

Article

Tradescantia pallida (Commelinaceae) Promotes Reductions in *Plutella xylostella* (Lepidoptera: Plutellidae) Populations

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Abstract: The feeding activity of *Plutella xylostella* in brassica crops can lead to large losses; thus, pesticides that prevent feeding during the larval stage or prevent the metamorphosis of this insect can be used for its control. In this study, the effects of two types of aqueous extracts of *Tradescantia pallida* on the different life stage of *P. xylostella* cycle were tested; neither of the two aqueous extracts, which were obtained by infusion and maceration, had been tested against *P. xylostella*. The biological variables evaluated were larval and pupal duration and viability, pupal weight, sex ratio, longevity of females, fecundity, fertility and oviposition period. There was no significant difference in the duration of the larval phase of *P. xylostella* between the bioassay treatments; however, larval viability was lower when the individuals were exposed to both types of *T. pallida* extracts. Reduced pupal viability was observed among the individuals treated with the application of the extracts. Treatment with the aqueous extract obtained by infusion caused the lowest pupal weight, fecundity, and fertility and longevity among females. The results obtained in this study allow us to propose the bioextract as an alternative for pest management, emphasizing the technique for small producers and/or organic.

Keywords: antibiosis; bioinsecticide; diamondback moth; organic production



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1. Introduction

Members of the Brassicaceae family have several nutritional and bioenergetic properties [1] and are widely cultivated in Brazil and worldwide; representative species of this family include cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), kale (*B. oleracea* var. *acephala*), broccoli (*B. oleracea* var. *italica*) and mustard (*B. juncea*) [2]. However, despite the vegetables of the genus *Brassica* being easily cultivated, the damage caused by *Plutella xylostella* (Linnaeus 1758) (Lepidoptera: Plutellidae), popularly known as the crucifer moth, affects crop productivity and generates large economic impacts by increasing management and control costs [3].

Pest control in brassica crops is usually achieved by the application of agricultural defence products, since they are practical, fast and efficient in controlling insect pests [4]. However, pesticides pose toxicological risks to the environment and to humans in addition to resulting in the evolution of insect resistance after continuous and incorrect applications [5,6]. In organic farming, the use of synthetic pesticides is not permissible, requiring the use of alternative control methods that allow the final product to be classified as organic. Therefore, due to the economic impacts caused by *P. xylostella* on brassica crops and problems related to control measures, alternative and selective control methods that rely on the techniques used in integrated pest management (IPM) have become necessary [7–9].

One of the alternatives for population reduction of this pest with less environmental impact is the application of botanical extracts. Promising plants, such as those in the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae, have chemical compounds with repellent or insecticidal potential, as reported by Jacobson [10]. Studies involving plant extracts with insecticidal properties indicate changes in the biological characteristics of insects, such as mortality [11–13] changes in food [14,15] or oviposition [15,16] preferences, deformities, and morphological and physiological transformations of *P. xylostella* [11,17–20].

We hypothesized that *Tradescantia pallida* var. Hunt. (Commelinaceae) exhibits insecticidal properties, considering that in a recent study, this species was included in annual environmental monitoring and no herbivory was observed at any point in the evaluated area [21]. Thus, we believe that the plant species contains some lethal or repellent compound that can be used in the population control of *P. xylostella*.

In this study, we evaluated the action of *T. pallida* aqueous extract at a concentration of 10% (*w/v*) from two different extraction methods on the life cycle of *P. xylostella* in a plastic tunnel.

2. Materials and Methods

2.1. Cultivation of *Plutella xylostella*

The individual used in the study design were sourced from the insect rearing facilities of Laboratory of Insect-Plant Interaction of the School of Biological and Environmental Sciences at the Federal University of Grande Dourados (Universidade Federal de Grande Dourados-UFGD) in Dourados, Mato Grosso do Sul, Brazil. The individuals were kept under controlled conditions of temperature (25 ± 2 °C), relative humidity ($70 \pm 5\%$) and photoperiod (12 h). The larvae were fed with organic leaves of *Brassica oleracea* L. var. *acephala* DC. until they reached the pupa stage. Adults were fed a 10% diluted honey solution [22].

2.2. Collection of Botanical Material and Preparation of Aqueous Extracts

The collection of botanical material was authorized by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) and the Council for the Management of Genetic Heritage (CGEN/MMA) under the number A1501D0.

Fully expanded leaves of *T. pallida* were collected from a garden in the Federal University of Grande Dourados, Dourados, MS ($22^{\circ}11'42.8''$ S $54^{\circ}56'06.7''$ W), between 7:00 am and 9:00 am during May 2019. The leaves were dried in a forced air oven for four days at a maximum temperature of 45 °C (± 1 °C). After this period, they were ground in a knife mill (Model 7Lab Micro 910) until a fine powder was obtained.

2.2.1. Maceration

An aqueous extract with a concentration (weight/volume) of 10%, conventional concentration, was obtained through the maceration technique and the addition of 10 g of the powdered plant material to 100 mL of distilled water by [11,19,23]. After manual stirring, the solution remained at rest for 24 h in a refrigerated environment (10 °C). At the end of this period, the liquid was strained with filter paper and the filtrate was used in the tests.

2.2.2. Infusion

To obtain an extraction by infusion, 100 mL of distilled water was heated to 100 °C, and when this temperature was reached, 10 g of the powdered plant material was added. The solution remained at room temperature under light protection for 2 h; after this period, the solution was filtered through filter paper and the filtrate was used in the tests.

2.3. Bioassay in a Plastic Tunnel

The experiment was conducted in a plastic tunnel measuring 21 m in length by 7 m in width and 3 m in height located in the nursery area of the School of Agricultural Sciences of the Federal University of Grande Dourados, Mato Grosso do Sul, Brazil.

Seedlings of cabbage (*B. oleracea* var. *acephala*) in the second phenological stage were planted in pots with a volume of 5 L filled with a dystrophic red latosol soil with a very clayey texture [24]. The properties of the 0.0–0.20 m layer were as follows: OM = 26.0 g dm⁻³; pH (CaCl₂) = 5.4; P (resin) = 25 mg dm⁻³; K⁺, Ca²⁺ and Mg²⁺ = 8.7, 36.0 and 22.0 mmolc dm⁻³, respectively; and S–SO₄²⁻ = 5.6 mg dm⁻³. The granulometric analysis revealed 644, 203 and 152 g kg⁻¹ of clay, silt and sand, respectively. The seedlings were acclimated in a plastic tunnel until they bore six to eight true leaves, characterizing the third phenological stage [25].

Ten plants were established for each treatment, namely, the control (distilled water), the aqueous extract of *T. pallida* obtained by maceration (ETPM) and the extract of *T. pallida* obtained by infusion (ETPI). Each plant represented one replicate, and each leaf represented a subsample, totalling 5 subsamples per replicate. A third-instar *P. xylostella* larva was placed on each kale leaf (subsample), and after 48 h, the treatments were applied. To prevent the escape of individuals, an iron frame with voile fabric was installed surrounding the entire vessel (Figure 1). The temperature and relative humidity were evaluated daily using an Underbody digital thermo-hygrometer. The plants were irrigated daily with 200 mL of water.

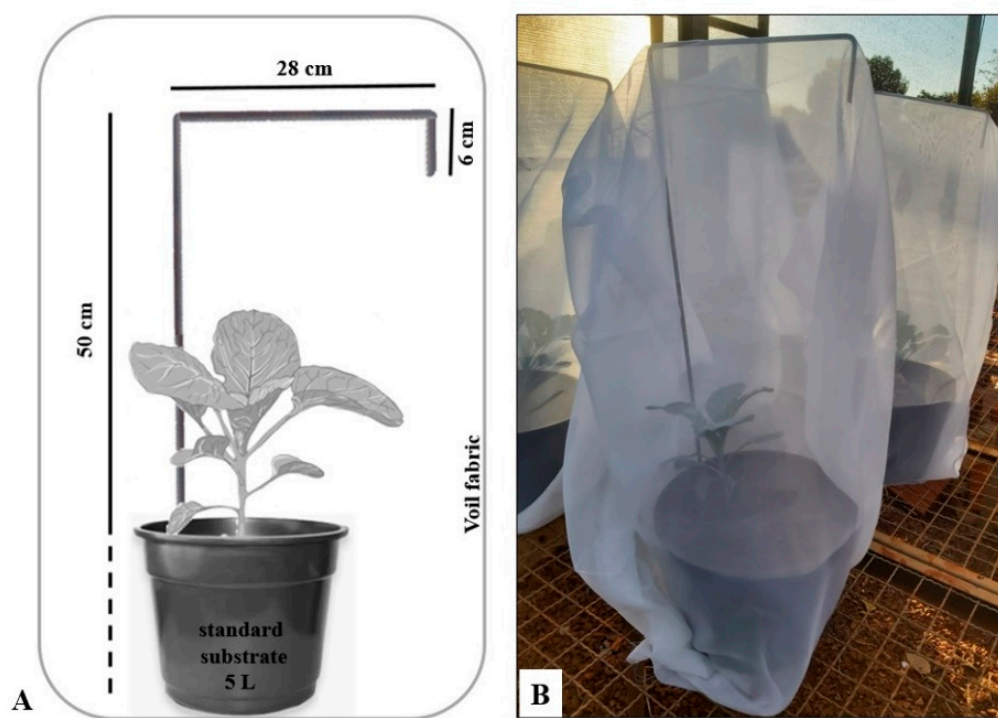


Figure 1. (A). Sketch of the experimental unit of the bioassay. (B). Execution of the bioassay in a semi-field situation.

A sprayer with a flow rate of 0.6 mL was used for each application of the extracts. Two applications of 0.6 mL per leaf (subsample) were necessary, totalling approximately 60 mL of extract per treatment.

The evaluation of the biological parameters, including the duration (days) and survival (%) of the larval and pupal stages and pupal weight (Bel Mark Analytical Balance (0.001 g)), was performed by daily monitoring inside the plastic tunnel, starting 24 h after the first application.

After adult emergence, the sexing process was performed, and the sex ratio [RS = female/(female + male)] was evaluated. Then, all the possible pairs for each treatment were formed, with 10 replicates for the control and extract obtained by maceration and 8 replicates for the extract obtained by infusion; the pairs were placed separately in plastic cages (measuring 24 cm in length, 19 cm in width, 10 cm height) to evaluate the reproductive stages of the insects. At the base of the cage, a disc of cabbage leaf (8 cm in diameter) was used as an oviposition substrate and replaced daily for egg counting. A 10% (*w/v*) honey solution was offered for feeding. The fecundity (number of eggs deposited on the leaf disc), fertility (number of newly emerged larvae), oviposition period [(days) = the period between the first and last laying] and female longevity were evaluated daily.

Larval, pupal and egg survival data were transformed to $\sqrt{x}/100$ arcsene and data on larval and pupal duration, sex ratio, female longevity, fecundity (number of eggs) and fertility (number of newly emerged larvae) were analyzed. The oviposition period was transformed to $\sqrt{x} + 0.5$. The results were submitted to variance analysis and the means were compared by the Tukey test ($p \leq 0.05$) using R program.

3. Results

There was no significant difference in the duration of the larval stage of *P. xylostella* between the treatments ($F = 3.80$; $DF = 2$; $p = 0.03$); however, larval survival was significantly lower ($F = 7.22$; $DF = 2$; $p = 0.03$) when the individuals were exposed to *T. pallida* extracts (Table 1). We observed that ETMP caused approximately 48% mortality in *P. xylostella* larvae, while ETPI caused mortality in 56% of the individuals (Table 1).

Table 1. Duration and larval survival (\pm standard error) of *Plutella xylostella* in plastic tunnels exposed to different treatments consisting of 10% *Tradescantia pallida* aqueous extract (Temp: 23 ± 8 °C, RH: $53 \pm 10\%$, photophase: $13 \text{ h} \pm 36 \text{ min}$).

Treatments	Larval Phase	
	Larval Duration (Days)	Larval Survival (%)
Control	7.28 ± 0.4 a $n = 50$	92.57 ± 4.9 a $n = 47$
ETPM	6.10 ± 0.3 a $n = 50$	52.01 ± 8.6 b $n = 26$
ETPI	6.04 ± 0.3 a $n = 50$	43.99 ± 8.7 b $n = 21$
C.V. (%)	7.98	38.50

Means followed by the same letter in a column do not differ according to Tukey's test at the 5% probability level ($p < 0.05$). ETMP = *T. pallida* extract obtained by maceration. ETPI = *T. pallida* extract obtained by infusion.

In pupal duration, treatments did not differ significantly from control ($F = 1.16$; $DF = 2$; $p = 0.32$) (Table 2). Regarding pupal survival, there was no significant difference between ETMP e ETPI treatments, but both treatments differed significantly from the control; ($F = 4.08$; $DF = 2$; $p = 0.02$). However, we noticed that the extracts had reduced the number of individuals that reached the adult stage, and the ETMP caused approximately 24% of incomplete pupae development and, consequently, did not emerge, and the ETPI had affected the pupae emergence of approximately 28% (Table 2). For pupal biomass, we observed that only ETPI differed from the control treatment, reducing the pupae weight by an average of 1.40 mg ($F = 4.03$; $DF = 2$; $p = 0.02$) (Table 2).

Table 2. Duration, survival and pupal weight (\pm standard error) of *Plutella xylostella* exposed in plastic tunnels to different treatments with 10% *Tradescantia pallida* aqueous extract (Temp: 23 ± 8 °C, RH: $53 \pm 10\%$, photophase: $13 \text{ h} \pm 36 \text{ min}$).

Treatments	Pupal Phase		
	Pupal Duration (Days)	Pupal Survival (%)	Pupal Weight (mg)
Control	6.59 ± 0.26 a <i>n</i> = 47	100 ± 0.00 a <i>n</i> = 47	5.70 ± 0.16 a <i>n</i> = 47
ETPM	4.67 ± 0.34 a <i>n</i> = 26	76.37 ± 9.95 b <i>n</i> = 19	5.50 ± 0.5 a <i>n</i> = 19
ETPI	4.83 ± 0.58 a <i>n</i> = 21	72.69 ± 12.35 b <i>n</i> = 14	4.30 ± 0.33 b <i>n</i> = 14
C.V. (%)	26.13	39.29	23.25

Means followed by the same letter in a column do not differ according to Tukey's test at the 5% probability level ($p < 0.05$). ETPM = *T. pallida* extract obtained by maceration. ETPI = *T. pallida* extract obtained by infusion.

As can be seen in Table 3, ETPI affected all parameters of the cruciferous moth reproductive phase, significantly differing from the control, while ETPM did not significantly differ from the control. Based on the results, it was verified that the females longevity from larvae fed with ETPI was affected when compared to the females control ($F = 4.71$; $DF = 2$; $p = 0.01$), in addition to the fecundity ($F = 7.56$; $DF = 2$; $p = 0.003$) and fertility ($F = 6.03$; $GL = 2$; $p = 0.007$). For fecundity, ETPI promoted an average laying of 81 eggs, and for fertility, only 21 larvae hatched, which represents a reduction of 73% and 76% when compared to the control, respectively.

Table 3. Longevity of females, and fecundity and fertility of adult *Plutella xylostella* in plastic tunnels exposed to different treatments with 10% *Tradescantia pallida* aqueous extract. (Temp: 23 ± 8 °C, RH: $53 \pm 10\%$, photophase: $13 \text{ h} \pm 36 \text{ min}$).

Treatments	Adult Phase		
	Longevity of Females (Days)	Fecundity (Number of Eggs)	Fertility (Number of Hatched Larvae)
Control	17.55 ± 1.13 a <i>n</i> = 10	111.51 ± 12.9 a <i>n</i> = 10	83.64 ± 13.9 a <i>n</i> = 10
ETPM	20.60 ± 1.61 a <i>n</i> = 10	65.31 ± 8.75 ab <i>n</i> = 10	41.00 ± 10.16 ab <i>n</i> = 10
ETPI	14.18 ± 1.18 b <i>n</i> = 8	30.05 ± 10.9 b <i>n</i> = 8	20.13 ± 9.2 b <i>n</i> = 8
C.V. (%)	26.13	39.29	23.25

Means followed by the same letter in a column do not differ according to Tukey's test at the 5% probability level ($p < 0.05$) (\pm standard error). ETPM = *T. pallida* extract by maceration. ETPI = *T. pallida* extract by infusion.

A way of showing *P. xylostella* damage and the effect of ETPI and ETPM is represented in Figures 2 and 3. Figure 2A shows healthy cabbage seedlings without *P. xylostella* larvae infestation and the Figure 2B shows the cruciferous moth larvae damage in the control treatment. However, throughout the experiment, we noticed that ETPM (Figure 2C) and ETPI (Figure 2D) reduced the cabbage leaves perforations caused by *P. xylostella*. Figure 3 shows the effect of the ex-treatment on the insect. Figure 3A,A' shows a healthy, greenish-greenish cruciferous moth larva from the control treatment. While in Figure 3B,B', the larva of *P. xylostella* can be seen dead after exposure to the *T. pallida* extract, with changes of the insect morphology and color.

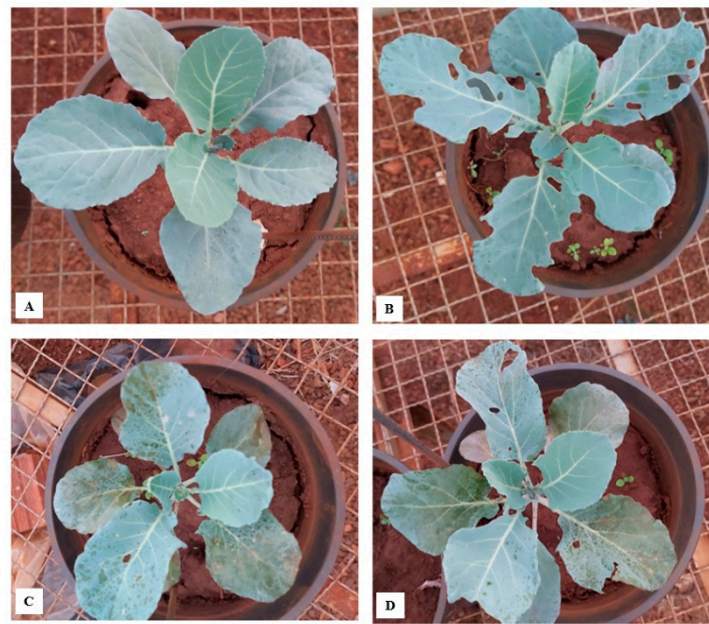


Figure 2. Overview of cabbages from different treatments. (A). Control sample unit and without *Plutella xylostella* infestation. (B). Sample unit, control infested. (C). Sampling unit with ETPM. (D). Sampling unit with ETPI.

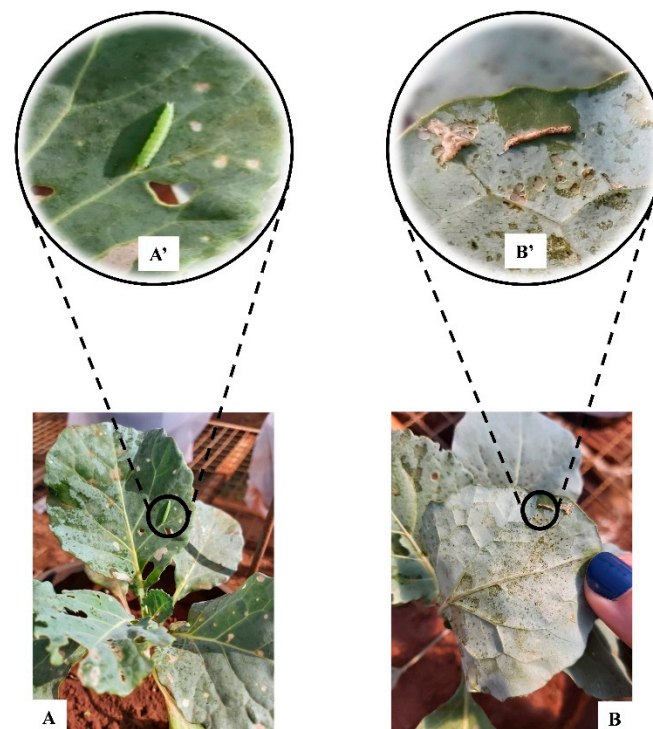


Figure 3. Development of the experiment. (A). Control: in evidence, larval stage of *Plutella xylostella*. (A'). Detail of the healthy larva. (B). In evidence, dead *P. xylostella* larvae after contact with the aqueous extract of *T. pallida*. (B'). Dead larvae detail.

4. Discussion

The present study is, to our knowledge, the first to evaluate the insecticidal potential of *T. pallida* aqueous extract on *P. xylostella* in organic crops using two extraction methods: infusion and maceration. We found that a 10% concentration of plant extract affected

the biology and development of the diamondback moth through lethal and sublethal effects, ranging from larvicidal effects to reduction in pupal weight and reproduction at the adult stage.

In this study, with both extraction methods, *T. pallida* caused significant lethal effects on larval and pupal stages. The *T. pallida* extract by infusion (ETPI) method affected pupal weight and was also reflected in the adult stage because females showed significant reduction in fecundity and fertility. Thus, ETPI showed better performance than the ETPM.

The chemical composition evaluation of the extract performed by Rocha et al. [26], indicated the presence of phenolic compounds and flavonoids, such as rutin, luteolin, and apigenin, in addition to anthocyanins and tannins. Of these, rutin is a flavonoid known to have an important role in plant protection against lepidopterans, demonstrating lethal and/or antinutritional effects during the larval stage [27,28]. In our study, the change in larval and pupal survival caused by both extracts, primarily by ETPI, may probably be owing to the presence of flavonoids, especially rutin. Some studies with *Alibertia* spp. in the control of *P. xylostella* showed a reduction in pupal survival, especially by *A. sessilis* [11]. Moreover, as highlighted by Tavares et al. [29], rutin contributes to the protection of plants through its antifeeding action against Lepidoptera. This flavonoid reduces growth and larval and pupal weights [27], in addition to decreasing survival [30]. The negative effect of rutin on the growth of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) [28] and biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) [30] has been reported.

Luteolin is a flavonoid that prevents insect oviposition on leaves [31,32], and this characteristic corroborates the results obtained in the present study of significantly reduced fecundity and fertility because of ETPI. In *Acyrtosiphon pisum* Harris (Homoptera: Aphididae), changes in fecundity were observed with different doses of luteolin [33]. Thus, these flavonoids may be directly related to changes in fecundity and fertility of *P. xylostella*, especially in the case of ETPI, reinforcing the hypothesis of a synergy between compounds. In contrast, as observed by Rocha et al. [26] tannins function as a phagodeterrent, causing insect death or several sublethal effects throughout the insect life cycle [13,19,34].

In our study, we observed a significant reduction in pupal weight, especially with the use of ETPI, which is directly related to insect performance in the larval stage [35] and clearly associated to the feeding activity performed at this stage. The presence of compounds with toxicity to the insect may lead to a reduction in pupal biomass, owing to the activation of the cytochrome P-450 mechanism. This activation is an important tool used by insects for defensive detoxification [36], in which the insects need to degrade possible allelochemicals of the extract and end up diverting resources that would be used to gain weight in the larval stage, i.e., there is greater energy expenditure to degrade the toxic compounds and lower conversion of ingested nutrients [37].

In general, in the present study, the aqueous extract of *T. pallida* obtained by the infusion method (ETPI) affected larval and pupal survival, pupal weight, female longevity, fecundity and fertility. This result may have been due to the role of temperature in the extraction of compounds present in the leaves of *T. pallida*, because, there may be an increase in the extraction of certain compounds and a reduction in the extraction of other secondary compounds, and the synergistic action between compounds may potentiate the insecticidal effect of an extract.

The choice of which method to use depends exclusively on the time and plant resource available to the farmer. We encourage the use of solvents and extraction methods for the development of affordable botanical insecticides, especially for the smallholder. In our study, both extracts were easy to manage, had low cost, could be prepared quickly, and were highly accessible, showing that simple extraction methods are applicable. Considering the results of the present study, we recommend the use of ETPI.

5. Conclusions

In our research, we observed that the *Tradescantia pallida* aqueous extract by the infusion method (ETPI) had the best results, affecting all insect development stages, especially the

adult stage, reducing the individuals number in the next generation, and, consequently reduction of damages in the diverse cultures of Brassicaceae in organic cultivation. The *T. pallida* aqueous extract by the maceration method (ETPM) should not be discarded if it is impossible to prepare and use ETPI. We emphasize that our research is unprecedented in the use of *T. Pallida* as a botanical insecticide and of easy and viable use by small producers and family farmers in field conditions, specially in developing countries.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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