LOUREIRO, E. S.; PESSOA, L. G. A.; PUTRICK, T. C.; PANTALEÃO, A. A.; DIAS, P. M. *In vitro* compatibility between insecticides and the commercial bioinsecticide Agree® WG. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 7, n. 1, p. 49-52, jan./mar. 2020. ISSN 2358-6303.

In vitro compatibility between insecticides and the commercial bioinsecticide Agree® WG

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Received: 03/06/2019; Accepted: 04/03/2020.

ABSTRACT

Compatibility studies are essential for the integration and simultaneous use of chemical and biological pest control methods since they are necessary for an Integrated Pest Management (IPM) program. In this work, the aim was to evaluate the compatibility of insecticides used in soybean and cotton crops for pest control with *Bacillus thuringiensis* (Bt). The *in vitro* inoculation technique was used with *B. thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *aizawai*, in culture medium containing the following insecticides: beta-cyfluthrin (Bulldock®), methomyl (Bazuka®), thiamethoxam + lambda-cialotrina (Engeo Pleno®), zeta-cypermethrin (Fury 200®), acetamiprid (Saurus®), bifenthrin + carbosulfano (Talisman®) and bifenthrin (Talstar®), in Petri dishes. The Petri dishes were taken to the B.O.D. (Biological Oxygen Demand), at a temperature of 30 ± 1 ºC, 70 ± 10% RH (relative humidity) and a photophase of 12 h, for 24 hours. Colony growth was measured, and Colony Forming Units (CFU) counted in the total area of the Petri dish. The product that allowed growth to be significantly equal to or higher than the control was established as compatible, and the one that did not allow growth or was significantly less than the control was incompatible. It was found that all insecticides were classified as incompatible with the bioinsecticide.

Keywords: selectivity, bioinsecticide, entomopathogenic bacteria.

Compatibilidade *in vitro* entre inseticidas e o bioinseticida comercial Agree® WG

RESUMO

Os estudos de compatibilidade são importantes para a integração e uso simultâneo de métodos de controle químico e biológico de pragas, uma vez que são necessários em um programa de Manejo Integrado de Pragas (MIP). Neste trabalho, objetivou-se avaliar a compatibilidade de inseticidas utilizados nas culturas de soja e algodão para o controle de pragas, com o *Bacillus thuringiensis* (Bt). Utilizou-se a técnica de inoculação *in vitro* do *B. thuringiensis* var. *kurstaki* e *B. thuringiensis* var. *aizawai*, em meio de cultura contendo os seguintes inseticidas: beta-ciflutrina (Bulldock®), metomil (Bazuka®), tiametoxam + lambda-cialotrina (Engeo Pleno®), zeta-cipermetrina (Fury 200®), acetamiprido (Saurus®), bifentrina + carbosulfano (Talisman®) e bifentrina (Talstar®), em placas de Petri. A placas foram levadas para a câmara de germinação tipo B.O.D. (Demanda biológica de Oxigênio), à temperatura de 30±1 ºC, 70±10% UR (umidade relativa) e fotofase de 12h, por um período de 24 horas. Foi realizada medição do crescimento das colônias e contagem de Unidades Formadoras de Colônia (UFC) na área total da placa de Petri. Foi estabelecido como compatível o produto que permitiu crescimento significativamente igual ou superior à testemunha, e incompatível o que não permitiu o crescimento ou este foi significativamente menor que o controle. Constatou-se que todos os inseticidas foram classificados como incompatíveis ao bioinseticida.

Palavras-chave: seletividade, bioinseticida, bactéria entomopatogênica.

1. Introduction

Integrated Pest Management (IPM) has emerged as a response to the incorrect use of phytosanitary products. Due to the wrong use of insecticides, problems occurred: raising the status of secondary for primary pests due to the reduction of natural enemies that acted on them, the resurgence of infestations, resistance to insecticides, and presence of residues in food (Panizzi, 2013).

According to Agostini et al. (2014), biological control plays a fundamental role in an IPM program, aiming sustainable agriculture. Batista Filho et al. (2001) claim that there is an improvement in the quality of food, reducing environmental and human health impacts, and it can be used concomitantly with agrochemicals if it is selective. It is of utmost importance to carry out compatibility studies for entomopathogens, with the use and conservation of microorganisms within agroecosystems being one of the strategies used in IPM since they are found naturally in these environments.

It is essential to know the action of phytosanitary products, determining their compatibility with entomopathogens to minimize impacts, both on the environment and the microbiota. The recommended dose of tested phytosanitary products can be an essential factor of compatibility between chemical and biological products (Agostini et al., 2014). According to Ramaraje et al. (1967), different doses of insecticides can influence the growth and sporulation of the entomopathogen in the compatibility tests.

The entomopathogenic bacterium *Bacillus thuringiensis* Berliner (Bt) is the most commercially used active ingredient in bioinsecticides, with a market ranging from 80 to 95% (van Frankenhuyzen, 2013). There are 25 products registered in the Brazilian market based on B. thuringiensis for the control of insects of agricultural importance, of the Coleoptera, Hymenoptera, Hemiptera, Lepidoptera and Orthoptera Orders, according to data from the Ministry of Agriculture, Livestock and Food Supply (MAPA). *B. thuringiensis* var. *kurstaki* (Btk) is used in approximately 9 million hectares in several crops of agricultural and forest importance (AGROFIT, 2020). The *B. sphaericus* is an entomopathogen that controls vectors of human disease etiological agents (Palma et al., 2014).

There are few studies on the compatibility of *B. thuringiensis.* It is not known whether the number of spores and the size of the colonies of this bacterium can be changed when used in conjunction with insecticide. This data is fundamental, since, in the case of incompatibility, the control of soybean defoliating caterpillars can be compromised (Schünemann et al., 2014).

Given the above, the present study aimed to evaluate the effect of insecticides on the germination of the colony-forming units (CFU) of the commercial bioinsecticide Agree®, formulated based on *Bacillus thuringiensis* var. *aizawai* GC 91 and *Bacillus thuringiensis* var. *kurstaki.*

2. Material and Methods

The experiment was carried out at the Entomology Laboratory of the Federal University of Mato Grosso do Sul - Chapadão do Sul Campus, MS, using the bioinsecticide Agree®; It has the active ingredient *Bacillus thuringiensis* var. *aizawai* (Btk) trans conjugated with toxins from *Bacillus thuringiensis* var. *kurstaki*. Commercial samples of insecticides whose information refers to the formulation, maximum and minimum dosage prescribed for soybean and cotton crops, chemical groups, and technical names are shown in Table 1.

In each Erlenmeyer flask containing 200 mL of PDA (Potato-Dextrose-Agar) culture medium at 45 °C, the insecticide was added at the average dosage of the concentrations recommended by the manufacturer for soybean and cotton crops (Table 1). Then, 10 mL aliquots of the mixture were poured into Petri dishes with 9 cm diameter, which was inside the laminar flow chamber (Batista Filho et al., 2001).

Table 1. Phytosanitary products used in the experiment.

Name		Formulation	Chemical group	Average dose (c.p.)	
Technical	Commercial				
Beta-Cyfluthrin	Bulldock®	SC	Pyrethroid	60	mL ha^{-1}
Methomyl	Bazuka®	SL	Oxime Methylcarbamate	1144	mL ha^{-1}
Thiamethoxam and Lambda Cyhalothrin	Engeo Pleno®	SC	Pyrethroid + Neonicotinoid	200	mL ha^{-1}
Zeta-Cypermethrin	Fury 200®	EW	Pyrethroid	175	mL ha^{-1}
Acetamiprid	Saurus®	SP	Neonicotinoid	100	g ha^{-1}
Bifenthrin and Carbosulfan	Talisman®	EC	Pyrethroid + Benzofuranyl carbamate	1025	mL ha^{-1}
Bifenthrin	Talstar®	CE	Pyrethroid	510	mL ha^{-1}

Source: AGROFIT (2020) * maximum and minimum dosage data.

The bioinsecticide Agree® was used in the dosage recommended by the manufacturer (0.5 kg c.p. ha^{-1}), which contains, per gram of product, 1.0×10^9 viable spores/g. For the installation of the bioassay, a suspension of 100 mL of sterile distilled water containing 1 gram of the bioinsecticide was used. After solidification of the culture medium, a 100µl aliquot of spray solution was inoculated with the aid of an automatic pipette and spread using the Drigalsky handle. The bioinsecticide Agree® was inoculated in a culture medium without the addition of phytosanitary products in the control treatment. Then, the Petri dishes were left open in the flow chamber for evaporation of excess water. After this procedure, the Petri dishes were incubated in B.O.D. (Biological oxygen demand) and maintained at a temperature of 30 ± 1 ºC, 70 ± 10% RH (Relative Humidity) and a photophase of 12h, for 24 hours.

After 24 hours, two plates from each treatment were separated. The colonies were scraped and transferred to glass tubes with sterile distilled water and adhesive spreader, and a suspension was prepared to allow spore counting using the Neubauer chamber. Four plates were also chosen at random to measure the growth of the colonies, using a leaf area meter. A classification was established concerning compatibility, based on the model proposed by Agostini et al. (2014). The product that allowed growth to be significantly equal or superior to the control was established as compatible, and the one that did not allow growth or was significantly less than the control was incompatible.

The experimental design was completely randomized, with 8 treatments and 12 replications, analyzed for a variance by the F test, and the Tukey test compared the means at 1% significance and transformation $(Y+0,5)^{0,5}$.

3. Results and Discussion

All insecticides tested inhibited or significantly reduced the size of the colony and the number of CFU (Colony-Forming Units). Among the products tested, it was found that Beta-Cyfluthrin provided the smallest reduction in the values of these parameters, differing significantly from the others (Table 2). According to the specialized literature, the insecticides Tiametoxan, Carbosulfan, Diafentiuron, Imidacloprid and Acetate in maximum concentration (Batista Filho et al., 2001) and the insecticides Actara® and Thiametoxan (in the medium and maximum doses tested), and Cyproconazol + Thiametoxan (in the minimum dose) were compatible, not affecting the bacterium B. thuringiensis (Dipel®) (Almeida et al., 2003).

The results observed in the present study differed from those obtained by Morris (1977). The author evaluated the compatibility of 27 pesticides with *B. thuringiensis* var. *kurstaki*, demonstrating that the insecticides belonging to the group of pyrethrins were highly bacteriostatic and those belonging to the group of compatible carbamates. According to the same author, this variation in results may be related to the formulation of products, where the presence of emulsifiers, inert, and other additives can directly negatively affect compatibility. This fact was also verified by Tamai et al. (2002), who concluded that toxicity might be related to the components of the formulation and not necessarily to the active ingredient.

Pinto et al. (2012) performed a compatibility test with Thiametoxan and Lambda-Cyhalothrin. They observed that when these products were tested using the recommended dose, they did not cause any inhibition in the growth of *B. thuringiensis* colonies. On the other hand, when using doses ten times higher than recommended, Thiametoxan had an inhibitory effect on the development of the bacterium, and the insecticide Lambda-Cyhalothrin did not have such an impact on *B. thuringiensis.* The recommended dose of tested phytosanitary products can be an essential factor of compatibility between chemical and biological products (Agostini et al., 2014). This variable can be used to promote compatibility (Batista Filho et al., 2001; Manachini, 2002).

All insecticides were incompatible with the bioinsecticide Agree® (Table 2). These results were similar to the experiments conducted by Agostini et al. (2014). They use the technique of mixing chemical phytosanitary products to the culture medium after carrying out of the entomopathogen inoculation provided more negative effects than the other compatibility assessment techniques.

Besides the toxicity found for the bacteria in this type of test, another factor can be considered. In this research, 100µL aliquots of the bioinsecticide Agree® in the spray solution were used, making bacterial cells very close to each other. A state is known as quorum sensi, allowing the exchange of signals through the production of secondary metabolic products, which can harm entomopathogens, such as reduced germination and spore viability. This result was verified by Agostini et al. (2014), using 5µL aliquots/spray solution of *B. thuringiensis*.

Even with the possibility of interference between the bacterial cells due to proximity, the technique for evaluating the effect of phytosanitary products mixed with solidified BDA (potato-dextrose-agar) medium is reliable, as it allows the quantification of the pathogen. The method used for insecticide evaluation showed many incompatible products, that is, that did not favor germination and growth of the bacteria on the medium used.

Table 2. Colony size (±EP) and CFU/mL (± EP) from cells of *Bacillus thuringiensis* var. *kurstaki* e *Bacillus thuringiensis* var. *aizawai* after incubation in PDA medium (30±1 ºC, 70 ± 10% RH and 12 h photophase) with the average dosage of the insecticides.

Treatments	Colony size (cm^2)	Average UFC/mL (10^7)	% UFC/mL concerning the control[2]
Beta-Cyfluthrin	58.51[1]±2.40 a	1.907±0.1 a	——
Methomyl	32.40±5.0 b	0.566±0.03 b	[2]-70.3
Thiamethoxam and Lambda Cyhalothrin	0.0±0.00 c	0.0±0.00 c	[3]——
Zeta-Cypermethrin	0.0±0.00 c	0.0±0.00 c	——
Acetamiprid	0.0±0.00 c	0.0±0.00 c	——
Bifenthrin and Carbosulfan	0.0±0.00 c	0.0±0.00 c	——
Bifenthrin	0.0±0.00 c	0.0±0.00 c	——
Beta-Cyfluthrin	0.0±0.00 c	0.0±0.00 c	——
CV (%)	15.70	22.00	

[1]Means followed by the same lowercase letter in the column do not differ significantly by the Tukey test (α = 1%).

[2]% Relation to the witness: [(Average CFU cm-2 of treatment/Average CFU cm-2 of the control × 100) - 100], with positive values for the increase of UFC and negative values for the reduction concerning the control.

[3]Calculation not performed because there is no formation of a Colony-Forming Unit (UFC).

For more conclusive results, further studies are needed concerning the colony size and CFU, spore germination, and evaluation of the bioinsecticide Agree® using other techniques.

4. Conclusions

None of the insecticides was compatible with the biological product Agree® (*Bacillus thuringiensis* var. *kurstaki* and *Bacillus thuringiensis* var. *aizawai*).

Acknowledgments

The present work was carried out with support from the Federal University of Mato Grosso do Sul - UFMS/MEC - Brazil and from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (Capes) - Finance Code 001.

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