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Homeopathic Solutions as a Tool for the Agroecological Management of Velvetbean Caterpillar

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HIGHLIGHTS

- Homeopathic preparations alter the biological aspect of *Anticarsia gemmatalis*.
- Homeopathy is efficient in the velvetbean caterpillar management.
- *A. gemmatalis* feed efficiency is influenced by homeopathic preparations.

Abstract: Soybean is the main oilseed crop in the world, and one of the main challenges for its production is the *Anticarsia gemmatalis* management, especially in agroecological production systems. In this study, it was analyzed the impact of dynamized solutions applied to soybean plants and pro-vided as food to *A. gemmatalis* on insect biology. Solutions tested include: *Calcarea carbonica* 4CH, *Carbo vegetabilis* 12CH, *Staphysagria* 4CH, *A. gemmatalis* nosode 12CH, Soybean nosode 4CH, and control of distilled water. Biological characteristics analyzed include: weight of caterpillars at the beginning and end of the 4th instar, dry weight of pupa, caterpillar length at 4th instar, duration of cycle, emergence of adults, fecundity of females, and ingestion, ingested food conversion efficiency, and efficiency of digested food conversion. At the beginning and at the end of the 4th instar, caterpillars fed soybean leaves treated with homeopathic substances showed reduced body weight relative to the distilled water control. The dynamized solutions tested are promising as an efficient alternative method for the control of *A. gemmatalis*, promoting lower conversion of food into biological efficiency and biomass.

Keywords: *Anticarsia gemmatalis*; biological efficiency; dynamized solutions; ecological pest control; *Glycine max*; soybean.

INTRODUCTION

Soybean [*Glycine max* (L) Merrill.] is an agricultural commodity of great representativeness in Brazilian economy. For the 2021/22 season, a production record is estimated (136 million tons), representing an increase of 8.9% compared to the previous season (2020/21). Exports should be around 84 million tons [1]. Thus, against expressiveness of this culture in national economy, any factor that interferes in production becomes of great importance.

In this context, one of the barriers that can reduce soybean productivity is insect pest attack. In most regions, the velvetbean caterpillar (*Anticarsia gemmatalis* Hübner 1818) is the main insect affecting soybean production and as such requires rapid attention and control procedures in order to avoid economic losses [2,3]. Chemical methods to control this insect are extensively used, however, instead of solving the problem, excessive use of insecticides without technical support can has brought about significant losses in crop productivity [4,5]. Moreover, because of the capacity pests' adaptability and resistance to chemical products used of irrational form due to successive applications and without rotation of the active principle, it can also has had a negative impact on both humans and the environment through human intoxication, ecological imbalance, and soil and water contamination [6].

In this context, agroecology searches for methods of managing this pest without harming the environment and social aspects, considering the agroecosystem sustainability. Agroecology meets the concerns of the United Nations Organization and society, prioritizing production without compromising future generations, through practices capable of promoting life and the balance between the various components of the agroecosystem [7].

Homoeopathic preparations are among the many agroecological methods of pest control. When these substances are applied in plants, they are able to alter their defense mechanisms by inducing the production of secondary metabolites with antibiotic action. And when provided as food for pests, these changes may interfere with insects' behavior regarding feeding, oviposition and shelter [8–10]. Developed as a science about 200 years ago by Samuel Hahnemann in Germany, the basic principle of homeopathy is based on the use of dynamized medicines, that is, medicines prepared from animal, vegetable, mineral, or diseased tissues [11]. According to Stangarlin [12] and Schmidt [11] homeopathy is based on experimentation of highly diluted and succussed preparations (using ascending and descending movements).

Homeopathy has been an important resource in agroecological production systems as it takes into account the equilibrium of agroecosystems [13,14]. When applying the homeopathic mixture *Spodoptera* 30CH to maize plants, Almeida and coauthors [15] found that the *Spodoptera frugiperda* population was maintained below the control level at the phenological stages of four, six, and eight completely developed leaves. Meanwhile, homoeopathic solutions prepared from the essential oil of *Eucalyptus cinerea* influenced the development of *Aedes aegypti* when applied to water where the larvae occurred, dynamizations 6CH, 9CH, and 12CH produced a lower average number of larvae in relation to the control (5% ethanol solution), and the 30CH dynamization showed the lowest average number of mosquitos [16].

Previous research has demonstrated that the use of homoeopathic solutions is efficient in insect control, having a negative impact on feeding or altering the biological development cycle. According to Mapeli and coauthors [8], the main effects of homoeopathic solutions on insects occurs through ingestion and contact. Thus, for insects, if food availability at immature phases is not adequate, it is possible that the adult will not emerge. If emergence does occur, it is likely to be of reduced size and without stored nutrients, among other characteristics. Meanwhile, slowed emergence can desynchronize the adult in relation to the host. A reduced size and the lack of stored nutrients can further prejudice the insects in relation to pheromone production, which will affect competition for sexual partners and production of ovules [8].

Thus, understanding the association between substances ingested by an insect through feeding and the impact that such an ingestion can have on its biology is very important. As such, the aim of this study was to verify biological alterations in *Anticarsia gemmatalis*, and life cycle of insects, altering ingestion, nutrition, development and reproduction, promoted by ingestion of soybean leaf treated with dynamized solutions.

MATERIAL AND METHODS

Site description

The experiment was conducted in Cáceres, Mato Grosso State, Brazil (16°04'14" S; 57°40'44" W; altitude of 176 m). The climate in region is tropical, hot and humid, with an annual average temperature of 24°C (highest maximum of 40°C) with average relative humidity of 80%. Annual rainfall is approximately 1.750mm, with maximum intensity from December to February.

Experiment conduction and treatments

Caterpillars used in the experiment were obtained from eggs donated by Embrapa-Soja and were reared under laboratory conditions at ambient temperature. Plastic containers with a capacity of 500 mL were used as recipients and covered with a fine cloth fixed with an elastic. Initially, feeding was based on a special diet prepared by Embrapa-Soja [17] and later using soybean variety P98Y11 irrigated with water and without any treatment.

Homoeopathic solutions were acquired from specialized pharmacy and dynamized solutions were prepared according to Farmacopeia Homeopática Brasileira [18]. Dynamizations were developed from the mother tincture (MT) using glass containers with 30 mL capacity to which 20 mL of 70% cereal alcohol and 0.2 mL of the MT solution were added. The solutions were subsequently shaken with ascending and descending movements 100 times to prepare 1CH. From this 1CH solution, 0.2 mL were removed and added to 20 mL of 70% cereal alcohol. It was again shaken 100 times to prepare the 2CH. We repeated this process until the appropriate CH was reached for each solution, to a maximum of 12CH.

Experimental treatments consisted of the following solutions: *Calcarea carbonica* 4CH, *Carbo vegetabilis* 12CH, *Staphysagria* 4CH, *A. gemmatalis* nosode 12CH, and soybean nosode 4CH. Distilled water was used as the control.

The treatments were applied to soybean plants (variety P98Y11) at stages R1, R2, and R3; and were planted in vases containing soil and organic compound (1:1). Flasks containing the treatments were labeled and coded so that neither the researcher nor the technician applying the treatments would know which solution was being applied, creating a double-blind process.

We mixed 2 mL of each solution, identified as T1, T2, T3, T4, T5, and T6; with 200 mL of distilled water in bottles for each solution. From this amount, 50 mL was sprayed manually on soybean leaves, and 150 mL was applied to the soil. From the 1st to the 25th of April, treatments were applied daily at 5:00 pm. After eclosion third generation caterpillars were separated into plastic containers with a slightly moistened filter paper and an untreated soybean leaf. Five first instar caterpillars were placed in each container, for a total of 60 containers and 300 caterpillars. Beginning from the second instar, the caterpillars were fed with leaves from treated soybean plants. The food surplus and feces were removed daily.

The food consisted of a tender and healthy leaflet from each plant. In the laboratory, leaves were weighed on a 0.001g precision scale to record the amount of food supplied. One leaf was placed in a paper bag and dried in a drying stove (64 ± 2°C) to determine the initial dry weight (IDW), while the other leaves were offered to the caterpillars as food. After 24 hours, the surplus and feces were removed from the containers and dried to record the surplus dry weight (SDW) and feces dry weight (FDW). This procedure was conducted daily for 25 days.

The following characteristics were analyzed: weight (g) of caterpillars at beginning and at the end of 4th instar; dry weight (g) of pupa (incorporated biomass); length (cm) of caterpillar at 4th instar; duration of cycle (days) from egg to adult; percent adult emergence; female fecundity (percent emerged eggs); and nutritional value of soybean leaves under treatment:

- Ingestion (ING)

$$ING\ (mg) = \frac{\text{IDW}-\text{SDW}}{\text{FDW}} \tag{1}$$

- Conversion efficiency of ingested food (CEI)

$$CEI\ (\%) = \frac{\text{Incorpored biomass}}{\text{ING}} x\ 100 \tag{2}$$

- Digestible feed conversion efficiency (DCE)

$$DCE\ (\%) = \frac{\text{Incorpored biomass}}{\text{ING}-\text{FDW}} x\ 100 \tag{3}$$

We consider as ingested food, the food eaten and not assimilated by the organism, and digested food, that food assimilated and converted into physiological activities, for the purpose of changes of instars, reproduction and life cycle [18].

Caterpillars fed with treated soybean leaves were weighed on a 0.001g scale at the beginning and at the end of the 4th instar and length (mm) was measured at the end of 4th instar. At 24 hours after verification of pupa presence, was weighed (g) their fresh mass. Subsequently, five pupas from each treatment were placed in the drying oven for three days (± 70 °C) and the dry weight was assessed to determine incorporated biomass.

Adult insects were transferred to pots (15 cm diameter x 6 cm height) which were separated by treatment. Two couples were placed in each pot at a time, for a total of 12 adults per treatment, and fed with a 10%

honey solution. Pots were inspected daily for retreat of the ovipositions and the food was changed every other day.

Experimental design an statistical analyses

A randomized experimental design was used, with six treatments and six replicates. Data were tested for homogeneity variances (Cochran and Bartlett) and normality of errors (Lilleifors test). When significant, an analysis of variance was performed. Was tested the averages using a Tukey test at 5% probability, except for cycle duration and ingestion, which were analyzed using the Kruskall-Wallis non-parametric test. Data expressed in percentage were converted into arcsine √(X/100), while the remaining data were analyzed without transformation.

RESULTS AND DISCUSSION

A. gemmatalis caterpillars fed with soybean leaves treated with dynamized solutions showed a higher level of leaf consumption (69.14%) when compared to those fed with the control treatment. However, higher levels of ingestion of treated soybean leaves resulted in lower caterpillar feed utilization (CEI). Of this ingested food, 40.05% was assimilated by the insect (CEI) and only 38.68% of digested food was transformed into biomass (DCE) (Table 1).

Table 1. Ingestion (ING), efficiency of ingested food conversion (CEI), and efficiency of the digested food conversion (DCE) of *Anticarsia gemmatalis* fed with homeopathized soybean leaves.

Dynamized solution	ING[1]	CEI[2]	DCE[2]
	mg	%	%
Calcarea carbonica 4CH	2.5184 A	0.1370 B	0.1376 B
Carbo vegetabilis 12CH	2.1878 A	0.1348 B	0.1358 B
Staphysagria 4CH	2.6332 A	0.1250 B	0.1255 B
Anticarsia gemmatalis 12CH	2.5894 A	0.1369 B	0.1377 B
Soybean 4CH	2.0954 AB	0.1108 B	0.1111 B
Disteled water	1.6595 B	0.2150 A	0.2112 A

Averages followed by the same letter in a column do not differ statistically based on Tukey test at 5% probability. [1] Data transformed into arcsine √(X/100) and not significant based on ANOVA F-test. [2] No significant differences based on Kruskall-Wallis test.

In comparison with the current study, similar results were observed by Mapeli and coauthors [8] when testing if homeopathic preparations applied to kale would result in food deterrence for *Ascia monuste orseis*. In their study, caterpillars fed with kale leaves treated with a Sulfur 12CH solution showed greater leaf consumption 56.55% compared to other treatments. Of this ingested food, only 40.99% was assimilated by the insect organism, with a CEI value of 87.69%. Meanwhile, of the food digested and transformed into biomass, the Sulfur 12CH treatment showed the lowest conversion of 48.11%. These results demonstrate that the amount and proportion of nutrients contained in food is important when analyzing insect consumption.

Considering these results, the high index of ingestion by the caterpillars of leaves treated with homeopathic preparations likely indicates a behavior acquired by the insect as a defense response to low nutritional value [9]. The homeopathic preparations may have somehow promoted changes to the nutritional value of soybean plants, since the caterpillars that were fed with the control leaves consumed less food and converted more (Table 1).

Efficiency of the ingested food conversion (CEI) and efficiency of the digested food conversion (DCE) by caterpillars were equal across homeopathic preparations (Table 1). Values of CEI and DCE reflect utilization of the food that was assimilated into the organism and spent on metabolism of the insect or even incorporated into biomass.

When metabolizing a food with low nutritional conversion, the insect tends to spend more energy and increase ingestion to compensate for the nutritional deficit and use the energy stored in its own biomass. At the beginning and at the end of the 4th instar, caterpillars fed leaves with homoeopathic solutions showed lower body weight in relation to those fed leaves with the distilled water treatment (Table 2). It is likely that this may have occurred due to increased metabolism and use of incorporated biomass in order to compensate for nutritional deficiencies that the homeopathic preparations may have brought to the soybean plants [8].

Table 2. Biological characteristics of *Anticarsia gemmatalis* influenced by different dynamized solutions.

Dynamized solution	Weight beginning 4th instar	Weight end 4th instar	Caterpillar length	Adult emergence[1]	Hatched eggs[1]	Cycle duration[2]
	g	g	cm	%	%	day
Calcarea carbonica 4CH	0.015 B	0.020 B	7.62 B	0.896	0.544	49.50
Carbo vegetabilis 12CH	0.013 B	0.023 B	9.20 B	0.906	0.573	48.50
Staphysagria 4CH	0.017 B	0.023 B	8.00 B	0.905	0.539	47.16
Anticarsia gemmatalis 12CH	0.010 B	0.020 B	8.89 B	0.881	0.559	47.00
Soybean 4CH	0.012 B	0.017 B	7.63 B	0.882	0.557	51.00
Disteled water	0.050 A	0.075 A	13.13 A	0.903	0.536	48.75

[1] Averages followed by different letters in the column are significantly different based on Krus-kall-Wallis test and non-parametric multiple comparisons. [2] Data transformed into arcsine √(X/100) and significant based on Tukey test at 5% probability level.

An increase in metabolic rate is necessary for individuals to maintain resistant defense mechanisms. When this does not occur, redistribution of energy to other physiological processes involved with development, reproduction, and maintenance of the insect can be affected [20,21].

Homoeopathic preparations can alter a plant's physiology and secondary metabolism, such as promoting an increase in defense metabolites [22]. Many substances found in soybeans are not easily digested by insects, such as the flavonoids rutin and genistein, which are produced as a chemical defense and can result in an antinutritional process in *A. gemmatalis* by altering column cells of the medium intestine [23]. Thus, the application of homeopathic preparations in this study may have stimulated the production of these substances in soybean plants, which were poorly utilized nutritionally and energetically during digestion by the insect.

Caterpillars fed leaves treated with homeopathic preparations showed a 75% decrease in body length compared to those receiving soybean leaves treated with distilled water (Table 2). This shorter length may be related to the limiting effect of food on nutritional storage during immature phases. Therefore, insects had variations in their body chemical composition, resulting in shorter lengths [20,23]. Based on these results, we can infer that the homoeopathic solutions interfered with the deposition of chitin during *A. gemmatalis* metamorphosis stages [21,25].

In a previous experiment carried out by Mapeli and coauthors [8], assessing the impact of homoeopathic solutions on *Ascia monuste orseis*, the authors found that caterpillars exposed to Sulfur 12CH and *Magnesia Carbonica* 30CH presented a shorter length at the 4th instar. According to the authors, the solutions may have inhibited the prothoracic hormone, which did not affect the metamorphic process but did affect growth.

This result might be an adaptation strategy in which the caterpillars begin to save energy in order to ensure that during the pupa phase there is sufficient energy to support the development of viable and fertile adults instead of spending it on growth. This hypothesis may be correct since the percent of adult emergence, percent-emerged eggs, and cycle duration were not significantly altered by the treatments in this research (Table 2).

CONCLUSION

Homeopathic preparations increase the consumption of leaves by the *Anticarsia gemmatalis*, but decrease the conversion of this food into biological efficiency and biomass. Resulting in caterpillars with shorter length and weight. Thus, the homeopathic preparations tested are promising tools for the agroecological management of the velvetbean caterpillar.

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Conflicts of Interest: The authors declare no conflict of interest.

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