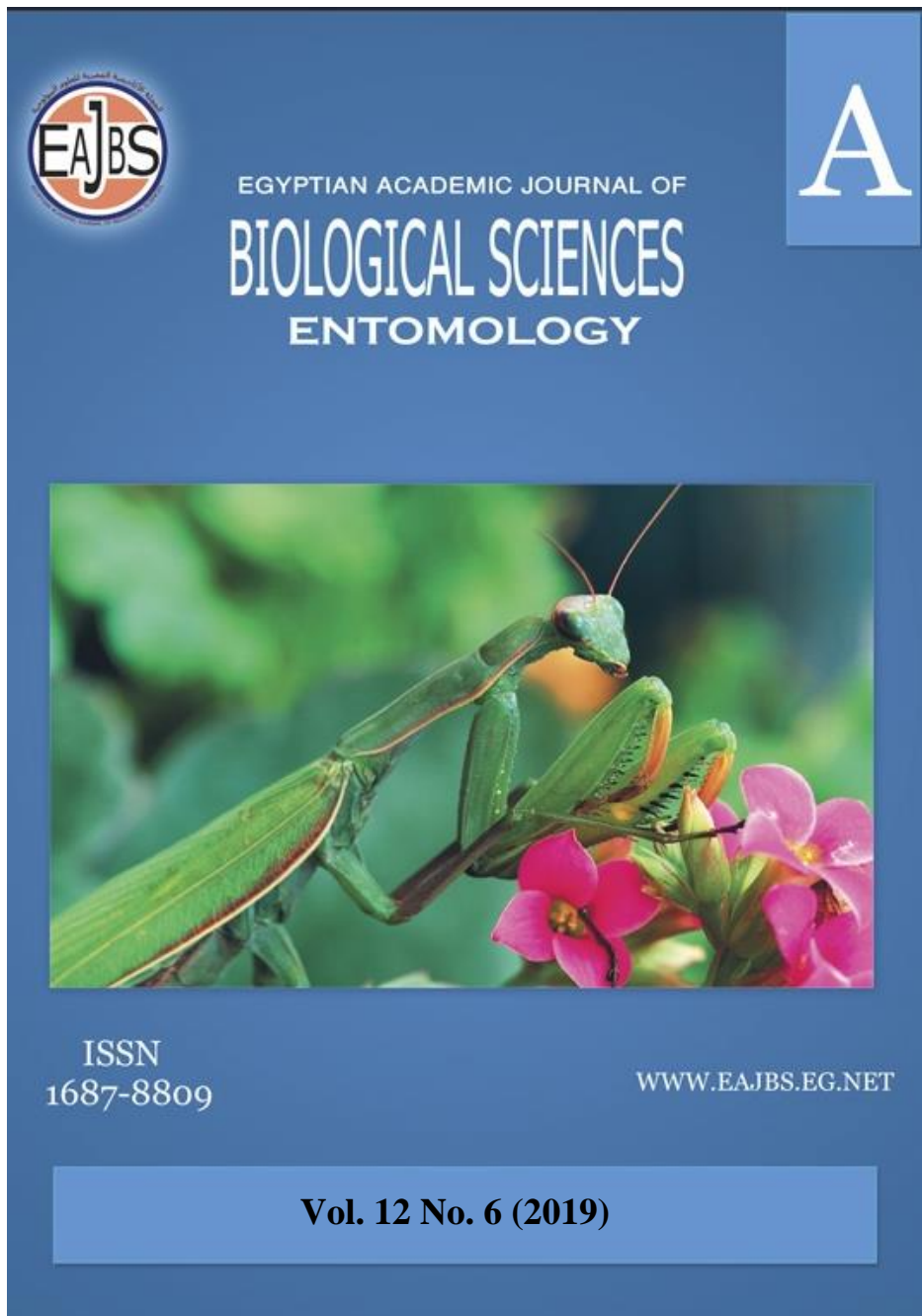
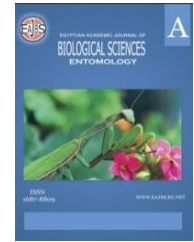


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Nanoparticulated Sulfur and Essential Orange Oil (Oro Solve[®]) Increases Efficiency of *Euschistus heros* Control In Soybean

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ABSTRACT

Soybeans stand out among the main crops of world economic interest, Brazil being one of the main producing and exporting countries. Thus, this work aimed to evaluate the efficiency of insecticides used alone and with the addition of adjuvant formulas based on orange oil and nanoparticulated sulfur (Oro Solve[®]) in the control of the neotropical brown stink bug in soybean. The experiment was carried out at the Experimental Farm of the State University of Maringá – UEM, located in the city of Umuarama – PR. Samples were collected and periodically evaluated at predetermined intervals: an analysis prior to application and analysis was performed at 1, 3, 5 and 10 days after application. On the 10th day after the beginning of the experiment, a new application was performed, with subsequent evaluations 1 and 3 days after reapplication. In the evaluations, the number of stink bugs found per sampling and the efficiency of control in each treatment was noted. It was evaluated by manual harvesting of 1.0 meters in three lines of the useful area of each plot, with a posterior trail, using a stationary trail. After weighing, the grains or values were transformed to kg.ha⁻¹, using the correction of recovery for the water content of 13%. Neotropical brown stink bug occurrence data, insecticide and mixtures evaluation, as well as soybean yield, were subjected to analysis of variance by the F test, and the means compared by the Duncan and Tukey test (P≤0.05), respectively. The insecticides based on acephate (Perito[®]), imidacloprid + bifenthrin (Galil[®]) and bifenthrin + carbosulfan (Talisman[®]) in addition to Oro Solve[®] were found to show greater bedbug control efficiency. There was a greater extension of the effective control period in the treatments that had adjuvant addition. The application to the tests that were sprayed with insecticides in addition to Oro Solve[®], and the insecticides used alone were used, but they all differed from the control and the percentage increase was always higher in the tests containing the mixture with Oro Solve[®].

INTRODUCTION

The main agricultural products in the Brazilian export basket are derived from soybean, a legume that has, for a very long time, been grown from the south to the far north. Due to the great expansion of cultivated area and management methods, several challenges have been observed for the cultivation of this crop.

Among the main limitations to an increase in yield and reduction in the cost of production stand out those provided by agricultural pests. The soybean crop is subject to pest attack from the germination stage to harvest (Hoffmann-Campo *et al.* 2000).

Phytophagous stink bugs of the family Pentatomidae are among the insects considered as primary pests of soybean because they feed on grains, pods, and branches, and may affect yield, grain quality and reproductive potential (Panizzi & Slansky 1985; Corrêa-Ferreira & Panizzi 1999; Panizzi 2013).

The neotropical brown stink bug, *Euschistus heros* (Fabr., 1794) (Hemiptera: Pentatomidae), the small green stink bug, *Piezodorus guildinii* (Westwood, 1837) (Hemiptera: Pentatomidae), and the green stink bug, *Nezara viridula* (Linnaeus 1758) (Hemiptera: Pentatomidae), are the three most abundant species that occur in soybean crop in most producing regions, with certain variations and emergence of secondary pests such as the green-bellied stink bug, *Dichelops* spp. (Ávila & Grigolli 2014; Ibrahim & Mahmoud 2017).

The colonization of soybean plants by stink bugs usually begins at the onset of the reproductive period, and its population peak can be observed with the end of pod development and the beginning of grain filling (Gazzoni 1998; Panizzi *et al.* 2000; Corrêa-Ferreira 2005).

Chemical control of pests that attack soybean is still the most efficient and rapid method, becoming, in most cases, a necessary practice to maintain positive production rates. However, the use of chemical methods in the context of integrated pest management methods as integrated pest management should be preceded by quantitative pest occurrence assessments and the adoption of safety levels for decision-making, procedures that are already fully developed for soybean cultivation (Bueno *et al.* 2013).

Even as the idea of integrated pest management has become popular, soybean farming has been experiencing increasingly serious problems with phytophagous stink bugs, so the indiscriminate use of broad-spectrum insecticides has caused serious damage to the natural enemy population and is sometimes not effective in controlling the target insect.

Inadequate use of pesticide application technology is another point that directly interferes with control, as poorly distributed spraying can lead to failures to reach the biological target. There are already known cases of resistance of phytophagous stink bugs to certain insecticides, in this condition, the use of selective products and different mechanisms of action is an interesting alternative for control, but the availability of these products is being increasingly restricted (Panizzi *et al.* 2000; Corrêa-Ferreira *et al.* 2010; Bueno *et al.* 2011).

One alternative to problems due to the use of chemical control may be the adoption of plant extracts that act as insecticides, as well as the addition of sulfur in the formulations. These control options develop some advantages when compared to synthetic products, namely: they are renewable, can have dislodging effect, are easily degradable and do not contaminate the environment (Guerreiro *et al.* 2013).

Among the products with dislodging effect, sulfur stands out, mainly due to the release of hydrogen sulfide gases, which act as insect irritants, resulting in greater movement and causing them to contact the insecticide more quickly thereby increasing pest control by insecticides, furthermore sulfur can have a direct suppressive effects on pests (Hanna *et al.* 1997; Prischmann *et al.* 2005; Guerreiro *et al.* 2013).

With such concerns, the objective of this work was to evaluate the efficiency of insecticides used alone and in addition to the essential orange oil and nanoparticulated sulfur

(Oro Solve[®]), in the control of stink bugs and the effect on soybean crop yield.

MATERIALS AND METHODS

The work was carried out at the Research Farm of the State University of Maringá, in the City of Umuarama – PR, during the 2016/17 crop year, in a 0.5 ha area cultivated with soybean, cultivar BRS 388 RR, with an indeterminate growth habit and a population of 300,000 plants ha⁻¹. Basal fertilization with 300 kg ha⁻¹ of the formula 2-20-8 (NPK) and 30 kg ha⁻¹ of K₂O in mulching 35 days after sowing.

The experimental design was randomized blocks with five replications, each repetition composed of a useful area of 4.0 m in length by 3.0 m in width (12 m²), with a row spacing of 0.45 m. To ensure the desired degree of infestation and control level for the start of the test, the crop was monitored to observe the level of pest occurrence. The treatments used were composed of insecticides mixed with the orange oil and nanoparticulate sulfur adjuvant (Oro Solve[®]- commercial product composed of orange essential oil + 45% nanoparticulate elemental sulfur, giving a concentration of 585 g L⁻¹).

The spray volume used was 150 L ha⁻¹. Insecticide applications were carried out with a costal spray, pressurized with CO₂ at a constant pressure of 3 kgf cm⁻², to which a 3.0 m long bar was fitted with 6 conical jet spray tips 0.5 m from each other. At the time of spraying the treatments, the relative humidity was close to 70%, the air temperature was at 25°C, with a cloudy sky and light winds ranging from 1.0 to 2.0 km h⁻¹.

A pre-evaluation of stink bugs counts was performed, and after the application of the treatments as mentioned in Table 1. After the spraying, 5 evaluations were performed 1, 3, 5, 7 and 10 days after the application (DAA). At 10 days after the start of the experiment a reapplication was performed and 2 subsequent new evaluations 1 and 3 days after the reapplication. Each sample was visually inspected with the cloth technique, which is a method that requires a piece of white cloth, 1 m long and 0.5 m wide, attached to two pieces of wood that are placed between two rows of cloth of soybean plants. Plants should be shaken so that the pests will fall down onto the cloth.

Data on the occurrence of stink bugs, according to the treatment adopted on each evaluation date were submitted to analysis of variance by the F test, and the averages compared by the Duncan test (P≤0.05). The control-adjusted efficiency percentages (% E) were calculated using the method of Henderson & Tilton (1955).

To determine the most efficient insecticide considering the total number of stink bugs by grouping the stink bugs occurrence values obtained on all evaluation dates, as well as the effect of the use of orange oil and nanoparticulate sulfur adjuvant (Oro Solve[®]), a 4×2 factorial analysis was used, and 4 insecticides (imidacloprid + bifenthrin - Galil[®]; bifenthrin + carbosulfan - Talisman[®]; thiametoxam + lambda-cyhalothrin - EngeoPleno[®] and acephate - Perito[®]) (Table 1) were adopted with or without the addition of orange oil and nanoparticulate sulfur adjuvant. The data were submitted to analysis of variance by the F test, with the average numbers of stink bugs compared by the Tukey test (P≤0.05).

Table 1. Trade name and active ingredients and their mixtures with Oro Solve[®] characterizing the treatments used in the experiment. Umuarama, 2017.

Treatments	Active Ingredient	Dosage application
		(g ou ml do p.c./ha)
Galil [®]	imidacloprid+bifentrin	400
Talisman [®]	bifentrin+carbosulfan	350
Engeo Pleno [®]	tiametoxam+lambda- cyhalothrin	200
Perito [®]	Acephate	800
Galil [®] +Oro Solve [®]	imidacloprid+bifentrin+(orange oil and sulfur)	400+1,5L/ha
Talisman [®] +Oro Solve [®]	bifentrin+carbosulfan+ (orange oil and sulfur)	350+1,5L/ha
Engeo Pleno [®] + Oro Solve [®]	thiametoxam+lambda- cyhalothrin + (orange oil and sulfur)	200+1,5L/ha
Perito [®] +Oro Solve [®]	Acefate+ (orange oil and sulfur)	800+1,5L/ha
Oro Solve [®]	(orange oil and sulfur)	1,5L/ha
Control	-----	-----

Yield data were estimated by hand-harvesting 1.0 meters in three rows of the useful area of each plot, with subsequent tillage using a stationary tiller. After weighing the grains, the values were transformed to kg ha⁻¹, using the yield correction for 13% water content. Data were subjected to analysis of variance by the F test, and yield averages were compared by the Tukey test (P≤0.05).

For all statistical treatments, Agroestat[®] (Barbosa & Maldonado Júnior 2015) was used. Normality (Shapiro-Wilks) and homogeneity (Levene) tests were performed and, as the number of stink bugs data did not show normality and homogeneity, they were transformed with the conversion factor $\sqrt{(x + 0.5)}$.

RESULTS AND DISCUSSION

The average number of total stink bugs (adults and nymphs) in the previous evaluation was above 3.3 per sampling, a value close to that considered as the limit indicated for stink bugs control in soybean, following the recommendation for the safe management of this pest for production of grains (Corrêa-Ferreira & Panizzi 1999). In addition, it was observed that the total stink bugs values were statistically similar and that the stink bugs population was evenly distributed in the experimental area, allowing the start of the experiment and avoiding possible experimental errors (Table 2).

Table 2. Average number of total (nymph and adult) stink bug before (preliminary assessment), days after application (DAA) and days after reapplication (DAR) of treatments. Umuarama, 2017.

Tratamentos	Evaluation Periods							
	Prior	1 DAA	3 DAA	5 DAA	7 DAA	10 DAA	1DAR	3DAR
1 Galil ^{®1}	3,3	1,9 bcd	1,7 bcd	1,8 cd	3,6 bcd	4,9 bcd	2,0 abc	1,5 b
2 Talisman ^{®2}	4,1	1,8 cd	2,0 bcd	2,5 bc	5,7 ab	6,9 bc	2,9 abc	4,7 a
3 Engeo Pleno ^{®3}	3,4	1,1 d	1,9 bcd	1,9 bc	4,5 abc	5,3 bcd	2,0 abc	1,0 b
4 Perito ^{®4}	2,2	0,6 d	0,7 d	0,6 de	3,7 bcd	4,1 cd	1,8 c	1,7 b
5 Galil [®] +Oro S ⁵	4,8	1,7 cd	1,2 bcd	2,2 bc	2,9 bcd	3,6 d	1,4 c	2,2 b
6 Talisman [®] +Oro S	5,2	2,7 bc	1,4 bcd	2,2 bc	2,1 d	5,1 bcd	1,3 c	2,0 b
7 Engeo [®] + Oro S	5,0	0,7 d	2,0 abc	2,4 bc	2,9 cd	4,7 cd	2,7 abc	1,2 b
8 Perito [®] + Oro S	4,3	0,6 d	0,9 cd	0,5 e	3,4 bcd	3,4 d	1,9 bc	0,9 b
9 Oro S	4,1	3,9 b	2,3 ab	3,9 b	5,4 abc	10,9 a	4,3 ab	5,2 a
10 Control	4,6	5,8 a	3,5 a	7,4 a	7,6 a	7,8 ab	4,8 a	5,7 a

Original averages (transformed into $\sqrt{(x+0,5)}$, for statistical analysis); means followed by the same letter in the column do not differ statistically from each other by the Duncan test 5% probability;¹ imidacloprid+bifentrin; ² bifentrin+carbosulfan; ³ thiametoxam+lambda-cyhalothrin; ⁴ acephate; ⁵ orange oil and sulfur

According to the averages of stink bugs occurrence provided in Table 2, it can be observed that on the date after the beginning of the experiment (1DAA), there was a significant decrease in the average number of stink bugs in all treatments in which insecticides were applied alone or in combination with Oro Solve[®].

It is noted that this trend continues at 3 days after application, but there is already a distinct control action for the insecticide acephate (Perito[®]), used alone or in conjunction with Oro Solve[®], and imidacloprid + bifenthrin (Galil[®]), and bifenthrin + carbosulfan (Talisman[®]), in addition with Oro Solve[®].

At 5 DAA, it was possible to observe that the smallest average of stink bugs remained as obtained for the insecticide acephate and that the highlight for this evaluation data was the mixture of acephate + Oro Solve[®] with an average of 0.5 stink bugs per cloth beat, during the entire experiment.

From 7 DAA onwards, the increase in the average number of stink bugs found in all treatments was observed. However, it should be noted that in the treatment with the addition of Oro Solve[®] in the spray solution, lower averages were observed under the conditions of the previous assessment.

After reapplication of treatments that occurred 10 days after the beginning of the average number of stink bugs sampled, again, the lowest averages were observed in the treatments in which Oro Solve[®] was added to the spray solution.

The efficiency percentage data shown in Table 3 ratify and better clarify those results already described that in most evaluation dates the most efficient treatments were those that had the addition of Oro Solve[®] in syrup spraying, emphasizing the result in stink bugs control, with emphasis on the mixture of acephate + Oro Solve[®].

The results obtained in this research are similar to those obtained by Cook and Gore (2018), who noticed higher efficiencies in red-banded stink bug control when the insecticide acephate was used.

Table 3. Percentage of total stink bug control efficiency (nymph and adult) according to the formula of Henderson-Tilton's. Umuarama, 2017.

Treatments	Evaluate Dates						
	1 DAA	3 DAA	5 DAA	7 DAA	10 DAA	1 DAR	3 DAR
1 Galil ^{®1}	54,34	32,29	66,09	33,97	12,43	33,67	58,11
2 Talisman ^{®2}	65,18	35,89	62,10	15,85	0,00	31,70	6,75
3 Engeo Pleno ^{®3}	74,34	26,55	65,26	19,89	8,07	38,68	74,18
4 Perito ^{®4}	78,37	58,18	83,05	0,00	0,00	28,66	43,26
5 Galil [®] +Oro S ⁵	71,91	67,14	71,51	63,43	55,77	36,81	16,38
6 Talisman [®] +Oro S	58,82	64,62	73,70	75,56	42,16	58,58	46,34
7 Engeo [®] + Oro S	88,90	47,43	70,16	64,89	44,56	6,65	65,06
8 Perito [®] + Oro S	88,93	72,49	92,77	52,14	53,38	9,19	63,78
9 Oro S	24,56	26,27	40,87	20,28	0,00	35,89	34,72
10 Control	-	-	-	-	-	-	-

Means followed by the same letter in the column do not differ statistically from each other by the Duncan test at 5% probability;¹ imidacloprid+bifenthrin; ² bifenthrin+carbosulfan; ³ thiametoxam+lambda-cyhalothrin; ⁴ acephate; ⁵ orange oil and sulfur

Although the acephate insecticide was the best insecticide in stink bugs control under the conditions of this study, Farias *et al.* (2006) observed only 43.58% efficiency of this insecticide in controlling the *P. guildinii* stink bug in soybean crop at 14 days after treatment, indicating it as less efficient, and that the results found here may indicate possible synergism of acephate mixed with Oro Solve[®], implying potentiation of its effect.

Thus, it was observed that the addition of Oro Solve[®] in the spray mixture provided

better responses in terms of stink bugs occurrence and consequent increase in the protection period of the plant against this pest.

The formation of sulfides may inhibit the activity of some enzymes that are involved in oxidative metabolism, as well as impairing respiration and developing vitamin deficit, causing a suppressive effect on Neotropical brown stink bug (Prischmann *et al.* 2005; Gammon *et al.* 2010)

It is noted that the addition of Oro Solve® increased efficiency while still providing longer-lasting protection to insecticides. Addition of adjuvants to insecticides aids and improves product deposition on the plant and targets, and may improve the efficiency of stink bugs control, as observed by Melo (2012) who evaluated the efficiency of adding adjuvants to the insecticide tiametoxam + lambacyhalotrin in stink bugs control, and concluded that Assist® and Naturo'il® adjuvants increased insecticide control efficiency.

When stink bugs averages are observed, and the reference values are those indicated for the level of pest control, either for seed production (2 stink bugs/beat cloth) or for grain production (4 stink bugs/beat cloth) (Corrêa-Ferreira & Panizzi 1999). It can be seen from Figure 1 that stink bugs averages in the assessments performed 1, 3 and 5 DAA were below or close to the level considered to be the most rigorous, adopted for stink bugs control in seed production fields.

From 7 DAA onwards there is an increase in the average, and it is observed that the level of occurrence of bed bugs for the treatments in which insecticides were used alone exceeded the value indicated for pest control, within the definition of integrated pest management for this culture (Bueno *et al.* 2013).

On the other hand, in the treatments using the insecticide + Oro Solve® mixture, the averages remained below or near the level indicated for the control of bed bugs for grain crops.

After the reapplication of the insecticides, isolated and in mixtures, the average number of stink bugs in the stricter levels was reduced again, with lower averages observed for acephate, bifenthrin + carbosulfan, thiamethoxam + lambda-cyhalothrin, and imidacloprid + bifenthrin Oro Solve® (Fig. 1).

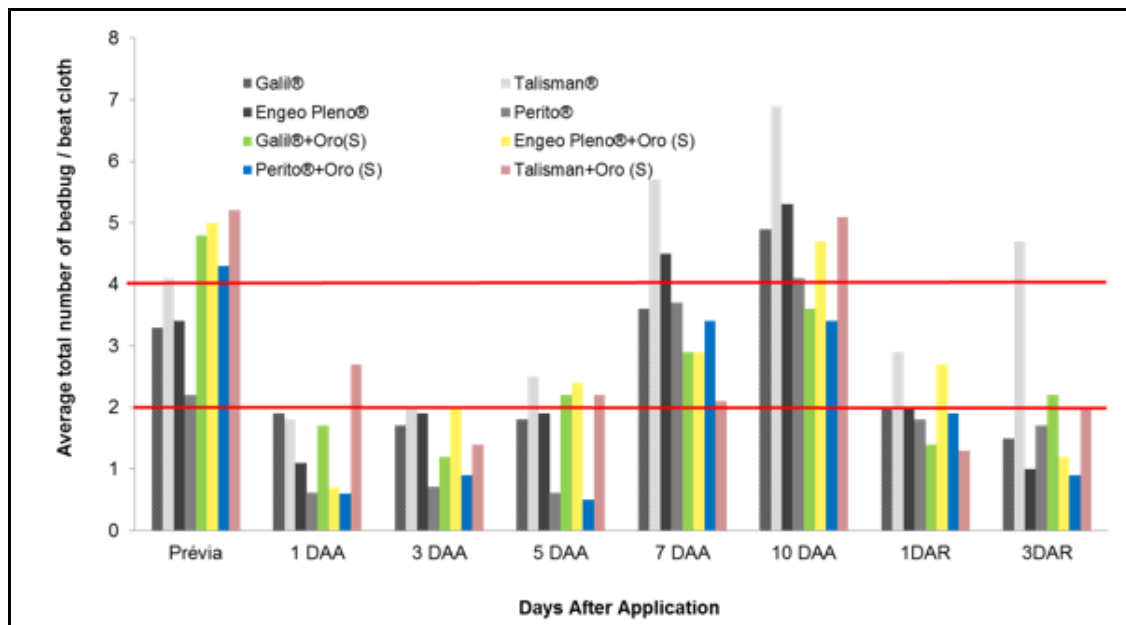


Fig. 1. Total average number (nymphs and adults) of stink bug /batting cloth (2 linear meters) considering whether or not sulfur is used for spraying, and reference values indicated for pest control level for seed production (2 stink bugs / cloth) or grain production (4 stink bugs / beat cloth). Umuarama, PR, 2017.

When comparing the findings of bed bugs found in the experiment when considering only the insecticide tested as treatment, it is possible to note that for the control of the nymphal phase the insecticides acephate and thiametoxam + lambda-cyhalothrin were the ones that stood out, already for bed bugs in the phase. In adulthood, higher efficiency was observed for the insecticide acephate. This result is repeated when considering the total number of insects observed (Table 4).

Table 4. Average number of stink bugs considering the insecticides used in the experiment, without comparison with the Oro Solve® addition. Umuarama, PR, 2017.

Active Ingredient	Nympha	Adulto	Total
1 imidacloprido+bifentrina (Galil ®)	0,70 ab	1,61 ab	2,33 ab
2 bifentrina+carbosulfano (Talisman ®)	1,21 a	1,89 a	3,09 a
3 tiametoxam+lambda-cialotrina (Engeo Pleno ®)	0,64 b	1,87 a	2,45 ab
4 Acefato (Perito ®)	0,61 b	1,16 b	1,77 b
CV %	15,10	12,09	14,20

Original averages followed by the same letter in the column do not differ statistically by Tukey test at 5% probability

However, when evaluating the use of adjuvant in mixtures alone and comparing them with the same active ingredients without the addition of adjuvant regardless of the type of insecticide, it is possible to notice a lower average of bed bugs in the young, adult, and bed bug totals. Oro Solve® was added to the spray solution (Table 5).

Table 5. Average number of stink bugs considering only insecticide-independent use of Oro Solve®. Umuarama, PR, 2017.

Treatments	Nympha	Adult	Total
Without Oro Solve®	0,91 a	1,79 a	2,67 a
With Oro Solve®	0,68 b	1,47 b	2,15 b
CV %	12,99	11,82	10,64

Original averages followed by the same letter in the column do not differ statistically by Tukey test at 5% probability

These results are evidenced by Figure 2, where it can be observed that the averages of bed bugs found varied according to the evaluation date, but it is possible to notice that when there was the addition of Oro Solve® in the syrup the averages were always lower and that only in the evaluation carried out at 10 DAA, the average bed bug level exceeded the level indicated for the use of bed bug control for grain crops.

Figure 3 shows the soybean crop yield data according to the treatment used, as well as the values related to the relative increase of treatment productivity in relation to the control.

It can be noted that all treatments that used insecticide alone or in addition to Oro Solve® differed from the control, but these did not differ from each other. In relative terms it can be said that the increase in productivity ranged from 12.38% to 43.42% in relation to the control. Furthermore, this value was always higher when the Oro Solve® adjuvant was added to the spray solution, especially bifenthrin + carbosulfan and acephate in addition to Oro Solve® treatments, which showed relative gains of over 40% in relation to the obtained productivity to the witness.

In agreement with Sosa-Gomes *et al.* (2001), the small number of active ingredients with different mechanisms and the irrational use of insecticides in soybean crops, to the detriment of IPM techniques, has increased the occurrence of and damage from bed bugs.

One possible solution to this problem is to use the chemical method within the MIP precepts, in conjunction with new control tactics, such as the joint adoption of plant extract products, so that control efficiency is increased, implying, in many cases, the synergistic effect of the mixture without damage to the beneficial entomofauna.

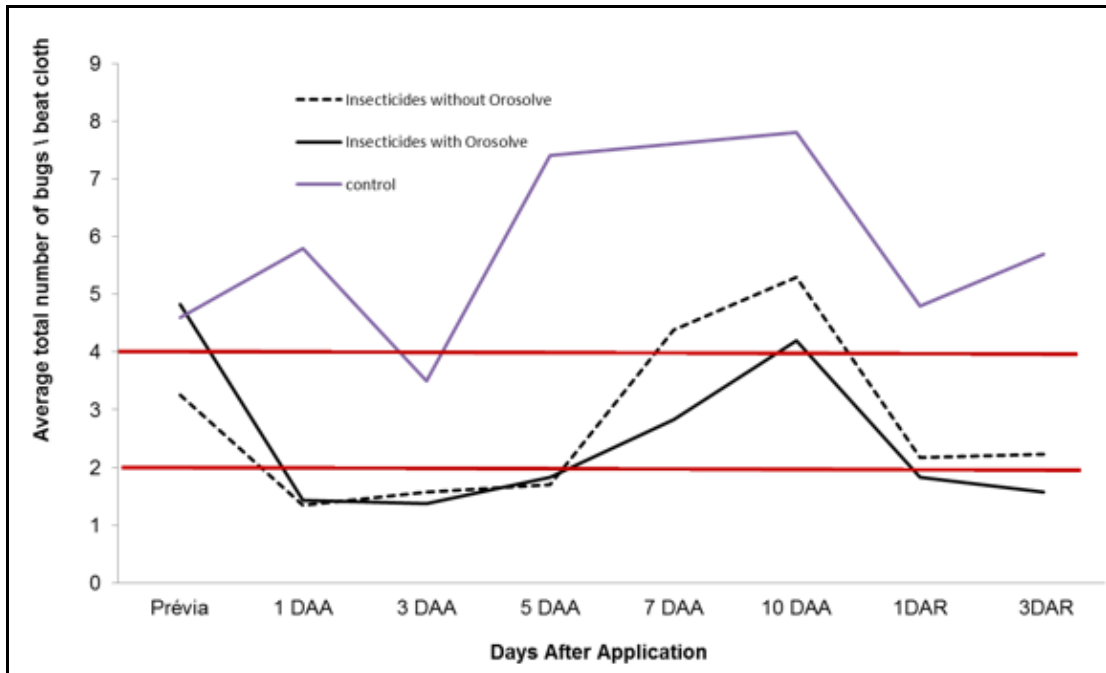


Fig. 2. Total average number (nymphs and adults) of stink bugs / /batting cloth (2 linear meters), varying according to the use of isolated insecticides or the addition of Oro Solve®. Umuarama, PR, 2017.

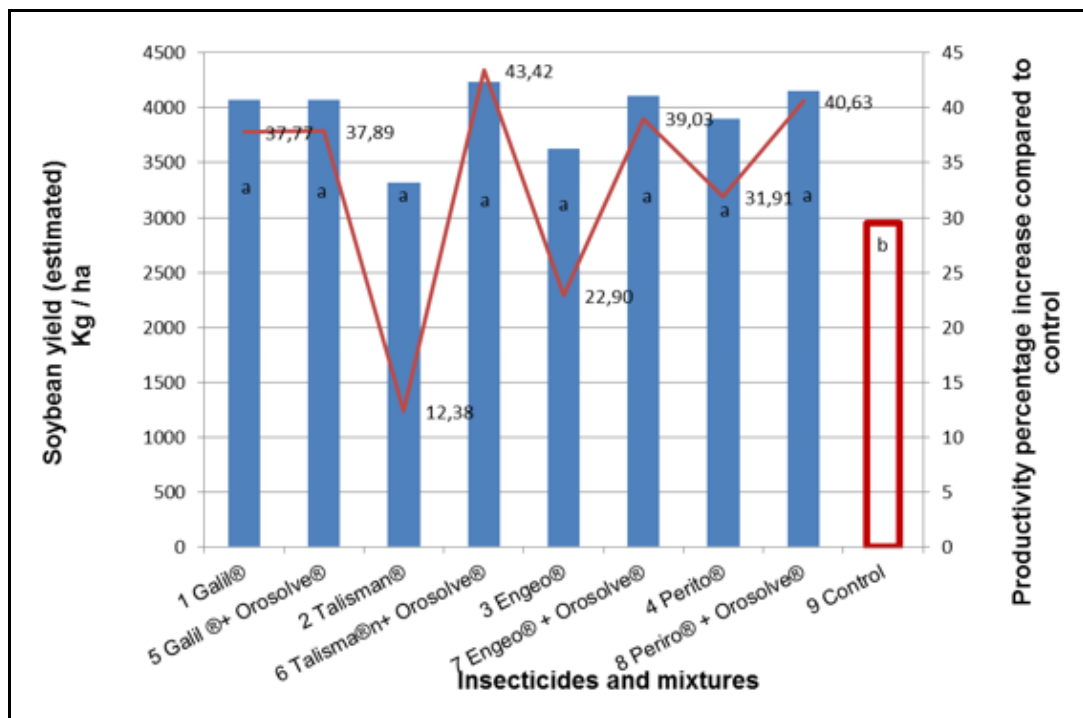


Fig. 3. Average yield and percentage increase of soybean crop yield in relation to the control, according to the insecticide used and its respective mixture with Oro Solve®. Umuarama, 2017.

There was a positive effect due to insecticide action even under strong pressure of bed bug occurrence, especially when the addition of the Oro Solve® adjuvant was adopted. Agreeing with Aguiar-Junior *et al.* (2011), the addition of adjuvants to the spray solution is one of the ways to improve chemical activity or application characteristics. It can generally be said that the addition of adjuvant maximized the action of insecticides.

In addition, soybean crop, due to its high population density and plant swelling, can make it difficult for insecticides to penetrate the middle and lower thirds, not reaching desirable nymphs and adults in these portions of the plant.

The use of Oro Solve® may have caused a smoking and dislodging effect on insects, causing nymphs and adults to walk more and reach plant sites with higher concentrations of insecticides, increasing the action of products, similar to the effect observed by Guerreiro *et al.* (2013), when sulfur was used in addition to insecticides to control *S. frugiperda* in maize.

CONCLUSIONS

The organophosphate insecticide group (acephate) was the best expressed positive results in the control of stink bugs.

The adjuvant Oro Solve® (orange essential oil + 45% nanoelemental sulfur, giving a concentration of 585 g L⁻¹), enhanced the efficiency of insecticides in the control of soybean stink bugs.

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