± 1.78 a | 38.50 ± 1.45 a |
| Thiamethoxam (250 g ha$^{-1}$) | 0.50 ± 0.57 b | 1.50 ± 1.73 b | 5.25 ± 2.37 a | 3.75 ± 1.50 b | 3.75 ± 1.89 b | 5.50 ± 1.08 b | 15.50 ± 2.07 b |
| Imidacloprid (700 g ha$^{-1}$) | 0.75 ± 0.95 b | 0.75 ± 0.97 b | 2.75 ± 1.43 b | 3.50 ± 1.38 b | 2.50 ± 2.08 b | 13.25 ± 1.75 a | 19.00 ± 1.44 b |
| *Metarhizium anisopliae* (*M.a*) (3×10$^{12}$ conidia ha$^{-1}$) | 1.00 ± 0.83 b | 1.25 ± 1.89 b | 4.00 ± 1.82 a | 2.75 ± 1.86 b | 2.25 ± 1.25 b | 10.50 ± 1.38 a | 17.50 ± 1.79 b |
| A1 (Rec. Dose of *M. a.* + 65 g ha$^{-1}$ of Thia.) | 0.75 ± 0.95 b | 1.00 ± 0.81 b | 2.00 ± 0.89 b | 1.75 ± 1.50 b | 1.25 ± 1.27 c | 3.25 ± 1.70 b | 7.00 ± 1.57 b |
| A2 (Rec. Dose of *M. a.* + 125 g ha$^{-1}$ of Thia.) | 0.00 ± 0.00 b | 0.25 ± 0.50 b | 1.00 ± 1.29 b | 1.00 ± 1.41 b | 5.00 ± 1.89 a | 3.50 ± 1.69 b | 17.25 ± 1.54 b |
| A3 (Rec. Dose of *M. a.* + 187.5 g ha$^{-1}$ of Thia.) | 0.75 ± 0.97 b | 3.00 ± 2.16 a | 2.50 ± 2.58 b | 2.50 ± 1.73 b | 1.75 ± 2.08 c | 6.25 ± 1.18 b | 8.00 ± 1.08 b |
| A4 (Rec. Dose of *M. a.* + 175 g ha$^{-1}$ of Imida.) | 0.00 ± 0.00 b | 1.25 ± 0.50 b | 3.00 ± 1.24 b | 3.25 ± 1.75 b | 3.25 ± 1.25 b | 7.00 ± 1.36 b | 18.00 ± 1.83 b |
| A5 (Rec. Dose of *M. a.* + 350 g ha$^{-1}$ of Imida.) | 1.75 ± 0.93 a | 0.50 ± 0.67 b | 4.50 ± 2.25 a | 2.25 ± 1.25 b | 1.50 ± 1.29 c | 6.00 ± 1.96 b | 16.00 ± 2.11 b |
| A6 (Rec. Dose of *M. a.* + 525 g ha$^{-1}$ of Imida.) | 0.50 ± 0.58 b | 2.00 ± 1.41 b | 2.25 ± 2.10 b | 2.50 ± 1.84 b | 4.25 ± 1.75 a | 10.75 ± 1.25 a | 13.50 ± 2.09 b |
| CV | 61.92 | 67.58 | 90.32 | 75.75 | 77.24 | 64.59 | 38.60 |

Means followed by the same letter in a column do not differ by the Scott-Knott test at 5% probability; CV- coefficient of variation, DAT- days after treatment; Dose Rec. of *M. a.* - Recommended dose of *M. anisopliae* (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$) for the control of *M. fimbriolata*; Thia. insecticide thiamethoxam; Imida. insecticide imidacloprid.

The high rainfall, average temperature, and relative humidity increased the efficiency of the combinations and the fungus *M. anisopliae* alone (Fig. 1 and Table 3). However, the greatest rainfall, mainly at 75 DAT, increased the population of nymphs of *M. fimbriolata* on the plots treated with insecticides (Fig. 1 and Table 3).

The values of TRS were higher with the combination A1 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 65 g ha$^{-1}$ of thiamethoxam), *M. anisopliae* (3 × 10$^{12}$ conidia ha$^{-1}$), thiamethoxam (250 g ha$^{-1}$), and imidacloprid (700 g ha$^{-1}$) and, consequently, the amount paid per tonne, and earnings per ha (Table 5). The costs of acquisition and application of the product in the following treatments: *M. anisopliae*, combination A1 and thiamethoxan (250 g ha$^{-1}$) were US$ 29.27, US$ 41.02, and US$ 64.31, respectively. In other treatments, the cost of *M. fimbriolata* nymph control ranged from US$ 38.46 to US$ 56.84. The treatments with the highest net earnings per hectare were in treatments A1 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 65 g thiamethoxam) and *M. anisopliae* (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$) alone at the recommended dose. Lower earning per ha were obtained the combinations A4 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 175 g ha$^{-1}$ de imidacloprid ), A2 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 125 g ha$^{-1}$ of thiamethoxam), A5 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 350 g ha$^{-1}$ of imidacloprid), and A3 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 187.5 g ha$^{-1}$ of thiamethoxam), respectively (Table 5).

## DISCUSSION

Almost all treatments had lower numbers of spittlebugs than the control at 15 and 30 DAT, which confirms the susceptibility of *M. fimbriolata* nymphs to the 2 insecticides, *M. anisopliae*, and combinations of the insecticides plus the fungus. However some anomalies occurred. For example treatment A5 appeared to be infective at 15 DAT and 45 DAT, but this treatment was quite effective at the other times. Likewise treatment A3 appeared to be ineffective at 30 DAT, but this treatment was effective at all other times. These anomalies in the data probably are the result of the highly aggregated distribution of the spittlebugs.

Furthermore, an additive interaction between each of the chemical insecticides and the fungus was observed in this experiment like that observed for *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae) (Quintela et al. 2013). However, this should not be generalized because the control efficacy depends on the weather, the mode of action of the chemical, and the *M. anisopliae* isolate used to control *M. fimbriolata* (Dinardo-Miranda et al. 2004a; Loureiro et al. 2005; James et al. 2011). On the other hand, the genetic constitution of the

TABLE 4. PERCENT CONTROL (ABBOTT - %) OF *MAHANARVA FIMBRIOLATA* (HEMIPTERA: CERCOPIDAE) BY OF THIAMETHOXAM, IMIDACLOPRID, *METARHIZIUM ANISOPLIAE* AND THE COMBINATION OF EACH INSECTICIDE WITH THE FUNGUS AT 15 DAY INTERVALS POST APPLICATION.

| | Days After Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | 15 | 30 | 45 | 60 | 75 | 90 | 105 |
| Thiamethoxam (250 g ha$^{-1}$) | 75 | 57.14 | 25.00 | 51.61 | 46.42 | 83.33 | 59.74 |
| Imidacloprid (700 g ha$^{-1}$) | 62.50 | 78.57 | 60.71 | 54.83 | 64.29 | 32.05 | 50.64 |
| *Metarhizium anisopliae* (*M.a*) (3 × 10$^{12}$ con ha$^{-1}$) | 50 | 64.28 | 42.85 | 64.51 | 67.86 | 46.15 | 54.54 |
| A1 (Rec. Dose of *M. a.* + 65 g ha$^{-1}$ of Thia.) | 62.50 | 71.42 | 71.42 | 77.41 | 82.14 | 85.89 | 81.81 |
| A2 (Rec. Dose of *M. a.* + 125 g ha$^{-1}$ of Thia.) | 100 | 92.85 | 85.71 | 87.09 | 28.57 | 82.05 | 55.19 |
| A3 (Rec. Dose of *M. a.* + 187.5 g ha$^{-1}$ of Thia.) | 62.50 | 14.28 | 64.28 | 67.74 | 75.00 | 67.95 | 79.22 |
| A4 (Rec. Dose of *M. a.* + 175 g ha$^{-1}$ of Imida.) | 100 | 64.28 | 57.14 | 58.06 | 53.37 | 64.10 | 53.24 |
| A5 (Rec. Dose of *M. a.* + 350 g ha$^{-1}$ of Imida.) | 12.50 | 85.71 | 35.71 | 70.96 | 78.57 | 69.23 | 58.44 |
| A6 (Rec. Dose of *M. a.* + 525 g ha$^{-1}$ of Imida.) | 75 | 42.85 | 67.85 | 67.74 | 39.29 | 44.87 | 64.93 |

Rec. Dose of *M. a.* - Recommended dose of *M. anisopliae* (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$) for the control of *M. fimbriolata* (3 × 10$^{12}$; Thia. Insecticide, thiamethoxam; Imida. Insecticide, imidacloprid.

pest population (Quinelato et al. 2012), adaptations, and mechanisms of insecticide resistance (Dubovskiy et al. 2013) may affect the efficacy of control methods.

Higher numbers of *M. fimbriolata* nymphs with thiamethoxam (60 DAT) and imidacloprid (75, 90, and 105 DAT) show that the time of application time affect the efficiency of the chemicals (Dinardo-Miranda et al. 2004a; George et al. 2007), because high rainfall can decrease their residual effects (Carvalho et al. 2011). In addition, the insecticides imidacloprid and thiamethoxam, at recommended doses, are not selective for some non-target insects (Zhao et al. 2012; Funderburk et al. 2013), which may explain the higher incidence of *M. fimbriolata* in the plots treated with these products. Imidacloprid can cause negative impacts on insects of the family Syrphidae (Easton & Goulson 2013) and can't be selective against the predator of *M. fimbriolata* nymphs, *Salpingogaster nigra* Schiner (Diptera: Syrphidae). On the other hand, low doses of thiamethoxam are selective against *Apis melifera* L. (Hymenoptera: Apidae) (El Hassani et al. 2007). This favors the maintenance of certain beneficial insects in the crop and can explain the efficacy of some combinations of *M. anisopliae* with this insecticide. In addition, low doses of thiamethoxam increased the susceptibility of *T. limbativentris* to *M. anisopliae* (Quintela et al. 2013).

The highest efficacy of controlling *M. fimbriolata* with the combinations A1, A2, and A3 at 30, 60, 75, and 90 DAT can suggest that the thiamethoxam is more suitable than imidacloprid for use with *M. anisopliae*. Contact of *M. anisopliae* with thiamethoxam did not affect the biological characteristics of the fungus, demonstrating compatibility between these two control agents (Botelho & Monteiro 2011; Akbar et al. 2012; Silva et al. 2013). Furthermore, this insecticide and the fungus *M. anisopliae* are most often used to control *M. fimbriolata* in sugarcane fields (Dinardo-Miranda et al. 2004 a, 2004 b).

The TRS was higher in group A1 (3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 65 g ha$^{-1}$ of thiamethoxam) with 96.19. The price per tonne of sugarcane is based on the TRS value, i.e., the higher the value obtained, the higher the price per tonne. The combination of 3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 65 g ha$^{-1}$ of thiamethoxam was found to be the most effective in increasing the price per tonne per ha. Similar values of TRS in the other treatments may be due to the combination *M. anisopliae* with the insecticides. The 2 chemical products kill insects faster than *M. anisopliae* (Dinardo-Miranda et al. 2002; Carvalho et al. 2011), and dead individuals are colonized by this fungus (Dinardo-Miranda et al. 2008; Jin et al. 2011). Thus, entomopathogenic fungi can increase their density in the crop by infecting healthy individuals with inoculum from carcasses (Bruck 2005). In addition, *M. anisopliae* has a high ability to persist in the field (Bruck & Donahue 2007; James et al. 2012), which decreases the probability of resurgence of pest (Guerrero-Guerra et al. 2013).

The price of the combinations ranged only from US$ 38.46 to US$ 64.31, indicating that it is affordable to control *M. fimbriolata* with a combination of *M. anisopliae* and a chemical insecticide. However, use of the combination of 3 × 10$^{12}$ *M. a.* conidia ha$^{-1}$ + 65 g ha$^{-1}$ of thiamethoxam produced lower costs, more tonnes of sugarcane ha$^{-1}$, the greatest estimated gross income per ha of US$ 1242.77 and the greatest net earnings per ha of US$ 556.16 (Table 5). On the other hand, the plant age can also influence the productivity of the sugarcane (Dinardo-Miranda et al. 2008), so that plants in a

TABLE 5. TOTAL RECOVERABLE SUGAR (TRS), QUOTATION OF TRS (COT. TRS), PRICE PER TONNE, GROSS INCOME ESTIMATE PER HA, MAINTENANCE COST OF SUGARCANE FIELDS PER HA (MCS), COST CONTROL OF *MAHANARVA FIMBRIOLATA* (HEMIPTERA: CERCOPIDAE) AND NET EARNINGS PER HA IN THE TREATMENTS WITH INSECTICIDES, *METARHIZIUM ANISOPLIAE* AND CERTAIN COMBINATIONS.

| Treatments | TRS tonnes $ha^{-1}$ | Cot. TRS (US$) | Price per tonne (US$) | Gross Income $ha^{-1}$ (US$) | MCS (US$) | Cost of Control $ha^{-1}$ (US$) | Net Earnings $ha^{-1}$ (US$) |
|---|---|---|---|---|---|---|---|
| Control (Untreated) | 78.00 | 0.19 | 14.98 | 1,007.76 | 645.59 | — | 362.17 |
| Thiamethoxam (250 g $ha^{-1}$) | 93.38 | 0.19 | 17.74 | 1,206.47 | 645.59 | 64.31 | 496.57 |
| Imidacloprid (700 g $ha^{-1}$) | 93.00 | 0.19 | 17.67 | 1,201.56 | 645.59 | 54.05 | 501.92 |
| *Metarhizium anisopliae* (*M.a*) ($3\times10^{12}$ con $ha^{-1}$) | 93.48 | 0.19 | 17.76 | 1,207.76 | 645.59 | 29.27 | 532.90 |
| A1 (Rec. Dose of *M. a.* + 65 g $ha^{-1}$ de Thia.) | 96.19 | 0.19 | 18.28 | 1,242.77 | 645.59 | 41.02 | 556.16 |
| A2 (Rec. Dose of *M. a.* + 125 g $ha^{-1}$ de Thia.) | 86.24 | 0.19 | 16.39 | 1,114.22 | 645.59 | 52.78 | 415.85 |
| A3 (Rec. Dose of *M. a.* + 187.5 g $ha^{-1}$ de Thia.) | 87.60 | 0.19 | 16.64 | 1,131.79 | 645.59 | 64.31 | 421.89 |
| A4 (Rec. Dose of *M. a.* + 175 g $ha^{-1}$ de Imida.) | 82.85 | 0.19 | 15.74 | 1,070.42 | 645.59 | 38.46 | 386.37 |
| A5 (Rec. Dose of *M. a.* + 350 g $ha^{-1}$ de Imida.) | 86.24 | 0.19 | 16.39 | 1,114.22 | 645.59 | 47.65 | 420.98 |
| A6 (Rec. Dose of *M. a.* + 525 g $ha^{-1}$ de Imida.) | 89.17 | 0.19 | 16.94 | 1,152.08 | 645.59 | 56.84 | 449.64 |

Plant age = 8 months; Cot. TRS (Price quotation by União dos Produtores de Bioenergia [UDOP]); Price per tonne = TRS × Cot. TRS (the price per tonne of sugarcane is based on the TRS value, i.e., the higher the TRS value, the higher the price per tonne); Gross income $ha^{-1}$ = value of 1 tonne × 68 tonnes (average production of Mato Grosso do Sul State); MCS = maintenance cost of sugarcane fields without costs of insect control products and application for control of *M. fimbriolata*; Cost of control = Costs of products and ground application; Profit per ha = Gross income - (MCS + Cost of control); Rec. Dose of *M. a.* = Recommended dose of *M. anisopliae* ($3 \times 10^{12}$ *M a.* conidia $ha^{-1}$) for the control of *M. fimbriolata*. Thia. = thiamethoxam; Imida. = imidacloprid.

more advanced developmental stage may have a higher yield of TRS. The value of the TRS was obtained with 8-month-old plants, but TRS of older plants may be higher.

Thus, the combination of the entomopathogenic fungus, *M. anisopliae,* with thiamethoxan or imidacloprid can reduce infestations of *M. fimbriolata* to sufficiently low levels to protect the sugarcane. Nevertheless, factor influencing the effectiveness of combinations of *M. anisopliae* with either thiamethoxan or imidacloprid need to be better understood, and further relevant studies should be conducted.

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