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Efficacy of Insecticides Applied to Soybean Seeds for Controlling Lepidoptera Caterpillars

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Abstract

The use of high-quality seeds is essential for the crop to express its full potential. The application of seed treatment constitutes a widely spread operation, every year new products are discovered and used for seed treatment, which can provide a protective effect against pests that attack the seeds, as well as physiological effects to favor the growth and initial development of plants, however, little is known about the influence of insecticides on seed germination and the emergence of soybean seedlings. The experiment was carried out in the Seed Quality Analysis Laboratory and in the experimental field of the Agrarian Sciences Faculty of the National University of Asunción-Paraguay. The objective consisted of evaluating the efficacy of chemical insecticides applied to soybean seeds to control lepidopteran caterpillars during the implantation of the crop, and the effect on the germination of seeds in the laboratory and the emergence in the field. Commercial soybean seeds were used and the treatments were the following (in cc of c.p./100 kg seeds): control (without treatment), thiamethoxam (200), cyantraniliprole + thiamethoxam (200), thiamethoxam + lambda-cyhalothrin (200), imidacloprid (400), imidacloprid + thiodicarb (500) and fipronil (200). The variables evaluated were: germination under laboratory conditions, number of larvae presented at 14, 21 and 28 days after emergence, seedling emergence and efficacy of chemical products in the field. The design used in the field was complete block to the random and in laboratory fully randomized, with seven treatments and 4 replications with a total of 28 experimental units for both trials. The data obtained were compared through the analysis of variance using the statistical software SASM-Agri and those variables that presented significant statistical differences between the means of the treatments were compared using the Scott-Knott test at 5% probability, while efficacy was determined by the Abbot formula. The data show that the chemical insecticides did not affect the germination of seeds in the doses tested. The highest percentages of emergence in the field were obtained with the insecticides thiamethoxam, cyantraniliprole + thiamethoxam, imidacloprid and imidacloprid + thiodicarb. At 28 day after seedling emergence the active ingredients cyantraniliprole + thiamethoxam and imidacloprid + thiodicarb achieved the greatest reduction in the lepidoptera population. Seed treatment with the active ingredients used in this study is an effective practice for the control of *Spodoptera frugiperda*, *Spodoptera cosmioides* and *Agrotis ipsilon* caterpillars during the initial stage of soybean cultivation.

Keywords: germination, vigor, insecticides, control efficacy, lepidoptera

1. Introduction

The main insect pests in Paraguay are *Agrotis ipsilon*, *Spodoptera eridania*, *S. cosmioides*, *S. frugiperda*, *Helicoverpa armigera*, *Anticarsia gemmatalis*, *Diabrotica speciosa*, and the stink bug complex (Hemiptera: Pentatomidae). *S. eridania*, *S. cosmioides* and *S. frugiperda* were registered through collections carried out in the departments of Alto Paraná, Itapúa, Canindeyú, Caazapá, San Pedro and Caaguazú (Cabral et al., 2018; Gómez et al., 2015).

The defoliation of caterpillars in soybean cultivation is the main reason for the application of insecticide products, which denotes difficult control in certain caterpillars. In addition to *S. frugiperda*, there are other species that cause crop damage, including *S. cosmioides* and *S. eridania* (Gómez et al., 2009).

The key to pest control is in the use of crops that have been genetically modified (GMO), however, entomofauna can naturally adjust to the point where those considered unimportant and called secondary pests can, in periods of time relatively short, turned into the main pests of crops (Gómez et al., 2015).

It is therefore necessary to evaluate the behavior of *Spodoptera* spp. in Bt soybean crops, since there are precedents regarding the natural tolerance of the species to the Cry1A protein and resistance to insecticides (Murúa et al., 2018). Insecticide resistance is an important factor that has influenced pest control for more than half a century (Sparks, 2015; Bialozor et al., 2020).

For this reason, the use of high quality seeds and the adoption of seed treatment techniques with insecticides and fungicides for the initial protection of the seedling are essential for the crop to express its maximum performance.

Seeds are the starting point for production, a good quality seed represents the strategic input par excellence that allows sustaining agricultural activities, contributing significantly to improving their production in terms of quality and profitability (Doria, 2010). High quality is directly reflected in the resulting crop, in terms of population uniformity and higher productivity (Popininis, 1985).

As the perception of the value of seed increases and the importance of protecting and / or improving its performance both physiologically and economically, the range of products available for seed treatment also increases (Avelar et al., 2011). The insecticides used for this purpose are characterized by their systemic action, low vapor pressure, solubility in water, slowly solubilized to be absorbed by the roots, constantly, giving the seedling an adequate protection period against insects (Silva, 1998).

Some insecticides can confer physiological effects on the growth and early development of plants, as well as the effect of protection against insects (Horii & Shetty, 2007). The positive effect on the growth and development of plants provided by the application of an active ingredient is known as the phytotonic effect (Castro et al., 2008).

Another aspect to consider is that seed treatment can reduce the number of insecticide sprays on soybeans, especially in the vegetative phase of the crop, reducing the impact on the agroecosystem, and not compromising the development of the complex of natural enemies present in the crop (Castro et al., 2008).

It should be noted the importance of guaranteeing the reduction of the possibilities of resistance development of existing products, since in many cases alternative compounds that have the same attributes or low cost may not be available (Sparks, 2015).

As an alternative to control in the early stages of development of soybean cultivation, knowing which insecticides for seed treatment represent the most appropriate technology for crop protection, knowing the effectiveness of currently available insecticides and new technologies applied to seeds, in order to generate relevant information on the effects and efficacy of the products in the field and laboratory, with the aim of achieving a better establishment of the seedlings.

The objective of this study was to evaluate the efficacy of chemical insecticides applied to soybean seeds for controlling pests during the implantation of the crop and the possible effects on seed germination in laboratory and field emergency conditions.

2. Materials and Methods

2.1 Location of the Experiment, Soil and Climate Features

The experiment was conducted at the Seed Quality Analysis Laboratory at a property located on the experimental field at the Faculty of Agricultural Sciences (FCA) in National University of Asunción (UNA), Central Department, Republic of Paraguay (25°19′ S. latitude and 57°31′ W. longitude), with an altitude of 144 m above sea level, the topography is undulating, with an approximate slope of 3.5% in the northeast-southwest direction, the soil is a Rhodic paleudult, with a loamy to sandy loam texture on the surface horizon. The climate is characterized by a minimum average annual temperature of 17.9 °C and an average rainfall of 1200 mm/year.

2.2 Treatments With Chemical Insecticides

For the seed treatment, insecticides were used individually and in combination of the active ingredients (in cc of c.p./100 kg seeds): thiamethoxam (200), cyantraniliprole + thiamethoxam (200), thiamethoxam + lambda-cyhalothrin (200), imidacloprid (400), imidacloprid + thiodicarb (500) and fipronil (200).

For each treatment, 1kg of commercial seeds of the SYN 1359S Ipro variety was used, which is most outstanding biological characteristics are: indeterminate growth, cycle of 130 days, average plant height 96 cm, high productive potential and optimal sowing time between the months of September and October (Syngenta, 2016).

The seeds were introduced into a treater machine brand Gustafson, rotary system and application cycle of 5 min (1 min injection, 4 min of mixing and drying) in order to obtain a suitable distribution of the products, using a device with air injection, sprayed with 30 ml syringes, mixing the active ingredient and a quantity of distilled water corresponding to each dose, obtaining good homogeneity and coverage. Then, the seeds were stored in cold for 24 h at 16 °C, until use.

2.3 Germination Tests

The analysis was performed according to the instructions described by the International Seed Testing Association (ISTA, 2017) using 4 replicates of 50 seeds for each treatment. The seeds were placed in previously disinfected plastic trays using sterilized washed sand moistened with distilled water as substrate and taking into account the water retention capacity in the substrate. Later, the trays were covered with plastic bags to maintain humidity and placed in germination chamber at a constant temperature of 25 °C with lighting for 24 hours for 8 days. The percentage of normal seedlings was evaluated which presented all the essential structures such as: root system, aerial part, terminal buds and cotyledons.

2.4 Emergence Seedling Tests

Each treatment was sown in plots 5 m long and 0.45 m between rows. The seeds were sown at a rate of 15 per linear meter, at a depth of 5 cm. 15 ml of irrigation were applied with a sprinkler system at the time of sowing and 5 days later. Evaluations were made 10 days after planting and recording the percentage of seedlings that emerged in relation to the total of seeds sown, according to the following formula described by Fakorede and Ayoola (1980):

$$E\ (\%) = \frac{\text{Number of plants emerged at 10 DAS}}{\text{Total number of seeds sown}} \times 100 \quad (1)$$

where, E (%) = Percentage of emergence; DAS = Days after sowing.

2.5 Efficacy of Insecticides Applied Alone and in Combination

The control efficacy of each insecticide was evaluated by determining the percentage of control using the formula adapted by Abbott (1925):

$$\text{Efficacy}\ (\%) = 1 - \frac{\text{n. in T. after treatment}}{\text{n. in Co. after treatment}} \times 100 \quad (2)$$

where, n = Number of live insects; T = Treatment; Co = Control, without chemical treatment.

The count of insects present and the identification of species were carried out at 14, 21 and 28 days after the emergence of seedlings, the evaluation was carried out at 1m from the central row, monitoring by direct observation at two points and recording the number of larvae present on each date.

2.5 Experimental Design and Statistical Analysis

The design used was complete randomized blocks for the field experiment and completely randomized for the laboratory, with 7 treatments and 4 repetitions including the control without chemical treatment, with a total of 28 experimental units. The data obtained were analyzed through the analysis of variance and the means between the treatments compared by means of the Scott- Knott test at 5% probability, using the statistical software SASM-Agri (Canteri et al., 2001).

3. Results and Discussion

3.1 Germination and Emergence of Seeds

The germination test did not show significant differences between treatments and not on the variable seedling emergence in field (Table 1).

Table 1. Power germinative (G) and seedling emergence (E) in soybean seeds, variety SYN 1359s Ipro, treated with insecticides in different doses. San Lorenzo, PY

Treatment	Dose (cc of c.p./100 kg seeds)	G (%)	E (%)
T1 Control without treatment	-	94 ns*	83 c
T2 Thiamethoxam	200	95	93 a
T3 Cyantraniliprole + thiamethoxam	200	94	95 a
T4 Lamdacyhalothrin + thiamethoxam	200	92	90 b
T5 Imidacloprid	400	95	94 a
T6 Imidacloprid + thiodicarb	500	95	93 a
T7 Fipronil	200	92	90 b
C.V. (%):		3.8	1.79

Note. * ns = not significant; c. p. = commercial product.

Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5%.

It can be observed in Table 1, that the insecticides used did not present negative effects on the germination of the seeds. The percentages obtained in all treatments were higher than those stipulated in the described standards for the marketing of seeds controlled in Paraguay, regulations of Seed (SENAVE, 2014) which determines a 75% minimum percentage of germination of soybeans.

Similarly, Dan et al. (2012), and Tavares et al. (2014) reported that they did not observe differences in the germination of soybeans treated with thiamethoxam, imidacloprid and fipronil, likewise, Bem Junior et al. (2019) found that the germination of soybeans of the BMX Potência RR variety is not negatively influenced by the insecticides cyantraniliprole, imidacloprid + thiodicarb and fipronil.

Unlike Amaral et al. (2008), indicated that soybeans treated with the active ingredients thiamethoxam presented a lower germination percentage compared to an untreated control. Such effects, according to Ludwig et al. (2011) could be related to the action of the active ingredient on the seeds, which can have a phytotoxic effect when high doses are used reducing germination thereof.

According to Carmona et al. (2010) phytotoxicity in seeds is manifested by reduced germination of seeds, abnormal seedlings, shorter, with thickened, vitreous and fragile hypocotyls. Such effects have not been observed in the present study.

As for the emergence of the seedlings, it can be seen (Table1) that with all the insecticides have emergence percentages higher than the control. The active ingredients thiamethoxam, cyantraniliprole + thiamethoxam, imidacloprid and imidacloprid + thiodicarb achieved the highest percentages of emergence in relation to the control and other chemical treatments.

In reference to the results obtained, we can mention the observations made by other authors such as Dan et al. (2012) who inform that there are insecticides that, in addition to providing control over pests, can act physiologically, assisting together in the initial growth and development of the plant. Similarly, Carvalho et al. (2011) point out that there are insecticides that acts in activating proteins transporting cell membrane, allowing the greatest ion transport, increasing mineral plant nutrition and promoting positive responses in the development and plant productivity.

Corroborating this assertion, Hossen et al. (2014) verified at best expression of the germination percentage and germination rate working with wheat seeds treated with the insecticide thiamethoxam in relation to the untreated seeds. In another investigation, it was found that soybeans treated with the same active ingredient presented higher amino acid content, enzymatic activity and synthesis of plant hormones that make the seedlings have a greater response to these proteins, allowing a better expression of the seedlings and greater tolerance for stressful situations.

Carvalho et al. (2011) point out that there are insecticides that can promote greater efficiency in enzymatic activation both in seeds and in adult plants, where the greater enzymatic activity would increase both the primary and secondary metabolism, thus increasing the synthesis of amino acids precursors of new proteins. And the endogenous synthesis of plant hormones, where the responses of plants to these proteins and hormonal biosynthesis would be related to significant increases in vigor.

It is known that the establishment of seedlings in the field constitutes the priority of the producer at the time of sowing, for this reason it is important to emphasize what is defined by Costa et al. (2018) who point out that germination is a factor of great importance, since once the lot has a low percentage of emergence, this causes failures in the population of plants in the field and probable economic damage for the producers.

Likewise, Marcos-Filho (2016) emphasizes that a rapid and uniform emergence, as well as the consequent establishment of seedlings constituted by vigorous seedlings, are essential manifestations to ensure adequate plant performance, uniformity of development, final crop yield and quality of the product.

Through seed treatment with chemical insecticides was achieved 7 to 10 percentage points higher than the percent seedling emergence obtained in the control. If is calculated the number of plants as per hectare using seeds treated with cyantraniliprole + thiamethoxam, would obtain a density of 316 663 plants by hectare (pl./ha.), taking into account the 95% seed emergence that managed to field.

Without the aid of chemical treatments effective for the control of pests initial could obtain a loss of 40 000 pl./ha. and consequently, a lower grain yield per ha.

3.2 Efficacy of Insecticide Control

3.2.1 *Spodoptera frugiperda*

In Table 2, it can be observed that there are significant statistical differences between the treatments on the population of caterpillars registered during the monitoring (Table 2), in addition, the efficacy of the insecticides on *S. frugiperda* is appreciated.

Table 2. Number of *S. frugiperda* caterpillars per linear meter in three sampling dates after emergence and insecticide control efficacy (%) in soybean cultivation variety SYN 1359s Ipro. San Lorenzo, PY

Treatment	14 DAE	21 DAE	28 DAE	Efficacy (%)
T1 Control without treatment	2.50 a	2.90 a	3.30 a	0
T2 Thiamethoxam	0.80 b	1.30 c	1.20 c	90
T3 Cyantraniliprole + thiamethoxam	0.80 b	0.90 c	0.70 d	97
T4 Lamdacyhalothrin + thiamethoxam	0.80 b	0.90 c	0.96 d	95
T5 Imidacloprid	0.90 b	0.90 c	1.50 c	89
T6 Imidacloprid + thiodicarb	0.80 b	0.70 c	0.96 d	97
T7 Fipronil	2.00 a	2.20 b	2.60 b	42
C.V. (%):	299	20.99	14.37	

Note. DAE = days after seedling emergence.

Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5%.

Regarding to the first two monitoring made at 14 and 21 days after the emergency (DAE), checks greater infestation of caterpillars in the control and with the insecticide fipronil, verifying one lower number of tracks recorded in the other chemical treatments.

At 28 DAE it was found that the insecticides cyantraniliprole + thiamethoxam, lambda-cyhalohtrin + thiamethoxam and imidacloprid + thiamethoxam had a lower population of caterpillars, while the control, on the contrary, was the one who presented the highest infestation of this pest.

Given effectiveness of control products, it can be verified that all insecticides reduced the number of caterpillars, except for the insecticide fipronil, which registered the lowest control efficacy. The insecticides used cyantraniliprole + thiamethoxam and imidacloprid + thiodicarb provided the highest percentages of control in caterpillars of *S. frugiperda*.

Results similar to those obtained in this study were recorded by Ceccon et al. (2004) in an experiment carried out in production plots in the state of Sao Paulo, Brazil. The authors demonstrated that there was a lower incidence of *Spodoptera frugiperda* in corn plots treated with the active ingredients thiodicarb and thiamethoxam, they also stated that those treatments in which these active ingredients are used higher percentages of control obtained 80%.

Triboni et al. (2019) highlight that the treatment of soybean seeds with insecticides from the chemical group of diamides, such as cyantraniliprole, constitutes the best option for the control of *Spodoptera frugiperda*, significantly reducing the consumption of foliar area and rapidly increasing the mortality of caterpillars.

Some authors like Yadav et al. (2012), and Pes et al. (2020) in studies on the application of insecticides chemicals in seeds mention that the diamide cyantraniliprole was effective for controlling species *Spodoptera*, obtaining 100% mortality in field conditions, they also highlighted the capacity absorption and redistribution of cyantraniliprole through the plant, conferring a residual action to prolonged and satisfactory control of the caterpillar.

Likewise, Selby et al. (2017), emphasizes the excellent spectrum of activity of this diamide to a wide range of insects, including lepidoptera. The high solubility in water of this insecticide was pointed out by (Barry et al., 2014). According to Selby et al. (2016), this feature will provide high mobility on the ground.

The translocation of insecticides is crucial for the control of insect pests in plants, since it allows the insecticide to be distributed homogeneously, reach all organs and be used as a food source by the insect (de Boer et al., 2014).

Thrash et al. (2013) emphasize that the mechanism of translocation of cyantraniliprole applied seed treatments was demonstrated to control *S. frugiperda* in soybean plants, under laboratory conditions.

Results similar to those obtained, but with *A. gemmatalis* in this study were demonstrated by Vieira et al. (2019) which state that in a trial of controlling the track, the last evaluation of leaf consumption and mortality made with plants soybean (35 DAE), the treatment fipronil, had the highest foliar consumption and the lowest mortality of caterpillars, while the mixture of cyantraniliprole + thiamethoxam had the lowest leaf consumption.

3.2.2 *Spodoptera cosmioides*

Table 3 shows that there are significant differences between the treatments on the caterpillar population and the efficacy of chemical insecticides on *S. cosmioides.*

Table 3. Number of *S. cosmioides* caterpillars per linear meter on three sampling dates after emergence and insecticide control efficacy (%) in the cultivation of soybean variety SYN 1359s Ipro. San Lorenzo, PY

Treatment	14 DAE	21 DAE	28 DAE	Efficacy (%)
T1 Control without treatment	1.14 a	3.75 a	2.37 a	0
T2 Thiamethoxam	0.83 b	0.7 c	1.09 c	83
T3 Cyantraniliprole + thiamethoxam	0.70 b	0,00 c	0.70 d	100
T4 Lamdacyhalothrin + thiamethoxam	0.70 b	0,00 c	0.96 c	95
T5 Imidacloprid	0.96 a	0.50 c	1.18 c	79
T6 Imidacloprid + thiodicarb	0.70 b	0.00 c	0.70 d	100
T7 Fipronil	1.05 a	2.00 b	1.90 b	38
C.V. (%):	20.12	60.09	20.24	

Note. DAE = days after seedling emergence.

Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5%.

In the three stages evaluated, the control was the one with the highest percentage of *S. cosmioides* infestation, followed by treatment 7 (fipronil), while the other chemical insecticides controlled this pest more efficiently. At 28 DAE, cyantraniliprole + thiamethoxam and imidacloprid + thiodicarb were the most efficient, standing out from the others.

Regarding the control efficacy, the insecticides applied in mixture cyantraniliprole + thiamethoxam and imidacloprid + thiodicarb presented the highest control percentages. The insecticides fipronil demonstrated the lowest percentage with 38% efficacy.

Kahl (2015) mentions that the use of insecticides from the chemical group of anthranilic diamides is on the rise, not only because of their long persistence once applied, but also because of their control effectiveness and low environmental impact.

Trash et al. (2013) point out that the insecticide cyantraniliprole applied to seeds, significantly reduced the survival of caterpillars of the genus *Spodoptera*, in a study carried out in laboratory, by obtaining data on pest control from the fourth day after the infestation, also mentions that this product could be useful to reduce the number of foliar applications necessary for lepidopteran pests as survival controls in the growth stage V3.

3.2.3 *Agrotis ipsilon*

Regarding to the *A. ipsilon* caterpillar, the number of individuals registered at 14, 21 and 28 days and the control efficacy of the insecticides, indicate that there are significant statistical differences between the treatments.

Table 4. Number of *A. ipsilon caterpillars* per linear meter in three sampling dates and effective control insecticides (%) in the soybean variety SYN 1359s Ipro. San Lorenzo, PY

Treatment	14 DAE	21 DAR	28 DAE	Efficacy (%)
T1 Control without treatment	1.25 a	5.50 a	2.68 a	0
T2 Thiamethoxam	0.70 b	0.75 c	0.83 d	93
T3 Cyantraniliprole + thiamethoxam	0.70 b	0.00 c	0.83 d	98
T4 Lamdacyhalothrin + thiamethoxam	0.70 b	0.00 c	0.96 d	96
T5 Imidacloprid	0.70 b	0.50 c	1.31 c	87
T6 Imidacloprid + thiodicarb	0.70 b	0.25 c	0.83 d	96
T7 Fipronil	0.83 b	1.50 b	2.12 b	58
C.V. (%):	23.91	39.17	17.81	

Note. DAE = days after seedling emergence.

The values correspond to the mean of the four repetitions per treatment. Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5%.

It can be seen that in the three monitoring carried out, all the active ingredients decreased the population of *A. ipsilon*, regard to the control. At 14 DAE, all chemical treatments behaved in a similar way, presenting lower populations of caterpillars with respect to the untreated control. Likewise, at 21 DAE it is verified again that the witness registered a greater number of caterpillars. On the other hand, all the chemical products managed to reduce the populations of the pest. At 28 DAE, thiamethoxam, cyantraniliprole + thiamethoxam, lambda-cyhalothrin + thiamethoxam and imidacloprid + thiodicarb presented lower populations of *A. ipsilon*.

Regarding the control efficacy of the products, cyantraniliprole + thiamethoxam obtained the highest control percentage with 98% efficacy. On the other hand, the fipronil insecticide presented the lowest percentage of control with 58% efficacy.

Zhang et al. (2019) obtained similar results as they found that the chemical treatment with the insecticide cyantraniliprole significantly reduced the infestation of *A. ipsilon* in maize. According to Clavijo (2008), the distribution of neonicotinoids in the seed and in the plant is slowly metabolized and remains available for a long period of time close to 30 days, as can be observed in the treatments with thiamethoxam and imidacloprid in this work.

4. Conclusions

The data obtained in the present test allow the verification of the efficacy of the active ingredients evaluated over 28 days, noting the effect of insecticides on seed germination, seedling emergence and percentage of control over the initial pests in soybean cultivation.

The chemical insecticide thiamethoxam, thiamethoxam + lambda-cyhalothrin, cyantraniliprole + thiamethoxam, imidacloprid, imidacloprid + thiodicarb and fipronil did not affect the germination of the soybean seeds at the evaluated doses.

In addition, through the use of insecticides, a better establishment of the field crop was achieved during the initial stages, favoring a better expression of the emergence of seedlings.

The insecticides thiamethoxam, cyantraniliprole + thiamethoxam, imidacloprid and imidacloprid + thiodicarb obtained the highest percentages of emergence in the field.

At 28 days after seedling emergence active ingredients cyantraniliprole + thiamethoxam and imidacloprid + thiodicarb achieved a greater reduction in population of lepidoptera regard to insecticides evaluated.

The smallest reduction in the lepidoptera population with regard to the insecticides evaluated was registered with the Fipronil insecticide.

Seed treatment with the active ingredients used in this study was practically effective for the control of *Spodoptera frugiperda*, *S. cosmioides* and *Agrotis ipsilon* during the initial stage of soybean cultivation.

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