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Bioassay

Insecticides Efficacy to Control the Neotropical Peanut Burrower Bug *Cyrtomenus mirabilis* (Perty, 1830) (Hemiptera: Cydnidae) Under Laboratory Conditions

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Abstract: In this study, we evaluated the effectiveness of insecticides sprayed by contact as well as seeds treatment for controlling the burrower bug *Cyrtomenus mirabilis* (Perty, 1830) (Hemiptera: Cydnidae) under laboratory conditions. In the first experiment, the insecticides were sprayed onto the insects (direct contact). Insect mortality evaluation started 24h after insecticide application and repeated every two days up to 20 days. In the second experiment, peanut seeds were treated with insecticides. The mortality of insects was evaluated at 10, 15, and 20 days after installation. The mixture of fipronil and alpha-cypermethrin in direct contact was the most efficient treatment to control nymphs and adults of *C. mirabilis*. Regarding the seed treatment, fipronil was the most efficient for controlling adults of *C. mirabilis* while for nymphs, all insecticides were highly effective.

Keywords: *Arachis hypogaea* L., soil pest, seed treatment, chemical control.

In the last peanut growing seasons (*Arachis hypogaea* L.), significant increases in the occurrence of damages caused by the Neotropical peanut burrower bug *Cyrtomenus mirabilis* (Perty, 1830) (Hemiptera: Cydnidae) were registered in this crop (Santos et al. 2013a; Michelotto et al. 2023). The damage caused by this insect in peanuts was attributed to attacks on pods containing developing kernels in which nymphs and adults inserted the stylets for feeding, directly affecting the quality of kernels (Chapin & Thomas 2003).

Adults of *C. mirabilis* are typical cydnids, black, short legs with strong spines that help them move in the soil. Nymphs are dark brown to almost black with white to cream colored abdomen. Both nymphs and adults pierce the pods and feed on the kernels (Schwertner & Nardi 2015). It is noteworthy that the species *Cyrtomenus bergi* Froshner, 1960 (Hemiptera: Cydnidae) was established as a junior synonym of *C. mirabilis*, and all information published for the first species should be considered as *C. mirabilis* (Avedaño et al. 2018).

Despite the importance of burrower bugs in peanuts, little is known about its control. Biological control with entomopathogenic fungi may be a possibility to control this species. For instance in Colombia, isolates of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina; Hyphomycetes) were evaluated under laboratory conditions, causing mortality of 61% of nymphs (5° instar) of *C. mirabilis* (Bellotti et al. 1999). In addition, Jaramillo et al. (2005) reported that applications of specific strains of *M. anisopliae* in association with low dosages of imidacloprid resulted in high mortality of *C. mirabilis* nymphs in a greenhouse study.

So far, there are no insecticides approved for controlling *C. mirabilis* in Brazil. As possible options, insecticides used to control soil pests in other crops, such as sugarcane and insecticides already registered for peanut crops (Brasil 2022) and entomopathogenic fungi should be tested. Xavier et al. (2020) in field experiments observed high mortality of nymphs and a reduction in damage caused by the burrower bug in peanuts using fipronil in seed treatment or in sowing furrows.

More than 85 % of the *C. mirabilis* population is found up to 10 cm, especially after plant fructification, and the number of nymphs increases as the number of maturing pods increases as well (Michelotto et al. 2023).

Thus, this study was designed to evaluate the efficacy of different insecticides in the control of adults and nymphs of the burrower bug under laboratory conditions, using the methods of direct contact and seed treatment.

The experiments were carried out in the laboratory of the Regional Research and Development Unit of Pindorama, Apta, in the State of São Paulo, Brazil.

The insects used in the essays were sampled in the field once there is no well-established rearing method for this insect. Adults of *C. mirabilis* were collected with white light traps (Michelotto et al. 2019), installed in an area previously cultivated with peanuts in Pindorama, São Paulo (21°13'16.1''S 48°55'37.7''W). The nymphs were collected in-built trenches of 30 x 30 x 15 cm (length, width, depth) using shovels and sieves (Michelotto et al. 2023).

The identification of the species was based on the morphological characteristics, using identification keys and by comparing them with previously identified specimens (Avendaño et al. 2017; 2018).

In the direct contact experiments with adults and nymphs, the products were tested by spraying onto the insects, using a completely randomized design with seven treatments and four replicates (Tab. 1).

Each experimental unit consisted of a Petri dish, in which 10 *C. mirabilis* (nymphs or adults) individuals were placed. Spraying was performed in a Potter tower, and the volume used in each treatment was 0.5 mL per application in each dish. After spraying, 40-45 g of sterile soil at a 10% moisture level were added to each Petri dish, together with peanut kernels (IAC OL3 cultivar), for feeding the insects.

In this experiment, adults of unknown age were used, while the nymphs were from the 4° and 5° developmental stages. Before using the peanut kernels, these were immersed in a sodium hypochlorite solution for 24h, for sterilization and removal of possible contaminants. Subsequently, the Petri dishes were closed and placed inside a BOD chamber at 25 ± 2 °C and relative humidity of 60 ± 10%, in the absence of light.

Table 1. Treatments (active ingredient), trade names, and doses tested to nymphs and adults of the burrower bug *C. mirabilis*, by spraying under laboratory conditions (Experiment 1).

Treatments/Active Ingredient	Trade Names	Chemical Groups	Rates (mL or g c.p. ha^{-1})
1. Non-Treated	Control		-
2. Imidacloprid	Provado® 200 SC	4A - Neonicotinoids	1,000
3. *Metarhizium anisopliae* (IBCB 425) + *Beauveria bassiana* (IBCB 66)	Metarhizium Oligos® + Beauveria Oligos®		1,000 + 1,500
4. Thiamethoxam + lambda-Cyhalothrin	Engeo Pleno™ S	4A - Neonicotinoids + 3A - Pyrethroids	400
5. *Metarhizium anisopliae* (IBCB 425) + *Beauveria bassiana* (IBCB 66) + Imidacloprid*	Metarhizium Oligos® + Beauveria Oligos® + Provado 200 SC	4A - Neonicotinoids	1,500 + 1,000 + 15 ppb**
6. Thiamethoxam + Chlorantraniliprole	Durivo	4A – Neonicotinoids + 28 - Diamides	300
7. Fipronil + alpha-Cypermethrin	Regent® Duo	2B – Phenylpyrazoles + 3A - Pyrethroids	1,000

*Imidacloprid was added in a sub-dose because it increases the efficiency of biological control agents, especially soil pests (Jaramillo et al., 2005); **ppb= parts per billion. *c.p.: commercial product*

The mortality of nymphs and adults was evaluated 24 h after applying the products (1DAA) and repeated every two days for 20 days.

In the seed treatment experiments with adults and nymphs of *C mirabilis,* a completely randomized design was used too, but with six treatments and five replicates (Tab. 2).

Each experimental unit was composed of a matte, black-painted plastic container, in which 10 specimens of *C. mirabilis* (nymphs or adults) were placed, in addition to 300 g of sterile soil at a 10% moisture level and five peanut kernels of the respective treatments for feeding the insects. In this experiment, adults of unknown age were used, while the nymphs were from the 2° and 3° developmental stages. The soil used in the experiments was a eutrophic Red-Yellow Argisol of sandy fine texture (Santos et al. 2013b), collected in a fallow area in a greenhouse with forced ventilation at 105 °C for 24 h.

Subsequently, the containers were closed and placed inside a BOD chamber at 25 ± 2 °C and relative humidity of 60 ± 10%, in the absence of light. The mortality of insects was evaluated at 10 and 15 DAI (days after infestation). This longer period between evaluations was adopted to avoid the movement and exposure of the seeds, making them more attractive to insects. The dead insects were usually found on the soil surface. In the evaluation at 20 DAI, the insects were separated from the soil and seedlings, and both the dead and the living ones were accounted in each treatment.

The obtained data were previously transformed for Arc sine $\sqrt{(x/100)}$ and submitted to a variance analysis. Means were compared by the Tukey test at a 5% probability level. The Control Efficacy (CE) of treatments was calculated using the formulae proposed by Abbott (1925).

In the spraying on insects, the mortality of the burrower bugs was different from 4 DAA, with the treatment fipronil + alpha-cypermethrin displaying mortality above 90%, thiamethoxam + lambda-cyhalothrin showing mortality close to 50%, and the other treatments displaying similar results in comparison to the control (Fig. 1).

In the subsequent evaluations, the same pattern was observed and at 20 DAA, the treatment thiamethoxam + lambda-cyhalothrin reached mortality close to 90%, not differing from the treatment fipronil + alpha-cypermethrin, with 100% mortality. The other treatments did not differ from the control, with mortalities ranging from 23% to 63% (Fig. 1).

When analyzing the effectiveness of nymphs control by the insecticides in relation to the control treatment, it is possible to observe the treatment fipronil+ alpha-cypermethrin reached an efficacy of 100% at 6 DAA, while the treatment thiamethoxam + lambda-cyhalothrin presented increased effectiveness throughout the evaluations, reaching 80% at 20 DAA. In relation to the treatments thiamethoxam + chlorantraniliprole and imidacloprid, partial effectiveness of 37.5% and 12.5% were observed, respectively. However, the other treatments were not statistically significant for the effectiveness in the control of the burrower bug nymphs (Fig. 1).

The mortality rate of spraying insecticide on adults was similar to those observed on nymphs. The treatment fipronil + alpha-cypermethrin reached mortality above 80% at 4 DAA, being the only one to display significant differences compared to the control. This pattern remained unaltered in the evaluation at 16 DAA. At 18 and 20 DAA, increased mortality of adults was observed in the treatment thiamethoxam + lambda-cyhalothrin (62.5%), not differing from the treatment fipronil+ alpha-cypermethrin (Fig. 2).

When evaluating the effectiveness of controlling adults by spraying the insecticides, only the treatment fipronil + alpha-cypermethrin presented an effectiveness above 90%. The other insecticides presented a controlling efficacy below 80%, which could be acceptable (Fig. 2). The treatment *Metarhizium* + *Beauveria* was ineffective in controlling the burrower bug's nymphs and adults. Conversely, the use combined with the sub-dose of imidacloprid presented effectiveness for controlling adults of 21.2%, which was still far from the expected (Figs. 1, 2).

Regarding the adult's death in the *Metarhizium* + *Beauveria* treatment, it is possible that the isolates used in the present experiment could not be effective because of the no infectivity in the burrower bug. Due to the low mortality, no sporulation test was performed to confirm the death by the fungi. Despite the low mortality, results obtained by Jaramillo et al. (2005) indicate the high potential for biological control

Table 2. Treatments (active ingredient), trade names, and doses used to control nymphs and adults of the burrower bug *C. mirabilis*, in the treatment of peanut seeds under laboratory conditions (Experiment 2).

Treatment/Active Ingredient	Trade Names	Chemical Groups	Rates (mL c.p./ kg seed)
1. Non-Treated	-		-
2. Thiamethoxam	Cruiser® 350 FS	4A - Neonicotinoids	2.0
3. Clothianidin	Inside® FS	4A - Neonicotinoids	2.5
4. Bifenthrin + Imidacloprid	Rocks®	3A - Pyrethroids + 4A - Neonicotinoids	2.0
5. Imidacloprid	Saluzi® 600 FS	4A - Neonicotinoids	3.0
6. Fipronil	Standak® Top*	2B – Phenylpyrazoles	3.0

* *Product also contains the fungicides Piraclostrobine and Methyl Thiophanate, which do not have insecticide action. c.p.: commercial product.*

of this insect. New tests with other isolates associated with adjuvants in specific experiments are necessary.

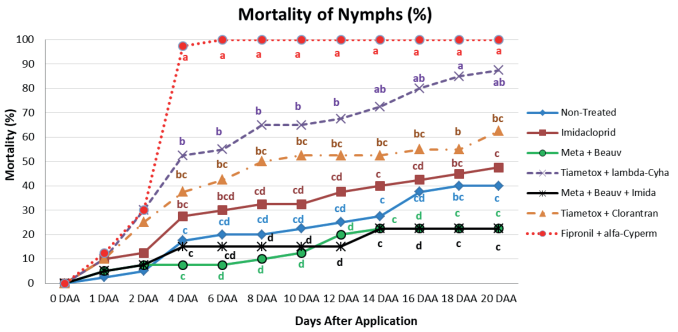

Figure 1. Mortality (%) of nymphs of the burrower bug by spraying insecticides in function of the days after application (DAA) in laboratory. Means followed by distinct letters indicate significant differences by the Tukey test at a 5% probability level.

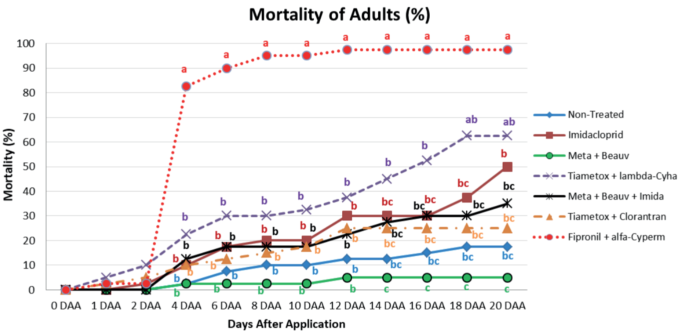

Figure 2. Mortality (%) of adults of the burrower bug by spraying insecticides in function of the days after application (DAA) in laboratory. Means followed by distinct letters indicate significant differences by the Tukey test at a 5% probability level.

In the experiment where peanut seeds were treated for the control of nymphs, the mortality caused by the insecticide did not differ among the evaluations at 10 and 15 DAI. At 20 DAI, it was possible to observe that the insecticides caused mortality of nymphs above 95%, not different from each other, but with significant differences from the control (Fig. 3).

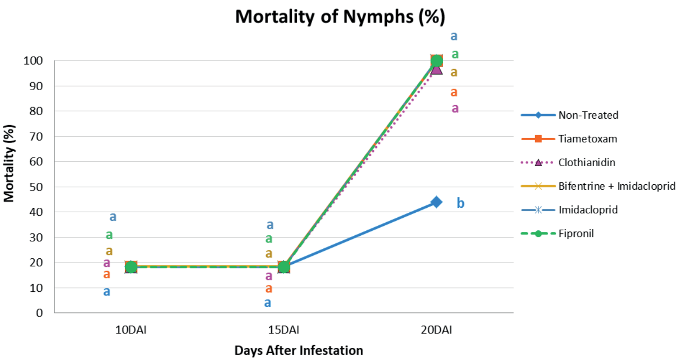

Figure 3. Mortality (%) of nymphs of the burrower bug when treating seeds in function of the days after infestation (DAI) in laboratory. Means followed by distinct letters indicate significant differences by the Tukey test at a 5% probability level.

When analyzing the control effectiveness, the treatments with imidacloprid and fipronil reached 4% and 2%, respectively, at 10 DAI, while the other insecticides were inefficient in controlling the insect. At 15 DAI, the results remained unaltered, but at 20 DAI, the treatments reached effectiveness above 95% (Fig. 3).

In the experiment with *C. mirabilis* adults, the treatments of seeds with different insecticides were different from each other in all evaluations (Fig. 3). At 10 DAI, the adults' mortality in treatments of imidacloprid and fipronil were significantly higher in comparison to the other treatments, with a mortality rate of 23% (Fig. 4). At 15 DAI, it was possible to observe increased mortality in the treatment fipronil (28.8%), even though it was not different from the treatment imidacloprid (23.0%). On the other hand, the treatments of thiamethoxam and clothianidin did not differ from the control (Fig. 4).

In the evaluation performed at 20 DAI, it was possible to observe that the treatment fipronil reached the highest mortality rate (59.6%), not differing from the insecticide imidacloprid (46.2%). The treatments bifenthrin + imidacloprid and clothianidin presented an intermediary mortality, while the thiamethoxam was similar to the control, with a 30.0% mortality rate (Fig. 4).

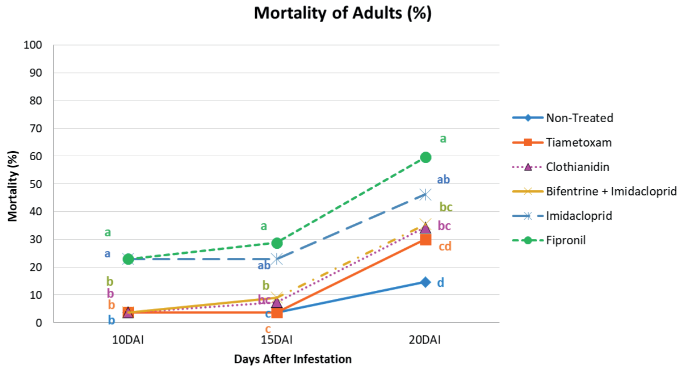

Figure 4. Mortality (%) of adults of the burrower bug when treating seeds in function of the days after infestation (DAI) in laboratory. Means followed by distinct letters indicate significant differences by the Tukey test at a 5% probability level.

When analyzing the control effectiveness of the insecticides in comparison to the non-treated, it was possible to verify that the treatments imidacloprid and fipronil reached an efficacy of 14% at 10 DAI, while the other insecticides were not effective in controlling adults of the burrower bug (Fig. 3). In the subsequent evaluation (15 DAI), the insecticide fipronil reached an efficacy of 22.4% in comparison to the non-treated. The insecticides imidacloprid, bifenthrin + imidacloprid, and clothianidin presented partial effectiveness (14%, 4% and 2%, respectively), while the treatment thiamethoxam was not efficient in controlling the adult of the burrower bug (Fig. 3).

In the last evaluation at 20 DAI, the highest effectiveness was observed in the fipronil treatment (72.0%). The insecticide imidacloprid presented an intermediary efficacy of 48%, while the insecticides bifenthrin + imidacloprid, clothianidin, and thiamethoxam presented partial efficacies (28%, 26%, and 20%, respectively) (Fig. 3).

Although the direct spraying of the insecticides on the insects does not necessarily reflect the reality of field conditions, since both nymphs and adults of the burrower bug remain most of their life in the soil (Michelotto et al. 2023), the exposure of the insects to these pesticides may indicate which ones are more effective. In this sense, the association of fipronil and alpha-cypermethrin resulted in high mortality of both nymphs and adults of the burrower bug. The combination between thiamethoxam and lambda-cyhalothrin caused satisfactory mortality of nymphs and adults, while the other insecticides used in this study were not efficient (Figs. 3, 4).

The entomopathogenic isolates of *M. anisopliae* and *B. bassiana* used in this experiment were not efficient in controlling both nymphs and adults. However, this is considered normal, given the host's specificity (Goettel et al. 2010), being therefore necessary to conduct further studies on this subject, in order to evaluate different isolates.

The use of insecticides in the treatment of seeds resulted in high mortality of nymphs. In adults, only the insecticide fipronil resulted in a satisfactory mortality rate (Fig. 3, 4). Seed treatment is considered one of the methods that provides effective protection with reduced dosages, mainly in the control of soil pests as an ecologically and economically justifiable measure (Vojvodić & Bažok 2021).

For the peanut burrower bug, this type of application can be extremely advantageous, once there is not an established sampling method as well as its threshold. Although this insect occurs in the field since the peanut sowing, its population increases in this crop, especially after the presence of mature pods, which occurs approximately 100 days after sowing (Michelotto et al. 2023), when the insecticides used in seeds might not be present anymore, especially the ones with high solubility.

Another important aspect to be evaluated when choosing the insecticides to be used in the peanut crop is the residual period that can be verified in the peanut kernels. Thus, for the insecticides and other pesticides to be used, it is important to respect the acceptable levels of residues established by the Brazilian legislation (Brazil), by the FAO's Codex Alimentarum (Codex), and by the European Union (EU), since part of the peanut produced in Brazil is exported.

Field studies should be carried out with the best insecticides to confirm the results obtained in the present study as well as the evaluation of residues in the peanut kernels harvested after using the products.

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Authors' Contributions

MDM and IJG planned, designed, executed experimental work, conducted, data analyses, and wrote the manuscript. ADPR, RMR and ROR executed experimental work and helped with data analyses. DJA and CFC wrote the manuscript. All authors read and approved the manuscript.

Conflict of Interest Statement

The authors declare no potential conflict of interest.

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