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Evaluation of the performance of *Spodoptera frugiperda* caterpillars after contamination by bacteria and yeast

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***Abstract*—** *The increase in the use of pesticides in the field has been bringing serious implications for the human health and the environment. In order for pesticides to be lethal only to target pests, the idea of biological control, which proposes eliminating pests from specific pathogens, avoiding causing harm to human beings and their environment, emerged. Baculoviruses, a virus that cause pathogenicity for specific insects, has been used as biological control. However, its laboratory production faces difficulties, one of which is the contamination of the caterpillars used (S. frugiperda - the main pest of soybean and maize) by undesirable microorganisms. The objective of this work was to evaluate the behavior of caterpillars used for the production of Baculovirus after contamination by previously identified microorganisms. For this, was identified the microorganisms present in the caterpillars: Staphylococcus aureus (22.5%), Staphylococcus sp. (42.5%), Klebsiella oxytoca (2%), Pseudomonas aeruginosa (8%), A. flavus and Penicillium sp. (52%) and yeasts (72%). Subsequently, these microorganisms were inoculated individually in healthy caterpillars, which were observed for a period of 12 days, in an ideal environment (temperature 25 º C and Humidity 70%). It was concluded that the bacteria Pseudomonas aeruginosa and Klebsiella oxytoca proved very harmful to the caterpillar, causing weight loss and early death. The caterpillars contaminated by fungi had similar behaviors. The caterpillars contaminated by the bacteria Staphylococcus aureus and Staphylococcus sp. until the 9th day showed no difference in the weight gain rate when compared to the control group. Among the caterpillars of the control group there was no death.*

I. INTRODUCTION

After the second world war, with the green revolution, which aimed to modernize agriculture and increase productivity, there was a major transformation in the field, such as the introduction of new technologies, machines, genetic improvement and agrochemicals. In Brazil, pesticides and chemical fertilizers gained ground in agribusiness in the 1970s, after the implementation of the National System of Rural Credit (SNCR). According to this system, the amount of loans granted depended on the percentage of money to be spent on pesticides. In this way, pesticide sales increased 945.51% between 1998 and 2008 [1].

While Brazil celebrates being a world leader in the agribusiness sector, Brazil also leads a growing dependence on pesticides. According to ANVISA [2]

Brazil is responsible for 1/5 of the world consumption of pesticides, using 19% of the pesticides produced in the world. According to FAO data [3], Brazil appears in the world ranking in first place in spending on pesticides and in seventh when analyzed the amount of pesticide used per area. In the last 40 years, Brazil increased its consumption of pesticides by 700%, while the agricultural area increased 78% in the same period [4].

Between 2002 and 2012, IBGE evaluated a 155% increase in the use of pesticides in Brazil, having jumped from three kilograms per hectare to seven kilograms per hectare. IBGE also classified that 30% of the pesticides used in Brazil are classified as very dangerous [5].

The introduction of pesticides and pesticides in the field includes many benefits such as increased food production due to reduced crop losses and consequently a considerable increase in economic potential. However, this unbridled introduction has serious implications for human health and the environment.

Among the problems caused by the intensive use of pesticides include: the contamination of food, soil, water, animals, the poisoning of farmers, the resistance of pests to active ingredients, the intensification of the emergence of new diseases; the biological imbalance, changing the cycling of nutrients and organic matter; the elimination of beneficial organisms and the reduction of biodiversity [4].

Based on studies, man, animals and the environment are in a dangerous route of contamination by pesticides, since they have great accumulative potential due to their solubility, adsorption, displacement, persistence and toxicity characteristics. Ideally, pesticides should be lethal to target pests but not to non-target species such as man [6; 7].

The damage to man and the environment has brought great concern to various segments of society and has led to a growing demand for new alternatives. The biological control, inserted in the integrated pest management, is one of the viable options to meet the desires of society in the constant search for sustainable solutions.

Biological control was defined [8] as "the action of parasitoids, predators and pathogens in maintaining the density of another organism at a lower level than would normally occur in their absences".

The basic idea of biological control is to control agricultural pests and insects that transmit diseases from the use of their natural enemies, which can be other beneficial insects, predators, parasitoids or microorganisms, such as fungi, viruses or bacteria. The main objective of applying biological control is to control healthy pests, without leaving residues that harm food, the environment and human health [4]. Embrapa has been researching on biological control since the 1980s, aiming at the sustainability of ecosystems by improving the quality of agricultural products, reducing environmental pollution and preserving natural resources. In one of the pioneer works, started in 1984, a survey of the main natural enemies of this pest was done in several corn producing regions of Minas Gerais state, including Sul de Minas, Vale do Rio Doce and Alto Paranaíba. During the survey, between 1984 and 1989, more than 14,000 caterpillars were collected, where several parasitoids of the base orders Diptera and Hymenoptera were found, including several caterpillars killed by virus [9]. This survey extended to the state of Paraná, where a high rate of parasitized and virus-killed caterpillars was detected [10].

Species of the genus Spodoptera are widely distributed worldwide and among the 30 described species, half are considered pests of various crops of great economic importance. Among them, *Spodoptera frugiperda* (J.E. Smith) stands out as it feeds on more than 80 plant species, including cotton, corn and soybean, and can cause losses of up to 73% of agricultural production [11]. Until 2007, this pest was controlled with the application of chemical insecticides. Later, transgenic plants were introduced, allowing genotypes with higher productivity, quality and resistance to insects to be obtained [12]. However, some factors such as tropical climate, massive production without intervals or harsh winters caused the infestation of caterpillars precisely in transgenic plants.

In order to contribute to the fight against the caterpillars *S. frugiperda*, the biological control was introduced, which has as main objective to control agricultural pests and insects from the use of their natural enemies. The baculoviruses are an option for biological control of caterpillars in corn and belong to a group of viruses that have pathogenicity to insects [13]. Baculoviruses are from the family Baculoviridae, have double circular single strand DNA and have great potential to affect arthropods. They have the genera nucleopolyhedrovirus (NPV) and granulovirus (GV) which act in the epithelial cells of the midgut of insects [14]. Among the types of isolates, I-18, I-19 and I-6NR, isolate 6-NR is the only one that does not cause liquefaction of the insect tegument immediately after death, which facilitates the collection of dead caterpillars and the production of biological insecticides on a large scale [15].

The emergence of a commercial formulation of *B. spodoptera* is seen by farmers as an important alternative for pest control, which can be combined with other control tactics. However, to become possible, some characteristics of Baculovirus spodoptera, such as, for example, the purity of the viral inoculum produced, are essential for

satisfactory development on an industrial scale. In this work, the performance of Spodopera frugiperda caterpillars will be evaluated when contaminated by other microorganisms, which is still one of the problems that reduce and hinder the industrial production, raising costs and decreasing quality. In the Baculovirus production process, the caterpillar *S. frugiperda* feeds on baculovirus-contaminated maize leaf and becomes infected by penetrating the viral bodies into its digestive system. The protective protein matrix of the virus is disrupted by the alkaline pH (approximately 11) of the caterpillar gut, and then the virions are released into the digestive lumen of the caterpillar. During the infection process, the insect becomes weakened, loses its motor and feeding capacity, and dies in approximately 5 to 8 days with a discolored body compared to healthy caterpillars. Two days after the death of the caterpillar, the insect's body ruptures, releasing a large amount of virus [16].

This material is purified and its biological activity is verified to evaluate the efficiency of the viral inoculum produced so that it can be used as biological control of pests. Despite the advance in research related to Baculovirus in recent years, its multiplication in laboratory still faces difficulties. According to Sousa [17], knowing the environment favorable to the development of *S. frugiperda* species is essential to optimize the virus production.

Among the factors that influence the virus production in laboratory are: the environmental conditions at the time of production (temperature, pH, humidity, air flows, photoperiod, pressure); the host biology (age, sex and development stages), the concentration and activity of the virus and the purification of the viral inoculum (decontamination by fungi, bacteria and other viruses).

Baculoviruses have been studied as biological control agents since the 1960s [18] and have been extremely promising for their specificity and for being harmless to other living beings and the environment.

II. MATERIALS AND METHODS

2.1 Isolation of contaminating microorganisms

Dead caterpillars of *S. frugiperda* (2nd instar) provided by a biological control industry of Uberaba were used in this study. Fifty samples of dead caterpillars were used, which were macerated in a sterilized pistil. After maceration, a 1 g sample was diluted in 9 ml of 0.1% saline water (10-1), followed by serial dilutions of 10 -2 and 10 -3.

To evaluate the growth of filamentous fungi and yeasts, the Spread Plate technique was used, where dilutions were inoculated on plates containing Potato Dextrose Agar (PDA) culture medium and were incubated at 25°C for 5 days. To evaluate the growth of bacteria, the Pour Plate technique was used, placing the dilutions (10 -1 to 10 -3) on Petri plates pouring Brain Heart Infusion (BHI) agar medium. The plates were incubated at 37°C for 48 hours and then the growth was counted per CFU (n°/dilution) [19].

2.1.1 Identification of Filamentous Fungi

For the identification of filamentous fungi, the microcultivation and macrocultivation techniques were used [19]. The plates were incubated at 25°C for 5 days.

2.1.2 Bacteria Identification

After growth in BHI Agar medium, Gram staining was performed to identify the bacteria for the first time.

For Gram-positive bacteria, the catalase and coagulase tests were used and for Gram-negative bacteria, the biochemical series: Motility (SIM- Sulfate, Indole, Motility), Triple Iron Sugar Medium (TSI), Urea and Citrate were used [2].

2.2 Inoculation of microorganisms isolated and identified in second-stage caterpillars

After isolation and identification of bacteria and fungi, these microorganisms were inoculated individually in "healthy" caterpillars (without apparent contamination) provided by the company, and later the development of contaminated caterpillars was evaluated. These microorganisms were divided into seven different groups, being formed by 50 caterpillars of each group. A group with 50 caterpillars was inoculated with Baculovirus as positive control and another group of the same amount of caterpillars were not contaminated by microorganisms being considered negative control. A total of 450 caterpillars were used.

For the inoculation of the caterpillars, new and organic castor oil plant leaves were immersed in hypochlorite solution with purified water at a concentration of 2% for approximately 20 minutes. After this procedure the leaves were washed in four (4) containers with purified water to remove all excess hypochlorite. Then the castor oil plant leaves were placed in clean sieves to remove excess

moisture and cut uniformly (Figure 1).

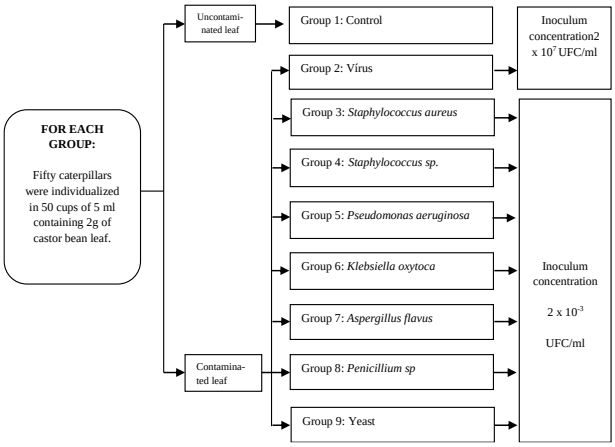

Fig.1. Schematic representation of the division of the inoculated groups in the study.

2.2.1 Monitoring the performance of caterpillars

The parameters observed in the caterpillars during the experiment were: the amount of food consumed by each one; the weight variation (in g); their color and survival.

The microorganism inocula were prepared at a concentration of 2x 10-5 CFU/ml in Brain Heart Infusion (BHI) broth. The hygienized castor oil plant leaves were individually passed through the inocula and after removing the humidity, they were placed in 50 ml coffee cups with PVC lids, as shown in Figures 02 and 03.

Fig.2. Caterpillar on castor bean leaf.

Fig.3: Trays with identified cups.

For the control group, 2g of hygienized castor bean leaf and one healthy caterpillar previously weighed and measured were placed in each of 50 cups. The cups were capped. The same procedure was repeated, but with leaves contaminated with each of the seven identified microorganisms (*Staphylococcus* sp., *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Aspergilus flavus* and yeast) and with the virus. From day 3, after feeding on the leaves, the caterpillars were fed Greene's artificial diet (Table 1), considered the most appropriate for the species [20].

Table 01: Composition of Greene's artificial diet.

Ingredient	Quantity
White beans	102,90g
Wheat Germ	82,30g
Soy Fareli	41,20g
Milk powder	30,90g
Brewer's yeast	51,40g
Ascorbic acid	4,90g
Sorbic acid	2,50g
Nipagin	4,10g
Vitamin solution	8,20ml
Tetracycline	0,10g
Formaldehyde (40%)	4.90ml
Agar	18,90g
Water	1400.00ml

Source: Busato, (2006).

The caterpillars were monitored for 12 days. On the odd days (1st, 3rd, 5th, 7th, 9th 11th days), they were weighed in order to check the weight gain of caterpillars. The diet was weighed to verify the consumption per caterpillar, and the characteristics coloration and survival were observed. On these days, after weighing, the diets and the cups were exchanged. On even days, only the caterpillars were observed (coloration and survival). The

observed colorations were divided into three parameters: brown, black and pinkish/clear.

During the experiment, humidity and temperature parameters were used, of 70% and 25° =/- 1°C, respectively, conditions considered ideal for the growth of *S. frugiperda* caterpillars [17].

2.2.2 Statistics for the evaluation of the weight gain of caterpillars

For the statistical analysis of the results, the weight gain rate of the 9 groups was considered: group of healthy caterpillars (control) and the groups of caterpillars contaminated with Virus, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Staphylococcus* sp., *A. flavus*, *Penicillium* sp. and Yeast. First, an exploratory analysis of the data was performed and the outliers (discrepant data) were excluded considering the non-commitment of the sample size. Afterwards, the comparison of weight gain rate averages was performed, and it was possible to evaluate the difference in weight gain averages of each group in relation to the control.

The ANOVA test was used for the analyses. The distribution of the weight gain rates of most groups was not normal, but when the sample size is greater than 20, the ANOVA test provides reliable data. In the present study, after eliminating the discrepant data, the sample sizes ranged between 45 and 50 for all groups. The statistical tests to compare the weight gain means of the nine groups were performed with confidence degrees of 95%, considering non-normal distribution and unequal variances. For different variances, Welch's ANOVA test was assumed. For simultaneous analysis of difference of means, Games Howell's test was used for different variances and Tukey's test for equal variances.

III. RESULTS

3.1 Identification of micro-organisms

In 2% (1/50) of the caterpillars no growth of microorganisms was observed. In 52% (26/50) of the caterpillars filamentous fungi grew, having been identified in 96% of the caterpillars the fungus *Penicillium* sp. and in 11% the fungus *A. flavus*. Growth of yeasts was observed in 72%. The growth of bacteria was observed in 75% of the caterpillars, being 22,5% identified as *Staphylococcus aureus*, 42,5% *Staphylococcus* sp., 8% *Pseudomonas aeruginosa* and 2.0% as *Klebsiella oxytoca*. The results of the identification of bacteria and fungi are shown in Figure 4.

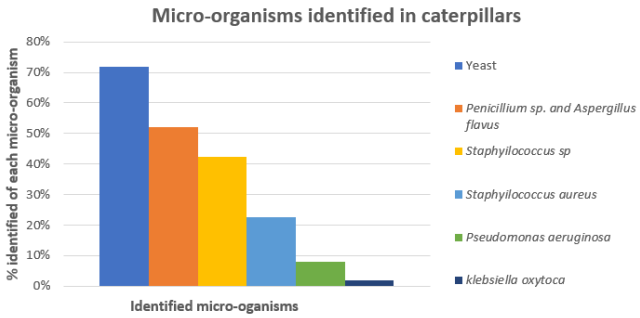

Fig.4. Microorganisms isolated and identified in S. frugiperda caterpillars.

As can be seen, a greater amount of fungi (yeasts and filamentous fungi) than bacteria *(Staphylococcus aureus, Staphylococcus* sp., *Pseudomonas aeruginosa* and *Klebsiella oxytoca*). Among the bacteria, a significant amount belonging to the genus *Staphylococcoccus* was identified and, to a lesser extent, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* were identified.

Rolim [21], in an analysis of the diversity of the gut microbiota of adults of *S. frugiperda* indicated the existence of at least six phyla of Bacteria, being Proteobacteria, Bacteroidetes and Firmicutes the most common. Most bacteria isolated in culture medium from *S. frugiperda* belong to the phylum Firmicutes with the genus Bacillus being the most abundant [22]. Caterpillars collected in corn cartridges showed abundance of *Klebsiella oxytoca* phylotypes [23].

Although a wide variety of bacterial phylotypes have been characterized and associated with lepdopteran intestines, their role in caterpillar physiology and adaptation is poorly known [24; 25].

3.2 Monitoring the performance of caterpillars after inoculation with microorganisms

3.2.1 Assessment of weight gain

The weight gain evaluation was performed on the odd days of the experiment. On day 3, the results of the simultaneous Games-Howel tests for the differences in mean weight gain rates from day 1 to day 3 indicated that: Statistically, the mean weight gain rates of the groups of caterpillars contaminated with *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Penicillium* sp. were significantly different from the mean weight gain rates of the control group. This result can be seen in Figure 05.

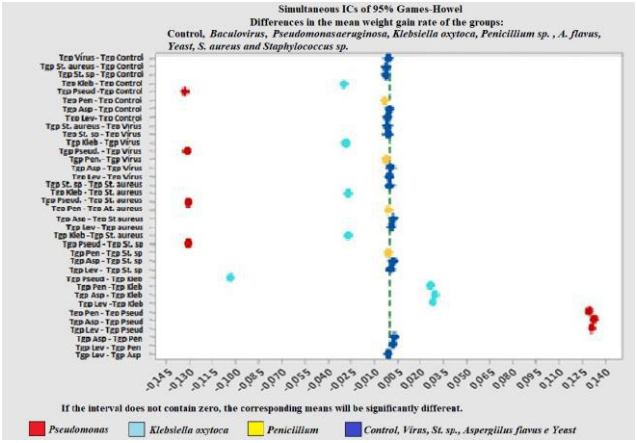

Fig.5. Difference in mean weight gain rate of the groups: Control, Baculovirus, Pseudomonas aeruginosa, Klebsiella oxytoca, Penicillium sp., A. flavus, Yeast, S. aureus and Staphylococcus sp.

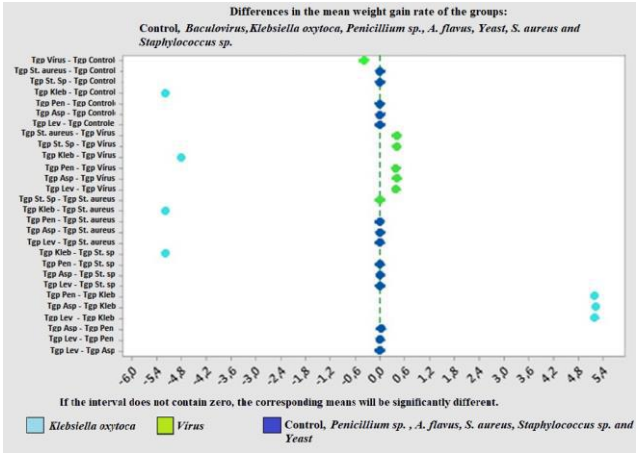

Fig.6. Difference in mean weight gain rate of the groups: Control, Baculovirus, Klebsiella oxytoca, Penicillium sp., A. flavus, Yeast. S. aureus and Staphylococcus sp.

It is also possible to state that the difference in the mean weight gain rate of the caterpillars contaminated with *Pseudomonas aeruginosa* was more significant than the difference in the mean weight gain rate of the caterpillars contaminated with *Klebsiella oxytoca* and the impact on the group contaminated with Penicillium sp. was even smaller.

The other groups of caterpillars (contaminated with *Baculovirus*, *Staphylococcus aureus*, *Staphylococcus* sp. *A. flavus*, and yeast) had no difference in the rate of weight gain in this period.

On day 5, 90% of the caterpillars contaminated with *Pseudomonas aeruginosa* bacteria, which had already lost weight from day 1 to day 3, died. Therefore, it was not possible to use the weight gain data of the caterpillars contaminated with *Pseudomonas aeruginosa* from day 5.

The results of the simultaneous Games-Howel tests for the differences in the mean weight gain rates from the third to the fifth day indicated that: Statistically, the mean weight gain rates of the groups of caterpillars contaminated with *Klebsiella oxytoca* and *Baculovirus* were significantly different from the means of the control group. This result can be seen in Figure 6.

It was possible to state that the difference in mean weight gain of the group contaminated by *Klebsiella oxytoca* bacteria in relation to the control group was more significant than the difference in mean weight gain of the group contaminated by virus. The other groups showed no difference in mean weight gain from the third to the fifth day compared to the control group.

On day 7, 95% of the caterpillars contaminated with *Klebsiella oxytocaque* bacteria had already shown significant weight loss from day 1 to 5, and died. Therefore, it was not possible to use the weight gain data of the caterpillars contaminated with *Klebsiella oxytoca* from day 7. Between days 5 and 7, 10% of the virus-infected caterpillars had died, but data were still available to continue evaluating the weight gain rate of these caterpillars.

The results of Tukey's simultaneous tests for the differences in the mean weight gain rates from day 5 to day 7 indicated that: Statistically, the mean weight gain rates of the groups of caterpillars contaminated with *Baculovirus* and the groups contaminated with the fungi *A. flavus*, *Penicillium* sp. and Yeast were significantly different from the means of the control group. The caterpillars contaminated with the bacteria S. aureus and *Staphylococcus* sp. showed no difference in relation to the control group. These results are shown in Figure 07.

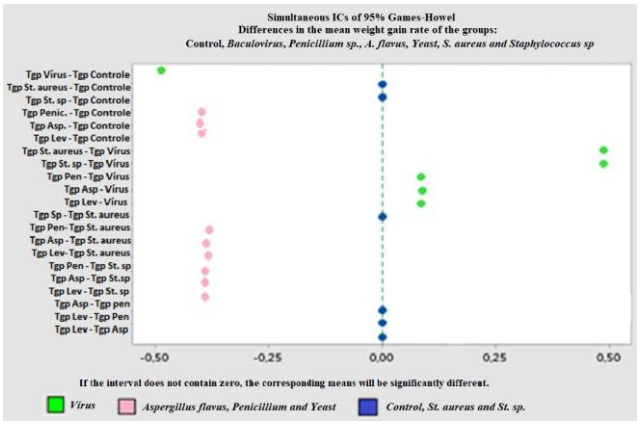

Fig.7. Difference in mean weight gain rate of the groups: Control, Baculovirus, Penicillium sp., A. flavus, Yeast, S. aureus and Staphylococcus sp.

The groups contaminated by *Baculovirus, A. flavus* and *Penicillium* sp. gained less weight than the control group and the groups contaminated by *S. aureus* and *Sthaphylococcus* sp. bacteria showed no difference in weight gain compared to the control group.

On day 9, it was observed that 90% of the *Baculovirus* contaminated caterpillars, which had already shown a significant decrease in weight gain relative to the control since day 5, died. Therefore, it was not possible to use the weight gain data of the caterpillars contaminated with Virus from day 9. Between day 7 and 9, also 84%, 80% and 90% of the caterpillars contaminated with the fungi *Penicillium* sp., *A. flavus* and yeast, respectively. Thus, the evaluations continued only with the control groups and the groups contaminated with bacteria *Staphylococcus aureus* and *Staphylococcus* sp., which until day 9 showed no difference in the mean weight gain.

The results of Tukey's simultaneous tests for the differences in the mean weight gain rates from day 9 indicated that: Statistically, the mean weight gain rates from day 7 to day 9 of the groups of caterpillars contaminated with *S. aureus* and *Staphylococcus* sp. bacteria were equal to each other and significantly different from the control group, as can be seen in Figure 8.

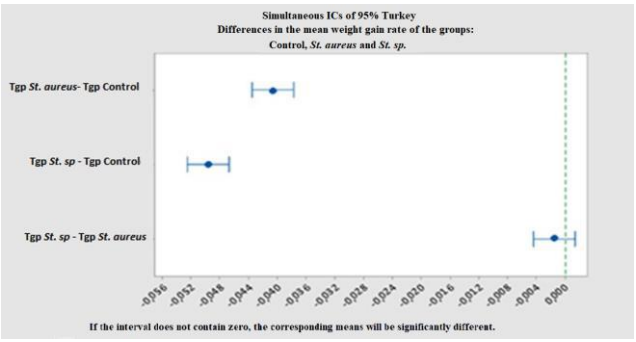

Fig.8. Difference in mean weight gain rate of the groups: Control, S. aureus and Staphylococcus sp.

On the 11th day, no caterpillar died. Again, the growth rate of the groups of caterpillars contaminated with *S. aureus* and *Staphylococcus* sp. were equal among themselves and statistically different when compared to the control group, as shown in Figure 9.

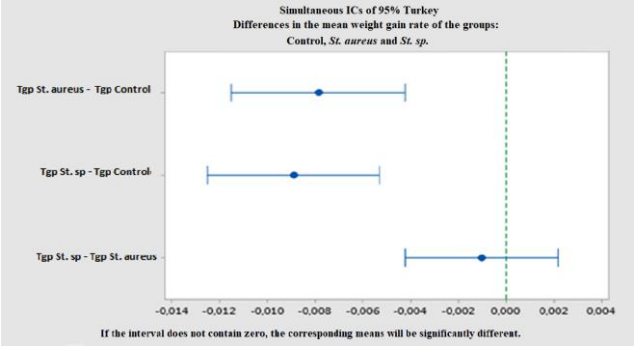

Fig.9. Difference in mean weight gain rate of the groups: Control, S. aureus and Staphylococcus sp.

3.2.2 Assessment of food intake by groups of caterpillars

For the evaluation of food intake by the caterpillar groups, the rate (%) of leaf and diet consumed during the experiment was used.

As observed in Figure 10 between days 1 and 3, the food intake was higher for all groups when compared to the other days, because the caterpillars fed on leaves. After the 3rd day, they began to feed on diet and therefore the average intake was lower.

In the injection rate observed on the 3rd day, a significant difference was observed only in the injection of diet by caterpillars contaminated by *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. The other groups did not show significant difference in the feed injection.

On day 5, we also observed a much lower dietary intake rate of the *Klebsiella oxytoca* and *Pseudomonas aeruginosa* contaminated groups and a significant decrease in intake by the virus contaminated group when compared

to the control group. On the 7th day, the caterpillars of the groups contaminated by *Klebsiella oxytoca* and *Pseudomonas aeruginosa* had died, so the evaluation continued to be done only with the other groups. On this day, there was a very significant decrease in diet injection by the group contaminated by viruses and a decrease in diet injection by the groups contaminated by fungi began to be observed.

On the 9th and 11th days, it was not possible to weigh the diet of the groups contaminated with *Staphylococcus* sp. and *Staphylococcus aureus* because they were pupating, and at this stage, the diet could not be removed because it would disturb the development process. Therefore, it was possible to evaluate only the food injection of the control group.

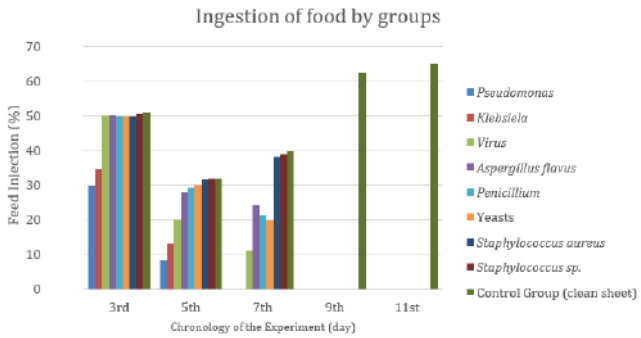

Fig.10. Evaluation of dietary intake by the contaminated groups.

3.2.3 Evaluation of the color and death of the caterpillars during the experiment

Color and life assessments of caterpillars were performed on even days.

By the second day, no difference in color could be observed, and no caterpillars had died.

On the 4th day, it was found that 60% of the group of caterpillars contaminated by *Pseudomonas aeruginosa* and 27% of the group of caterpillars contaminated by *Klebsiella oxytoca* had died. The dead caterpillars showed black coloration when compared to the live caterpillars.

On day 6, 65% of the group of caterpillars contaminated by bacteria *Klebsiella oxytoca* and 5% of the group of caterpillars contaminated by *Baculovirus* were found to have died. The caterpillars killed by the bacteria *Klebsiella oxytoca* showed black coloration when compared to the live caterpillars and the caterpillars killed by viruses showed light coloration when compared to the live caterpillars.

By day 8, 79% of the *Baculovirus* contaminated group of caterpillars had died. The caterpillars killed by viral contamination had a light pinkish coloration. Also on this day, 60% of the fungus-contaminated caterpillars died. The caterpillars killed by fungus were brown in color.

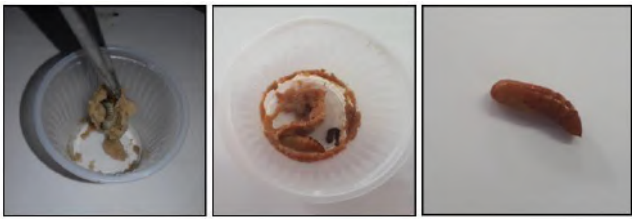

Fig.11. Pupal transformation process of the group contaminated by Staphylococcus aureus and Staphylococcus sp.

Fig.12: Coloration of the caterpillars according to the inoculated. A) Control group, color: brown; B) Virus contaminated group, color: light pink; C) Pseudomonas aeruginosa contaminated group, color: black; D) Klebsiela oxytoca, color: black; E) Aspergillus flavus contaminated group, color: dark brown; F) Penicillium sp. contaminated group, color: dark brown.

By day 10, 90% of the *Baculovirus* contaminated group of caterpillars, 84%, of the *Penicillium* sp. 80% of the caterpillars contaminated with *A. flavus* and 90% of the caterpillars contaminated with yeast had died. Visually, as shown in Figure 11, the caterpillars contaminated with

Staphylococcus sp. and *Staphylococcus aureus* showed accelerated development because some of them began the process to pupate, while no caterpillar in the control group showed this behavior.

On the 12th day no death of caterpillars was observed and 40% of the caterpillars contaminated by *Staphylococcus aureus* and 30% of the caterpillars contaminated by *Staphylococcus* sp. became pupae, while no caterpillars of the control group became pupae.

It was observed that each microorganism when developed in the caterpillar showed different coloration (Figure 12).

There was no death of any of the caterpillars in the control group.

3.2.4 General Evaluation

Table 02. Monitoring of the development of caterpillars after contamination with the microorganisms isolated in this study.

Days of observation	Performance of caterpillars compared to the control group
2	There was no difference with respect to color, size, or amount of food ingested.
3	A significant difference was observed in the rate of weight gain among caterpillars contaminated with *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Penicillium* sp. A significant difference was observed in the rate of diet intake in groups contaminated with *Pseudomonas aeruginosa, Klebsiella oxytoca*.
4	Death of 60% of the group of caterpillars contaminated by bacteria *Pseudomonas aeruginosa* and 27% of the group of caterpillars contaminated by *Klebsiella oxytoca*. Death by bacteria showed a black coloration.
5	Death of 90% of the caterpillars contaminated with bacteria *Pseudomonas aeruginosa*. Significant difference in weight gain and dietary intake of the groups contaminated with *Klebsiella oxytoca* and virus.
6	Death of 65% of the group of caterpillars contaminated with *Klebsiella oxytoca* bacteria and 5% of the group of caterpillars contaminated with viruses. Caterpillars of the *Klebsiella oxytoca* group died with black coloration and the caterpillars killed by viruses had a light pinkish coloration.
7	Death of 95% of the caterpillars contaminated with the *Klebsiella oxytoca* bacterium. Very significant decrease in diet intake and weight gain by the virus-contaminated group. Groups contaminated with fungi began to show a decrease in weight gain and dietary intake.
8	Death of 79% of the group contaminated by virus. Death of 60% of the caterpillars contaminated by fungus. Caterpillars killed by virus were light pink and fungus-contaminated caterpillars were brown
9	Death of 90% of the group contaminated by *Baculovirus*, 84%, 80% and 90% of the caterpillars contaminated with the fungi *Penicillium* sp. *A. flavus* and yeast, respectively. Caterpillars killed by fungi showed brown coloration and caterpillars killed by *Baculovirus*, light coloration. It was not possible to evaluate the diet intake of the groups contaminated by *Staphylococcus aures* and *Staphylococcus* sp.
10	The caterpillars contaminated by bacteria *Staphylococcus* sp. and *Staphylococcus aureus* showed acceleration in development, since some of them started the process for pupal transformation.
11	The caterpillars contaminated by bacteria *Staphylococcus* sp. and *Staphylococcus aureus* showed difference in weight gain rate and continued the process of transformation into pupa.
12	There was no death. 40% of the caterpillars in the *Staphylococcus aureus* group and 30% of the caterpillars in the *Staphylococcus* sp. group pupated, while no caterpillars in the control group pupated.

IV. DISCUSSION

It was possible to observe that the decrease in the food intake rate corroborates with the results of weight gain and death presented, that is, as the groups had a decrease in their food intake rate, they also decreased their weight gain rate and as a consequence presented early death in relation to the control group.

Regarding the group contaminated by Baculovirus, the results of death and color go in agreement with Moscardi and Souza [16] who state that after the process of infection of the caterpillar by *Baculovirus*, the insect becomes debilitated, losing its motor and feeding capacity and dies between the 5th and 8th days with discolored body.

Considering the development of groups contaminated by fungi, the genus *Aspergillus* presents some species that are pathogenic to insects, but also has members that are pathogenic to vertebrates. *A. flavus* has been considered for use as entomopathogen, but the use of these fungi has not been proposed due to safety issues. Infections by *Aspergillus* in birds, other animals and humans have been widely reported [26]. No specialized literature has reported the presence of *A. flavus* as an entomopathogen to *S. frugiperda* caterpillars, but several species of Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Isoptera have been reported as susceptible to this fungus [27]. Senna Nunes et al [28] evaluated, in vitro, the action of *A. flavus* on adults of *Musca domestica* Linnaeus (Diptera: Muscidae) and obtained up to 100% mortality of insects in seven days of evaluation and with a concentration of 10^8.mL^{-1} conidia.

The transformation of the caterpillars contaminated with *Staphylococcus aureus* and *Staphylococcus* sp. into pupae may have occurred due to some biological requirement. Busato et. al [29] found that populations collected in different locations and hosts had different larval stage durations, possibly due to different nutritional requirements due to adaptation to the host of origin or due to different environmental requirements regarding temperature and relative humidity.

According to Sarmento et al [30], at the end of their larval period, the caterpillars penetrate the soil, where they become reddish pupae measuring approximately 15mm in length. Therefore, it can be stated that the pupae formed in these groups had normal development conditions, as can be seen in Figure 6.

V. CONCLUSIONS

It is concluded that the bacteria *Pseudomonas aeruginosa* and *Klebsiella oxytoca* were the most harmful to the caterpillar *S. frugiperda*, causing weight loss after only 3 days of contamination.

The caterpillars contaminated by fungi (*A. flavus*, *Penicillium* sp. and Yeast) had similar behaviors, having started the decrease in weight gain around day 5 and died around day 8.

The groups of caterpillars contaminated with bacteria S. aureus and *Staphylococcus* sp. up to day 9 showed no difference in weight gain rate when compared to the control group. Between the 10th and 12th day, the caterpillars in these groups showed a decrease in weight gain and visually it was possible to observe an acceleration in their development as they became pupae before the caterpillars in the control group.

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