

## Development of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on spontaneous plant species

Desenvolvimento de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) em espécies de plantas espontâneas

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The tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a major insect pest in tomato cultivation (*Solanum lycopersicum* L.), which can cause losses of up to 100% in production. This study aimed to assess the development of *T. absoluta* in different spontaneous plant species and the insecticidal effects of plant species in which *T. absoluta* does not develop. Nine species of spontaneously growing plants were studied: *Ipomoea purpurea*, *Commelina benghalensis*, *Amaranthus viridis*, *Bidens pilosa*, *Solanum viarum*, *Richardia brasiliensis*, *Cenchrus echinatus*, *Conyza bonariensis*, and *Solanum americanum*. The larval and pupal survival, attractiveness for oviposition, feeding, and leaf area consumed by *T. absoluta* were greater for *S. americanum*, *S. viarum*, and *I. purpurea*. Thus, these species can be considered host plants for *T. absoluta*, given that the pest has completed its life cycle, and serve as a green bridge for insect maintenance during the tomato cultivation off-season. *C. benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus*, and *C. bonariensis* exhibited deleterious effects on *T. absoluta* as they did not host or support development in these plants. The essential oil extracted from *C. bonariensis* was effective at reducing the egg viability (at a concentration of 0.10%) and causing caterpillar mortality (at concentrations of 0.10%, 0.07%, 0.04%, and 0.01%) of *T. absoluta*. This indicates the potential of the essential oil from *C. bonariensis* for managing this pest, both at the egg and caterpillar stages.

Keywords: essential oils, tomato leaf miner, weeds.

A traça-do-tomateiro *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) é uma das principais pragas na cultura do tomate (*Solanum lycopersicum* L.), o que pode causar perdas de até 100% na produção. Este estudo teve como objetivo avaliar o desenvolvimento de *T. absoluta* em diferentes espécies de plantas espontâneas e os efeitos inseticidas de espécies vegetais nas quais *T. absoluta* não se desenvolve. Foram estudadas nove espécies de plantas de crescimento espontâneo: *Ipomoea purpurea*, *Commelina benghalensis*, *Amaranthus viridis*, *Bidens pilosa*, *Solanum viarum*, *Richardia brasiliensis*, *Cenchrus echinatus*, *Conyza bonariensis* e *Solanum americanum*. A sobrevivência larval e pupal, a atratividade para oviposição, a alimentação e a área foliar consumida por *T. absoluta* foram maiores em *S. americanum*, *S. viarum* e *I. purpurea*. Assim, essas espécies podem ser consideradas plantas hospedeiras de *T. absoluta*, uma vez que a praga completou seu ciclo de vida, além de servirem como uma ponte verde para a manutenção do inseto durante o período de entressafra do tomateiro. Por outro lado, *C. benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus* e *C. bonariensis* exibiram efeitos deletérios sobre a *T. absoluta*, pois não hospedaram ou suportaram o desenvolvimento dessa praga. O óleo essencial extraído de *C. bonariensis* reduziu a viabilidade dos ovos (em concentração de 0,10%) e causou mortalidade de lagartas (em concentrações de 0,10%, 0,07%, 0,04% e 0,01%) de *T. absoluta*. Isso indica o potencial do óleo essencial de *C. bonariensis* no manejo dessa praga, tanto nas fases de ovo quanto de lagarta.

Palavras-chave: óleos essenciais, traça-do-tomateiro, plantas daninhas.

## 1. INTRODUCTION

The tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a species of moth native to South America [1]. Its presence is significant in the major tomato-producing countries in Latin America (Brazil, Mexico and Chile) [2]. Currently, this pest is considered a major threat to global tomato production because of its recent occurrence in European production centers [2, 3].

In Brazil, *T. absoluta* is considered the primary insect pest affecting tomato cultivation, leading to significant reductions in productivity and losses of up to 100% [2, 3]. Damage from *T. absoluta* is caused by caterpillars that feed and develop in the soft tissues of plants, including the leaves, shoots, and fruits of the aerial parts of tomato plants, during any stage of plant growth. This infestation weakens the plant, leading to wilting, necrosis (tissue death), and leaf drop. Additionally, it damages fruits, making them unsuitable for consumption or commercialization [4].

The predominant method of controlling *T. absoluta* is the use of insecticides [4, 5]. However, since the 1990s, evidence has indicated limited efficacy of the active ingredients used to combat this pest [6]. Excessive and exclusive use of synthetic insecticides, coupled with the intrinsic biological characteristics of *T. absoluta*, has led to the selection of populations resistant to multiple classes of insecticides, including pyrethroids, avermectins, thiocarbamates, and diamides [4, 5, 7, 8].

In nature, plant species manifest in two distinct forms: as cultivated plants, intentionally planted by humans, and as spontaneous plants that emerge and develop without human intervention. While the former results from intentional actions, the latter emerges and grows autonomously [2, 3]. *Tuta absoluta* is not only a pest in tomato cultivation; it is a polyphagous pest that can develop on various cultivated and uncultivated crops within the Solanaceae family, as well as on other non-solanaceous host plants. Potatoes (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and black nightshades (*Solanum nigrum* L.) are susceptible to *T. absoluta* infestation [2, 9, 10]. Additionally, non-solanaceous host plants for *T. absoluta* have been reported, including *Chenopodium album* L. from the family Amaranthaceae, *Convolvulus arvensis* L. from the family Convolvulaceae, *Vicia faba* L. from the family Fabaceae, and *Malva* spp. from the family Malvaceae [11-13].

Many uncultivated host plants can be found in various environments such as fields, wastelands, cultivated areas, and urban spaces. The survival of *T. absoluta* larvae fed on spontaneous plant species, such as *Solanum tuberosum* L. (67%) and *Solanum dulcamara* L. (52%), was statistically higher than *Nicotiana rustica* L. (23%) and *Lycium halimifolium* Mill. (17%), while all larvae died in the case of *Nicotiana tabacum* L., *Malva sylvestris* L., and *Vicia faba* L. [14]. These diverse host plants can act as reservoirs or refuges for *T. absoluta*, allowing the pest to survive and multiply even when preferred hosts, such as tomato plants, are not available.

The identification of host plants is a crucial step in the risk assessment process and can be used to determine whether spontaneous plant species should be eradicated from the field [15]. Little is known about the development and reproductive capacity of *T. absoluta* on most agricultural and uncultivated plants reported as potential hosts in the literature [15, 16]. Understanding the potential host plants of *T. absoluta* can support the development of more targeted and effective management strategies. By prioritizing the most susceptible host species, it becomes possible to implement more precise monitoring approaches, as these plants serve as primary foci of *T. absoluta* infestations. This knowledge enables the rapid identification of high-risk areas and supports preventive actions, thereby reducing the likelihood of pest dispersal. Furthermore, focusing on the most heavily attacked hosts facilitates the implementation of specific control measures, such as the strategic application of selective insecticides, the use of bioinsecticides, and the synchronized release of parasitoids.

Identifying plant species on which *T. absoluta* does not develop allows for studies on insecticidal action against pests [16]. The use of vegetables and products derived from constituents such as extracts and essential oils as alternatives for insect control has increased in industrialized countries, including Brazil. These substances can act as repellents, growth

inhibitors, ovicides, and larvicides against various insects including *T. absoluta* [16, 17]. This represents a promising alternative to the conventional chemical control methods. Furthermore, the diversity of compounds present in different plants offers various options for pest control, contributing to the adoption of more specific and selective approaches [17].

Therefore, this study aimed to assess the development of *Tuta absoluta* on various spontaneous plant species and to investigate the natural insecticidal effects of species in which *T. absoluta* cannot establish.

## 2. MATERIAL AND METHODS

### 2.1 Research site and facilities

The experiments were carried out in the laboratory of the Integrated Pest Management Research Group in Agriculture (AGRIMIP), affiliated with the Department of Plant Protection at São Paulo State University "Júlio de Mesquita Filho" (UNESP), located on the Botucatu Campus, São Paulo, Brazil. The experiments were conducted in climate-controlled facilities, maintaining a constant temperature of  $25 \pm 2$  °C, photoperiod of 12 hours, and relative humidity of  $70 \pm 10\%$  [18]. These controlled conditions are essential for ensuring the accuracy and reliability of the test results.

### 2.2 Breeding and maintenance of *T. absoluta* in the laboratory

Adult *T. absoluta* moths, originating from commercial tomato plantations in the state of São Paulo, Brazil, were maintained under controlled conditions within rectangular cages measuring 0.4 x 0.4 m at the base and 0.5 m in height [18]. These cages were constructed with side openings covered with nylon mesh. Plastic pots containing soil and 30-day-old tomato plants (with six leaves) were placed inside the cages; no insecticides were applied to the plants. Egg-bearing plants were later transferred to other cages of identical size to allow hatching. During the larval stage, plants damaged by the caterpillars were replaced every three days. When the caterpillars reached the pupal stage, they were maintained in cages with dried plants until emergence. The adults were fed a 10% honey solution, whereas the caterpillars were provided with Santa Clara tomato plants for nourishment [18].

### 2.3 Development of *T. absoluta* on spontaneous plant species

For the experiments, nine species of spontaneous plants commonly found in commercial tomato crops were utilized: purple morning glory (*Ipomoea purpurea* L.), family Convolvulaceae; Benghal dayflower (*Commelina benghalensis* L.), family Commelinaceae; slender amaranth (*Amaranthus viridis* L.), family Amaranthaceae; hairy beggarticks (*Bidens pilosa* L.), family Asteraceae; tropical soda apple (*Solanum viarum* L.), family Solanaceae; Brazilian pusley (*Richardia brasiliensis* L.), family Rubiaceae; southern sandbur (*Cenchrus echinatus* L.), family Poaceae; hairy fleabane (*Conyza bonariensis* L.), family Asteraceae; and American black nightshade (*Solanum americanum* L.), family Solanaceae. Seedlings were cultivated in pots (40 cm diameter x 20 cm height) in a greenhouse. Spontaneous plants were used in this study when they reached 15 days after seedling emergence because this signified their complete development with the necessary leaf area for conducting the tests. This ensured that the plants were at an appropriate growth stage for the tests to be conducted accurately, allowing the acquisition of relevant and reliable results [18].

Tomato plants were not used as control specimens in the tests because of their preferential nature as hosts for *T. absoluta*. Preliminary tests were conducted in choice tests, where the caterpillars migrated to the tomato leaves, significantly influencing the results. This approach became irrelevant when the research focused on periods when tomato cultivation was absent. During these times, the presence of the pest in the field was observed on other host plants.

Therefore, the spontaneous plant species *S. americanum* was chosen as a control, as it has been widely investigated, in which *T. absoluta* can complete its life cycle [10, 12, 13].

## 2.4 Preference tests for oviposition of *T. absoluta*, with choice and no-choice tests

Two experiments were conducted following a completely randomized design for both the choice and no-choice tests. Each experiment consisted of nine species of spontaneous plants, with each species serving as a treatment (species described in section 2.3). There were five replicates for both choice and no-choice tests.

The preference test for oviposition with choice was conducted in rectangular cages, measuring  $1.0 \times 1.0$  m at the base and 0.6 m in height (Figure 1). These cages were constructed using a plastic frame and side openings covered with nylon mesh [18]. In each repetition of this test, spontaneous plants were arranged equidistantly (ten centimeters apart) in a circular layout and two pairs of newly emerged adult *T. absoluta* were released at the center of the cage for each spontaneous plant species (Figure 1). During the test, adults were provided with 10% honey solution for feeding, prepared with 90% distilled water and 10% pure wild honey, which was absorbed by cotton placed in a Petri dish (90 x 15 mm) at the center of the cage [19].



Figure 1: Cage used (A) and arrangement of nine spontaneous plant species (B) offered for oviposition of *Tuta absoluta* in a choice experiment. Botucatu, São Paulo, Brazil, 2022.

The no-choice oviposition assay was conducted in acetate cages (smooth and transparent plate made of sodium and acetic acid, more resistant than plastic), measuring 10.5 cm in height and 9.0 cm in diameter. The cages were completely closed at the bottom and top using acrylic Petri dishes with a diameter of 9.0 cm. One plant of each spontaneous species was placed in each cage and two pairs of newly emerged adult *T. absoluta* were released. During the experiment, adults were provided with a 10% honey solution prepared with 90% distilled water and 10% pure wild honey, which was absorbed by cotton.

The number of eggs per plant was counted in both analyses. This was performed with the assistance of a stereoscope model EZ4 (Leica Microsystems Ltd., Brazil) 24, 48, and 72 hours after the release of the adults [19]. At each egg counting moment, a spontaneous plant was individually removed from inside the cage. The eggs were counted, and the plant was carefully transferred back to the cage, ensuring the moths remained inside.

## 2.5 Preference tests for feeding of *T. absoluta*, with choice and no-choice tests

The experimental design adopted for the choice tests was a randomized block design, whereas a completely randomized design was used for the no-choice tests. The experiment involved nine species of spontaneous plants as treatments (described in section 2.3), with five

replicates for each type of test (with choice and no-choice), totaling two distinct trials. Non-preference feeding tests were carried out using fourth-instar caterpillars, aged 12 days.

The choice test was conducted in aluminum trays with a diameter of 30 cm and height of 5 cm (Figure 2). The bottom of each tray was lined with moistened filter paper. Leaves from each plant of the spontaneous species were collected and each leaf had a surface area of 14–17 cm<sup>2</sup>. Leaves were evenly arranged in a circle (five centimeters apart) inside the arenas (trays). In the center of each arena, two caterpillars per spontaneous species were released, and the arena was sealed with plastic wrap, following the protocol described by Boiça Junior et al. (2012) [19] and Oliveira et al. (2009) [20].

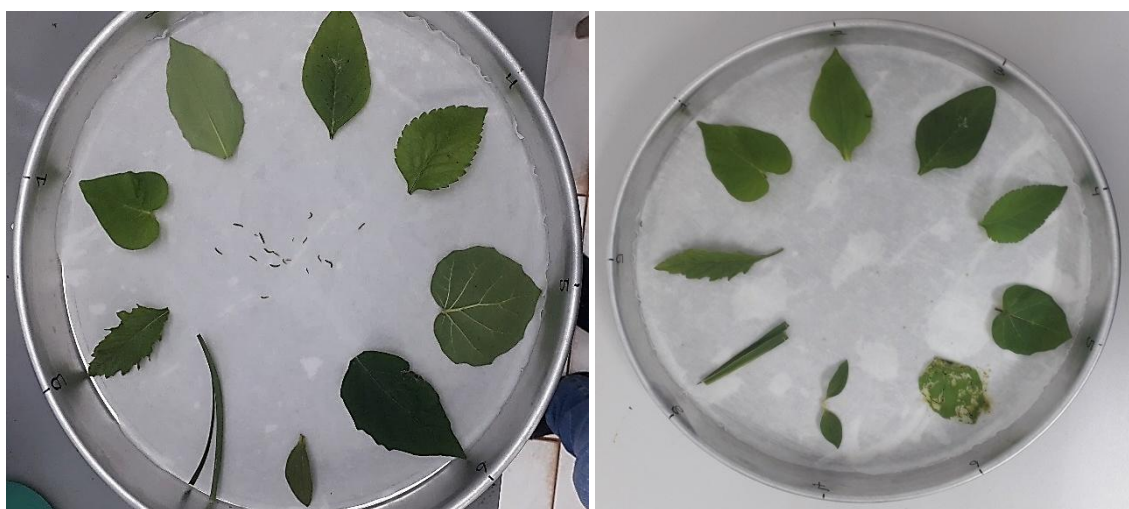


Figure 2: Arrangement of nine spontaneous plant species offered to *Tuta absoluta* in a choice feeding experiment. Botucatu, São Paulo, Brazil, 2022.

The no-choice test was conducted in 9.0 cm diameter Petri dishes. The bottom of each dish was lined with moistened filter paper. In each Petri dish, a single leaf from the corresponding plant was placed in the center and two caterpillars were released for the test.

In both tests, the attractiveness of the caterpillars to the treatments was assessed in each repetition at intervals of 15, 30, 60, 120, 360, 720, and 1440 min after caterpillar release. Additionally, to calculate the percentage of leaf area consumed (%), the initial leaf area was subtracted from the final leaf area for each leaf of all spontaneously grown plant species tested. This calculation was performed using the "Easy Leaf Area" app [21].

## 2.6 Antibiosis test

For the antibiosis test, the experimental design was completely randomized. This experiment included nine species of spontaneous plants as treatments with five replicates per treatment. Each replicate included five newly hatched *T. absoluta* caterpillars, totaling forty-five experimental units and 225 caterpillars for evaluation.

Firstly, *T. absoluta* eggs were obtained and carefully transferred to Petri dishes (9 cm diameter x 1.5 cm height) using a brush until hatching. Subsequently, the newly hatched caterpillars were placed in Petri dishes containing slightly moistened filter paper (1 ml of distilled water) at the bottom and leaves corresponding to each species of spontaneous plants. Notably, the leaves provided to the caterpillars were completely developed. Every two or three days, new leaves were obtained from the middle and apical parts of the plants [22].

The Petri dishes were monitored daily to record pupal formation. After 24 hours of pupation, the pupae were individually weighed and placed in glass tubes (8.5 cm height x 2.5 cm diameter). Adults emerged in these tubes and were later identified based on their sex. The evaluated parameters included the duration and viability of the larval and pupal stages

(equation 1), duration and viability from caterpillar eclosion to adult emergence (total) (equation 1), adult longevity without food, pupal mass at 24 h, and the sex ratio (equation 2) [22].

$$\text{Viability (\%)} = \frac{\text{Number of caterpillars, pupae, or adults obtained}}{\text{Initial number of insects in each stage of development}} \times 100 \quad (1)$$

$$\text{Sex ratio} = \frac{\text{Number of females}}{\text{Number of females} + \text{Number of males}} \quad (2)$$

## 2.7 Insecticidal effect of essential oil extracted from *C. bonariensis* on *T. absoluta*

*Tuta absoluta* mortality was evaluated using the essential oil of *C. bonariensis*. This was not feasible for the other plant species on which *T. absoluta* did not develop (*C. benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, and *C. echinatus*) as not enough essential oil was obtained to conduct the experiment.

The essential oil extracted from *C. bonariensis* was obtained from the AGRIMIP laboratory using the steam distillation method. A steam distillation apparatus (model MA 480, Marconi Equipamentos para Laboratórios Ltd., Brazil) was used for this procedure. The plant material was placed in a perforated metal basket, which was positioned in the apparatus to maintain it above the distilled water (five liters) in the distillation vessel. Subsequently, the water was heated and the material underwent a steam current directed through the condenser of the apparatus. As this mixture of oil and water vapor condenses, it separates into layers owing to the difference in density, resulting in the extraction of the essential oil.

The essential oil was extracted from fresh plant material collected on the same day. All plant materials, including leaves and stems, were used [23]. The plant material was cut into 10 cm long pieces and filled with a perforated metal basket. To ensure an adequate quantity of essential oil, three separate extractions were performed. For each extraction, 4.0 kg of the plant material was placed in an extractor. The extraction period was approximately 8 hours, and afterward, the essential oil was stored in amber containers sealed under refrigeration temperature ( $4 \pm 0.5$  °C) [24].

The breeding of *T. absoluta*, cultivation of *C. bonariensis* seedlings, and the soil used, as well as the insects employed in the bioassays, were obtained following the methodology described in sections 2.2 and 2.3 [18].

Preliminary tests were performed to determine the concentrations required for the bioassays. The maximum concentration was determined to be 0.10% (above this, it caused detrimental effects on tomato leaves) and the minimum concentration was set at 0.005% (below this, there was no mortality at any stage of *T. absoluta*). Each bioassay included six treatments: acetone PA control, and 0.005%, 0.01%, 0.04%, 0.07%, and 0.10% of essential oil from *C. bonariensis* diluted in acetone PA (99.7%) to facilitate homogenization of the solution [25].

### 2.7.1 Ovicidal action bioassay on *T. absoluta* eggs

For the bioassays with *T. absoluta* eggs, the pupae were sexed, placed in groups of five pairs in cages measuring  $30 \times 30 \times 15$  cm, and covered with an anti-aphid mesh. A tomato seedling was placed inside the cage as an oviposition substrate to eliminate the need to remove the leaves from the plant. This strategy aims to prevent stress on plants for translocation purposes and reduce egg damage caused by handling individual leaves.

A period of 48 hours was allowed for oviposition to obtain eggs within two days of development. This approach facilitated the bioassay with eggs that had completed two days of incubation [26].

Subsequently, the plants were inspected, leaving only 50 eggs per plant, which were then sprayed with the treatments. Spraying was performed using a calibrated airbrush at an



application rate of 1,000 L ha<sup>-1</sup>, with a pound force per square inch (psi) pressure that allowed the deposition of 1.5 mg cm<sup>-2</sup> of each solution/concentration, as measured using a precision electronic balance [27]. The spray solution was applied using an air compressor (model EL 504, Eletrolab Indústria e Comércio de Equipamentos para Laboratório Ltd., Brazil) and deposition was confirmed on water sensitive paper. For spraying, the treatments were separated using an insulin syringe with a matrix solution volume of 1.13 ml, equivalent to the application rate for which the equipment was previously calibrated. This volume was placed in 2.0 ml Eppendorf® tubes and sprayed onto the target. To prevent the influence of one treatment on the other, the spraying system was cleaned with 100% alcohol after each application [27].

The experiment was conducted using a completely randomized design consisting of seven treatments (as described in section 2.7) with five replicates. Each plot contained 15 eggs. The ovicidal effects and the number of hatched caterpillars per treatment were evaluated. The assessment was conducted 96 hours after the application of the treatments, which coincided with the sixth day of egg incubation (144 h). This time was chosen to ensure that no eggs hatched at that time, given that the hatching process typically begins around the fourth day of incubation. The percentage of mortality was calculated using equation 3:

$$\text{Mortality (\%)} = \frac{\text{Number of dead eggs}}{\text{Total number of eggs}} \times 100 \quad (3)$$

The reduction percentages of hatched eggs, or efficiency percentages, were calculated for each treatment based on Abbott's formula (equation 4) [28], considering that the caterpillars hatched from treatments that did not cause mortality in the eggs.

$$\text{Efficiency (\%)} = \frac{\text{Number of caterpillars (control group)} - \text{Number of caterpillars (treatment group)}}{\text{Number of caterpillars (control group)}} \times 100 \quad (4)$$

### 2.7.2 Bioassay for the control of *T. absoluta* caterpillars

Bioassays with *T. absoluta* caterpillars were conducted after generating the respective populations under laboratory conditions and caterpillars of the same larval instar were available. Caterpillars of the same age (second instar) were placed on the leaves of a tomato crop in a greenhouse without a history of insecticide application, specifically designated for this purpose. The composite leaves were removed from the plants and transported to the laboratory. Subsequently, the leaflets were detached and, if necessary, the edges were trimmed to have a diameter smaller than that of a Petri dish. The leaves selected for the bioassay were those located at the apex of the plant, between the third and fourth expanded leaves, because they were less lignified, easier to handle, and more suitable for feeding laboratory-reared caterpillars that were later transferred to this new substrate.

Before detachment and trimming, the leaflets were sprayed with the treatments described in section 2.7. The tomato plants were air-dried at room temperature and only after this process the leaves were detached and offered to the caterpillars.

Dried leaflets were individually placed in Petri dishes lined with moistened filter paper. A second instar caterpillar was confined to each leaflet inside each Petri dish. Evaluations were conducted on the first day and ended on the sixth day after confinement. Bioassays were conducted according to methods described by Branco et al. (2001) [29]. One second instar *T. absoluta* caterpillar was confined to each leaflet. The experiment was performed using a randomized complete block design with 30 replicates, totaling 30 caterpillars for each treatment. After treatment, the caterpillars were kept in climate-controlled rooms at 25 ± 2 °C, 70 ± 5% relative humidity, and a photoperiod of 14 hours.

The live and dead caterpillars were evaluated using a stereoscope. Caterpillars that did not exhibit agility or movement were considered dead. To confirm mortality on the last day of the evaluation, the leaves were desiccated to observe the caterpillars inside the mine. During the

intermediate evaluations, the caterpillars were not removed from the mine to avoid injuring the insects with the brush. Only mortality during feeding or contact with the treated surface was assessed. The caterpillar mortality percentage was calculated using equation 5:

$$\text{Mortality (\%)} = \frac{\text{Number of dead caterpillars}}{\text{Total number of confined caterpillars}} \times 100 \quad (5)$$

The population reduction percentages of the pest or efficiency percentages were also calculated for each treatment using Abbott's formula (equation 4) [28].

## 2.8 Statistical analysis

In all tests, the data were subjected to analysis of variance using the F-test, and when significant, the means were compared using Tukey's test at a 5% significance level. The computational program SISVAR version 5.8 was used for the data analysis [30]. The data for the number of eggs and number of *T. absoluta* insects attracted were transformed using  $(x + 0.5)^{1/2}$ , and the original data were tabulated.

## 3. RESULTS AND DISCUSSION

### 3.1 Preference tests for oviposition of *T. absoluta*, with choice and no-choice tests

In the choice oviposition test of *T. absoluta*, there were differences in all the evaluations, that is, the number of eggs laid at 24, 48, and 72 h after the release of the adults (Table 1).

Table 1: *Tuta absoluta* egg numbers at 24, 48, and 72 hours after adult release on different spontaneous plant species in a choice oviposition test (mean  $\pm$  standard deviation).

Spontaneous plant species	Number of eggs after 24 hours	Number of eggs after 48 hours	Number of eggs after 72 hours
<i>Ipomoea purpurea</i>	2.00 $\pm$ 1.55 a	8.60 $\pm$ 2.73 ab	20.6 $\pm$ 4.68 b
<i>Commelina benghalensis</i>	3.80 $\pm$ 1.53 ab	13.2 $\pm$ 6.49 ab	37.4 $\pm$ 11.9 b
<i>Amaranthus viridis</i>	0.00 $\pm$ 0.00 a	1.80 $\pm$ 1.11 a	3.20 $\pm$ 1.53 a
<i>Bidens pilosa</i>	0.00 $\pm$ 0.00 a	0.20 $\pm$ 0.20 a	1.40 $\pm$ 1.17 a
<i>Solanum viarum</i>	9.20 $\pm$ 3.60 ab	25.4 $\pm$ 6.02 b	36.0 $\pm$ 8.46 b
<i>Richardia brasiliensis</i>	0.60 $\pm$ 0.60 a	1.60 $\pm$ 1.17 a	3.80 $\pm$ 2.01 a
<i>Cenchrus echinatus</i>	0.00 $\pm$ 0.00 a	0.40 $\pm$ 0.40 a	0.40 $\pm$ 0.40 a
<i>Conyza bonariensis</i>	0.00 $\pm$ 0.00 a	0.20 $\pm$ 0.20 a	0.60 $\pm$ 0.24 a
<i>Solanum americanum</i>	38.4 $\pm$ 21.8 b	91.2 $\pm$ 16.3 c	165 $\pm$ 11.8 c
F	3.83*	28.9*	58.7*
C.V. (%)	35.6	22.0	18.6

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

Within the first 24 h, *S. americanum* was seen to have the highest average number of eggs laid (38.4 eggs), distinguishing it from the other species. After 48 h, *S. americanum* exhibited the highest oviposition rate (91.2 eggs), followed by *S. viarum* (25.4 eggs). After 72 h, *S. americanum* maintained the highest oviposition rate for *T. absoluta* (165 eggs), followed by *C. benghalensis* (37.4 eggs), *S. viarum* (36.0 eggs), and *I. purpurea* (20.6 eggs). These significant differences indicated that *S. americanum*, *C. benghalensis*, *S. viarum*, and *I. purpurea* had higher oviposition rates than the other species studied (Table 1).

In the no-choice oviposition test for *T. absoluta* (Table 2), differences were observed in all evaluations (eggs deposited 24, 48, and 72 hours after the release of adults). This oviposition pattern was consistent with that observed in the choice test, in which *S. americanum* had the



highest mean number of eggs deposited in all evaluations. After 72 hours, *S. americanum* presented 49.2 eggs deposited, followed by *C. benghalensis* (42.2 eggs), *S. viarum* (26.2 eggs), and *I. purpurea* (15.4 eggs). The other species showed lower mean egg deposition (Table 2).

A lower oviposition preference was observed among the *T. absoluta* adults in both the choice and no-choice oviposition tests for the spontaneous plant species *C. echinatus*, *C. bonariensis*, *R. brasiliensis*, *B. pilosa*, and *A. viridis*. In addition to the antibiosis identified in a study on the effect of spontaneous plant species on insect development, antixenosis or non-preference for oviposition has also been shown to play a significant role in the resistance of these species to *T. absoluta* [31]. The higher oviposition rates of *S. americanum* and *S. viarum* are consistent with the fact that these species belong to the same botanical family as the tomato plant, Solanaceae, making them more attractive to pests [32].

Table 2: *Tuta absoluta* egg numbers at 24, 48, and 72 hours after adult release on different spontaneous plant species in a no-choice oviposition test (mean  $\pm$  standard deviation).

Spontaneous plant species	Number of eggs after 24 hours	Number of eggs after 48 hours	Number of eggs after 72 hours
<i>Ipomoea purpurea</i>	5.00 $\pm$ 2.17 ab	7.40 $\pm$ 3.66 ab	15.4 $\pm$ 3.63 bc
<i>Commelina benghalensis</i>	10.6 $\pm$ 4.40 ab	28.4 $\pm$ 6.98 c	42.2 $\pm$ 7.36 cd
<i>Amaranthus viridis</i>	2.80 $\pm$ 1.24 ab	5.80 $\pm$ 3.02 ab	7.40 $\pm$ 3.78 ab
<i>Bidens pilosa</i>	0.00 $\pm$ 0.00 a	0.40 $\pm$ 0.40 a	0.60 $\pm$ 0.40 a
<i>Solanum viarum</i>	7.80 $\pm$ 2.80 ab	19.2 $\pm$ 4.62 bc	26.2 $\pm$ 5.30 bcd
<i>Richardia brasiliensis</i>	0.00 $\pm$ 0.00 a	0.20 $\pm$ 0.20 a	0.40 $\pm$ 0.24 a
<i>Cenchrus echinatus</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.80 $\pm$ 0.80 a
<i>Conyza bonariensis</i>	4.20 $\pm$ 1.83 ab	7.00 $\pm$ 1.97 ab	8.80 $\pm$ 1.66 ab
<i>Solanum americanum</i>	16.4 $\pm$ 6.50 b	37.8 $\pm$ 10.9 c	49.2 $\pm$ 14.3 d
F	3.64*	10.0*	14.0*
C.V. (%)	23.3	23.4	22.3

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

The resistance of certain spontaneous plant species to *T. absoluta* is related not only to the intrinsic plant characteristics affecting insect development, but also to their ability to be non-preferential hosts for oviposition [32]. This understanding is crucial for developing management strategies aimed at reducing the effects of *T. absoluta* on tomato fields and other related crops by leveraging the natural resistance of certain host plant species. For instance, the strategic selection of intercrops that are less suitable for *T. absoluta* development. Similarly, integrating resistant spontaneous species as cover plants or in border strips may function as “push” components in push–pull strategies, reducing oviposition on the main crop and slowing larval establishment. Examples include tomato intercropped with coriander (*Coriandrum sativum*), which reduces oviposition rates by interfering with the pest's host-location behavior; the use of cowpea (*Vigna unguiculata*) or crotalaria (*Crotalaria juncea*) as green manures and intercrops, which enhance natural enemy abundance; and maize or sorghum border rows, which act as physical and chemical barriers, decreasing tomato attractiveness.

The oviposition preference of *T. absoluta* may be closely related to the chemical and morphological characteristics of the spontaneous plant species. For instance, species within the Solanaceae family, such as tomato plants, often have a higher sugar content, measured by Brix degrees ( $^{\circ}$ Brix), and can emit attractive odor characteristics of this family, which lures *T. absoluta* [33, 34]. Furthermore, the morphological features of plants play a fundamental role in their interactions with pests. Trichomes, microscopic structures on leaf surfaces, can hinder insect access to leaf surfaces or restrict movement. Additionally, some glandular trichomes secrete toxic or sticky exudates that can trap pests. These structures store chemical components that act as repellents or toxic agents against arthropods [33].

Volatile compounds, such as sesquiterpenes, exert adverse effects on both the survival (antibiosis) and preference (antixenosis) of herbivorous insects on tomato plants. This is due to

the influence of these compounds on the flight behavior and oviposition of female *T. absoluta* during mating [31]. Remarkably, females showed a higher propensity to land and deposit eggs on species that were more susceptible to these volatile compounds, thus establishing a direct relationship between the presence of these compounds and female preference for egg-laying [31].

Another source of resistance against *T. absoluta* moths involves the use of plants containing specific compounds, such as 2-tridecanone, acylsugars (which are allelochemicals), and zingiberene [35]. These compounds have demonstrated repellent or toxic properties against *T. absoluta*, making plants containing them less attractive or even harmful to pests. 2-tridecanone is a chemical compound that has shown repellent activity against herbivorous insects, including *T. absoluta* [35]. Acylsugars are allelochemicals that can affect the behavior and survival of insects, whereas zingiberene has demonstrated toxic activity against pests [35, 36].

High survival rates, short development times, and high fecundity rates of herbivorous insects are all correlated with a suitable food quality source. *T. absoluta* females depend on plant volatile organic compounds to assess host quality, which could have misled them during the host selection process. Oviposition on a suitable host, in response to plant cues, is the beginning of a new generation cycle and is of utmost importance for the insect to maximize its fitness [14].

### 3.2 Preference tests for feeding of *T. absoluta*, with choice and no-choice tests

In the choice feeding tests, significant differences were observed in all assessments (time: minutes after caterpillar release) concerning the attractiveness to *T. absoluta* (Table 3).

Table 3: Population density of *Tuta absoluta* caterpillars attracted to the spontaneous plant species at different time intervals after release and the consumed leaf area in the choice feeding test.

Spontaneous plant species	Population density							Consumed leaf area (%)
	Time after caterpillar release (mean, minutes)							
	15'	30'	60'	120'	360'	720'	1440'	
<i>Ipomoea purpurea</i>	0.80 ab	0.80 ab	1.00 abc	0.60 ab	1.00 a	1.00 ab	1.00 ab	3.82 a
<i>Commelina benghalensis</i>	3.00 bc	3.00 bc	2.60 c	2.20 b	1.60 a	1.80 b	2.20 b	0.70 a
<i>Amaranthus viridis</i>	1.20 ab	1.00 ab	0.40 ab	1.40 ab	0.60 a	0.20 ab	0.20 a	0.00 a
<i>Bidens pilosa</i>	0.80 ab	1.00 ab	0.80 abc	0.60 ab	0.60 a	0.40 ab	0.60 ab	0.00 a
<i>Solanum viarum</i>	1.80 ab	2.80 bc	1.80 bc	2.40 b	1.80 a	1.60 ab	1.80 ab	3.24 a
<i>Richardia brasiliensis</i>	0.40 a	0.20 a	0.40 ab	0.20 a	0.20 a	0.20 ab	0.20 a	0.00 a
<i>Cenchrus echinatus</i>	0.20 a	0.20 a	0.00 a	0.20 a	0.00 a	0.00 a	0.00 a	0.00 a
<i>Conyza bonariensis</i>	1.00 ab	0.80 ab	0.80 abc	0.60 ab	0.40 a	0.80 ab	0.80 ab	0.00 a
<i>Solanum americanum</i>	5.60 c	6.00 c	6.40 d	6.40 c	7.60 b	8.00 c	9.20 c	37.0 b
F	9.17*	10.1*	17.7*	13.2*	18.0*	18.6*	26.5*	24.0*
C.V. (%)	6.09	6.41	5.16	5.86	5.88	6.05	5.71	17.0

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

*Solanum americanum* was the most preferred species for feeding, attracting 9.20 caterpillars 1440 min after caterpillar release. *Commelina benghalensis* was the second most preferred species, attracting 2.20 caterpillars after 1440 min. In contrast, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus*, and *C. bonariensis* showed low attractiveness to caterpillars in all assessments (Table 3).

Differences were observed in the leaf area consumed by the caterpillars in the choice feeding test (Table 3). The spontaneous plant species *S. americanum* had the highest leaf area consumed compared to the other species, totaling 37.0% leaf consumption (Figure 3). *Ipomoea purpurea* (3.82%), *S. viarum* (3.24%), and *C. benghalensis* (0.70%) also showed some degree of leaf consumption. The caterpillars did not feed on the leaves of the other species.

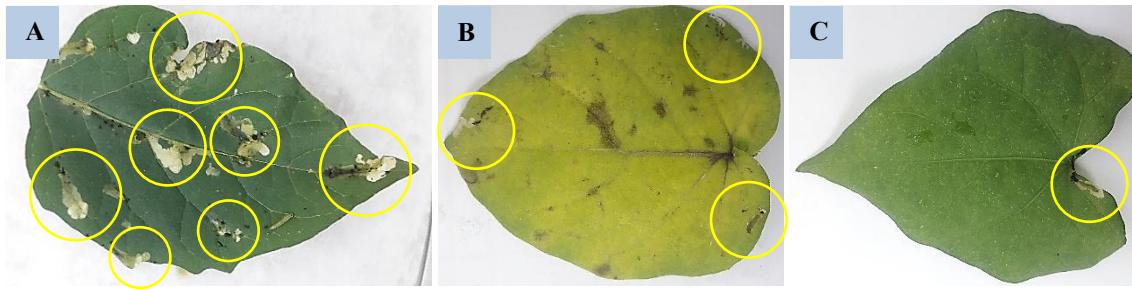


Figure 3: Feeding and development of *Tuta absoluta* on spontaneous plant species. (A) *Solanum americanum*; (B) *Solanum viarum*; and (C) *Ipomoea purpurea*. Botucatu, São Paulo, Brazil, 2022.

Regarding attractiveness and feeding preference in the no-choice scenario, differences were observed only in the assessments conducted 360 and 1440 minutes after caterpillar release (Table 4). *Solanum americanum* and *S. viarum* were more attractive to *T. absoluta* caterpillars than other spontaneous plant species, with 2.00 and 1.80 caterpillars attracted 1440 min after caterpillar release, respectively (Table 4).

Table 4: Population density of *Tuta absoluta* caterpillars attracted to the spontaneous plant species at different time intervals after release and the consumed leaf area in the no-choice feeding test.

Spontaneous plant species	Population density							Consumed leaf area (%)
	Time after caterpillar release (mean, minutes)							
	15'	30'	60'	120'	360'	720'	1440'	
<i>Ipomoea purpurea</i>	0.80	0.80	0.40	0.60	0.20 a	0.40	0.60 ab	4.74 bc
<i>Commelina benghalensis</i>	1.20	1.20	1.60	1.40	0.60 ab	1.00	1.00 ab	0.00 a
<i>Amaranthus viridis</i>	1.00	1.00	0.80	1.40	0.60 ab	0.80	0.40 a	0.00 a
<i>Bidens pilosa</i>	1.00	1.40	0.80	1.20	1.40 ab	0.80	1.20 ab	0.00 a
<i>Solanum viarum</i>	1.00	0.80	1.20	1.20	1.20 ab	1.00	1.80 b	2.34 ab
<i>Richardia brasiliensis</i>	0.60	0.60	0.60	0.40	0.80 ab	1.40	0.60 ab	0.00 a
<i>Cenchrus echinatus</i>	0.40	0.60	0.80	0.60	1.00 ab	1.20	0.60 ab	0.00 a
<i>Conyza bonariensis</i>	1.00	1.00	1.20	1.20	1.00 ab	1.20	1.20 ab	0.00 a
<i>Solanum americanum</i>	1.00	1.60	1.60	1.60	2.00 b	2.00	2.00 b	10.8 c
F	0.55 <sup>ns</sup>	1.12 <sup>ns</sup>	1.56 <sup>ns</sup>	1.82 <sup>ns</sup>	3.03*	2.04 <sup>ns</sup>	3.73*	7.11*
C.V. (%)	4.19	4.06	4.23	3.88	3.73	3.87	3.61	13.3

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. <sup>ns</sup> = Not significant. F = F-test: significance test. C.V. = Coefficient of variation.

In the no-choice feeding test, *S. americanum* exhibited the highest leaf area consumption, reaching 10.8%, followed by *I. purpurea* (4.74%) and *S. viarum* (2.34%). Conversely, the remaining species did not show any leaf area consumed by caterpillars (Table 4). These results confirmed the feeding preference of *T. absoluta* caterpillars for *S. americanum*, *I. purpurea*, and *S. viarum* compared with the other species tested in the experiment.

In the choice feeding test, a high movement of caterpillars among species was observed during the initial minutes of the evaluation. Shortly after their release, they dispersed among the selected species and established a greater presence in *S. americanum*. This observation suggests that volatile compounds released by the leaves affect caterpillar behavior, considering that this initial phase is critical for the selection of host plants for feeding and/or oviposition by phytophagous insects [19].

Several plant species are resistant to *T. absoluta* [20]. Resistance to damage caused by *T. absoluta* can be indicated by the presence of many small minerals on the leaves, suggesting that the insects do not feed on the plant [37]. The resistance of these species may be associated with the presence of compounds that inhibit or deter *T. absoluta* from feeding [38].

*Bidens pilosa* extract is effective in controlling pests, such as *Sitophilus oryzae*, *Oryzaephilus surinamensis*, and *Acanthoscelides obtectus*, because of its deterrent effect on

insects through the volatile compounds present in the plant [39]. This was supported by the results of the present study as *T. absoluta* exhibited low oviposition and did not feed on *B. pilosa*.

Similar to many plant species belonging to the family Gelechiidae, *T. absoluta* caterpillars have an internal feeding habit on various parts of the host plant, making them challenging to control [3]. Caterpillars consume the mesophyll layers of leaves, stems, and fruits. In addition to tomatoes, *T. absoluta* feeds on other species of the family Solanaceae, including eggplant (*Solanum melongena* L.), American black nightshade (*Solanum americanum* L.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and jimsonweed (*Datura stramonium* L.) [2, 40, 41]. Alternative host plants play a crucial role as pest reservoirs, allowing the survival of *T. absoluta* when tomato crops are unavailable. The ability to persist and reproduce on other host plants contributes to the maintenance of vigorous pest populations throughout the year. Consequently, this can lead to more intense infestation pressure on the next tomato crop, as the pest can survive and proliferate in other crops during periods when tomatoes are not cultivated [3].

### 3.3 Antibiosis test

There were differences in the development of *T. absoluta* among the different spontaneous plant species, as shown in Tables 5 and 6. Caterpillars fed *S. americanum* leaves had a longer larval duration, averaging 13.1 days, and a high larval survival rate of 56.0%. Additionally, the pupal stage had an average duration of 9.70 days and a pupal survival rate of 78.0%. Although *S. viarum* and *I. purpurea* exhibited low larval and pupal survival rates, these numbers were sufficient for *T. absoluta* development. In contrast, *T. absoluta* did not survive on *C. benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus*, and *C. bonariensis*, indicating an unfavorable environment for pest development (Table 5).

Table 5: Duration and survival of the larval and pupal stages of *Tuta absoluta* reared on the leaves of spontaneous plant species (mean  $\pm$  standard deviation).

Spontaneous plant species	Larval stage		Pupal stage	
	Duration (days)	Survival (%)	Duration (days)	Survival (%)
<i>Ipomoea purpurea</i>	6.12 $\pm$ 0.98 c	16.0 $\pm$ 7.48 b	8.33 $\pm$ 2.10 b	30.0 $\pm$ 20.0 a
<i>Commelina benghalensis</i>	2.04 $\pm$ 0.10 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Amaranthus viridis</i>	2.00 $\pm$ 0.06 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Bidens pilosa</i>	1.88 $\pm$ 0.05 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Solanum viarum</i>	4.88 $\pm$ 0.49 b	12.0 $\pm$ 4.90 ab	9.33 $\pm$ 1.93 b	40.0 $\pm$ 24.5 ab
<i>Richardia brasiliensis</i>	1.96 $\pm$ 0.04 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Cenchrus echinatus</i>	1.88 $\pm$ 0.08 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Conyza bonariensis</i>	1.96 $\pm$ 0.04 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Solanum americanum</i>	13.1 $\pm$ 0.89 d	56.0 $\pm$ 7.48 c	9.70 $\pm$ 0.37 b	78.0 $\pm$ 10.2 b
F	99.2*	22.1*	16.1*	7.18*
C.V. (%)	3.21	21.8	9.60	37.3

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

When reared on tomato leaves (Santa Clara cultivar), *T. absoluta* had a larval duration of 17.0 days [19]. This represents a 22.9% increase in larval duration compared to caterpillars reared on *S. americanum* leaves, a 64.0% increase compared to caterpillars reared on *I. purpurea*, and a 71.3% increase compared to caterpillars reared on *S. viarum* leaves. Similarly, when reared on tomato leaves (Santa Clara cultivar), the pupae had a duration of 9.70 days [19]. This value is similar to the findings of the present study, with caterpillars reared on *S. americanum* leaves that also exhibited a pupal duration of 9.70 days. Pupae reared on

*I. purpurea* had a duration of 8.33 days, while those reared on *S. viarum* had a pupal duration of 9.33 days. This pattern can be explained by the fact that most of these species are unsuitable hosts for *T. absoluta*, exhibiting low attractiveness and lacking essential nutrients, while producing allelochemicals (such as phenols, flavonoids, or alkaloids) that impair larval feeding. Under these conditions, larvae often initiate feeding but quickly cease ingestion due to poor nutritional quality or toxicity, leading to shortened and artificial developmental cycles and, in most cases, complete mortality before reaching the pupal stage (e.g., *Commelina benghalensis*, *Amaranthus viridis*, and *Bidens pilosa*). These results emphasize the significant influence of host plants on the biology and development of *T. absoluta*. The host plant can affect the life cycle of the pest, which is an important aspect to consider in the integrated pest management of tomato crops and other related cultures.

Adult longevity, which was assessed based on the total period from eclosion to adult emergence, as well as survival throughout this period, showed a consistent pattern. *Solanum americanum* exhibited the highest values in these parameters. While *S. viarum* and *I. purpurea* had lower values, *T. absoluta* was able to complete its life cycle in all three species, indicating that they are susceptible hosts for the pest. In contrast, no *T. absoluta* development was observed on *C. benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus*, or *C. bonariensis*, suggesting that these plants are not favorable hosts for the pest (Table 6).

Table 6: Adult longevity, duration, and survival from larva to adult, average pupal mass, and sex ratio of *Tuta absoluta* reared on leaves of the spontaneous plant species (mean  $\pm$  standard deviation).

Spontaneous plant species	Adult longevity (days)	Larval-adult duration (days)	Larval-adult survival (%)	Average pupal mass (mg)	Sex ratio
<i>Ipomoea purpurea</i>	5.50 $\pm$ 1.36 b	22.0 $\pm$ 5.44 a	8.00 $\pm$ 4.90 a	3.43 $\pm$ 0.86 b	0.30 $\pm$ 0.20 ab
<i>Commelina benghalensis</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Amaranthus viridis</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Bidens pilosa</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Solanum viarum</i>	6.25 $\pm$ 1.84 c	22.1 $\pm$ 5.47 a	8.00 $\pm$ 4.90 a	3.70 $\pm$ 0.76 b	0.60 $\pm$ 0.24 b
<i>Richardia brasiliensis</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Cenchrus echinatus</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Conyza bonariensis</i>	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a
<i>Solanum americanum</i>	6.50 $\pm$ 0.92 bc	29.3 $\pm$ 1.09 b	44.0 $\pm$ 7.48 b	3.90 $\pm$ 0.14 b	0.53 $\pm$ 0.12 b
F	8.73*	12.8*	17.6*	16.3*	4.99*
C.V. (%)	8.33	20.1	21.4	4.59	1.51

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

Adverse effects on pests indicate that antibiosis is a factor responsible for lower susceptibility. The underlying causes of this phenomenon can be associated with several host plant characteristics, including the presence of glandular and non-glandular trichomes as well as features associated with leaves, midribs, fruits, crystal idioblasts, or plant growth habits. The complex interactions among these factors can contribute to the natural resistance of host plants to *T. absoluta*, hindering pest feeding, development, and survival, and consequently resulting in lower susceptibility to damage [42].

In addition to spontaneous plant species, the age of the plant and leaf development stage can also play crucial roles in pest susceptibility. This is due to variations in volatile compound concentrations, which can affect the behavior of *T. absoluta* [42]. Based on the results of the present study, it is evident that spontaneous plants provide a solid foundation for further studies. However, considering the variations in volatile compounds throughout plant development, it is crucial to consider germination at different stages. This comprehensive exploration of changes in plant volatile compounds at different growth stages offers valuable insights for future research and the development of more effective agricultural management strategies.

Chemical compounds that affect the nervous and enzymatic systems of insects can induce behavioral changes, reduce their reproductive and behavioral performance, and, in some cases,

cause death. These compounds are often employed in pest control strategies because they can have significant adverse effects on insects by impairing their vital functions [43].

Numerous studies conducted in different regions around the world report damage caused by *T. absoluta* on *S. americanum*, which is consistent with this study. It becomes evident that *S. americanum* is a highly favorable host for this pest [2, 32, 44, 45].

Populations of *T. absoluta* can be maintained at specific locations, surviving on various tomato plants that grow alongside corn when cultivated in rotation. Additionally, this pest can be found in wild solanaceous plants, such as *S. viarum* Dunal and *S. americanum* Mill., as well as in other cultivated solanaceous plants, such as potatoes and eggplants [46]. The dispersal ability of *T. absoluta* allows it to spread to new areas cultivated with tomatoes, often aided by the wind. Furthermore, the transport of infested fruits containing caterpillars during commercialization or processing plays a significant role in the geographic spread of insects [46].

Although *S. viarum* and *I. purpurea* may not be the preferred hosts of *T. absoluta*, the oviposition, feeding, and presence of a small number of pest survivors observed in this study highlight the significance of these species for the survival of *T. absoluta* at all stages of development in the field. Therefore, it is crucial to consider effective control measures for these host plants to prevent increases in pest populations of tomato crops. The implementation of integrated pest management strategies that address these alternative hosts plays a vital role in safeguarding crops.

Predicting insect host range from laboratory experiments can be challenging, especially with spontaneous host plants, as these results did not include field constraints. Factors such as climatic variations, temporal conditions, and hence phenological and physiological state of the plants may have an influence on host selection patterns for the insects [46]. Genetic variability in plants and insects, in addition to local selective pressures on plant traits, also play a role in this scenario [44]. Considering climatic conditions as significant barriers to the insect spread, and plant availability, it is possible that *T. absoluta* spreads from an infested tomato production area to neighboring favorable environments, not only focusing on agricultural ecosystems [14]. Further studies in open-field environments are needed to validate these assumptions.

### 3.4 Insecticidal effect of essential oil extracted from *C. bonariensis* on *T. absoluta*

#### 3.4.1 Bioassay of ovicidal action on eggs of *T. absoluta*

Significant effects were observed in the assay evaluating the mortality and efficacy of different concentrations of essential oil from *C. bonariensis* in controlling *T. absoluta* eggs (Table 7).

Table 7: Efficacy of different concentrations of hairy fleabane essential oil (*Conyza bonariensis* L.) in controlling *Tuta absoluta* eggs (mean  $\pm$  standard deviation).

Treatment	Concentration (%)	Efficacy (%)
1. <i>Conyza bonariensis</i> essential oil	0.10%	94.8 $\pm$ 0.03 a
2. <i>Conyza bonariensis</i> essential oil	0.07%	55.2 $\pm$ 0.03 b
3. <i>Conyza bonariensis</i> essential oil	0.04%	34.5 $\pm$ 0.06 c
4. <i>Conyza bonariensis</i> essential oil	0.01%	24.1 $\pm$ 0.03 c
5. <i>Conyza bonariensis</i> essential oil	0.005%	1.72 $\pm$ 0.02 d
6. Control (Acetone)	-	1.72 $\pm$ 0.02 d
F	-	55.6*
C.V. (%)	-	9.47

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

A concentration of 0.10% exhibited the optimal control efficacy (94.8%), followed by 0.07% (55.2%). Intermediate values were observed for concentrations of 0.04% and 0.01%, with

control efficacies of 34.5% and 24.1%, respectively. In contrast, 0.005% concentration demonstrated a lower efficacy (1.72%), which did not differ significantly from the control using only acetone (Table 7).

In general, lepidopteran eggs possess a lipid or waxy layer inside the egg chorion, which prevents insecticide penetration [47]. It is possible that the active compounds in the 0.10% concentration of *C. bonariensis* oil could penetrate this lipid layer and affect the embryonic development of *T. absoluta*, leading to embryo death [48].

### 3.4.2 Bioassay for controlling *T. absoluta* caterpillars

A significant effect was observed in the assay that evaluated the mortality and efficacy of different concentrations of *C. bonariensis* essential oils in controlling *T. absoluta* caterpillars (Table 8). Concentrations of 0.10%, 0.07%, and 0.04% exhibited the highest control rates (100%), followed by 0.01% (82.1%). The 0.005% concentration displayed low efficacy (32.1%). All concentrations differed from the control, which used only acetone (Table 8).

Table 8: Efficacy of different concentrations of hairy fleabane essential oil (*Conyza bonariensis* L.) in controlling *Tuta absoluta* caterpillars (mean  $\pm$  standard deviation).

Treatment	Concentration (%)	Efficacy (%)
1. <i>Conyza bonariensis</i> essential oil	0.10%	100 $\pm$ 0.00 a
2. <i>Conyza bonariensis</i> essential oil	0.07%	100 $\pm$ 0.00 a
3. <i>Conyza bonariensis</i> essential oil	0.04%	100 $\pm$ 0.00 a
4. <i>Conyza bonariensis</i> essential oil	0.01%	82.1 $\pm$ 0.05 b
5. <i>Conyza bonariensis</i> essential oil	0.005%	32.1 $\pm$ 0.06 c
6. Control (Acetone)	-	0.00 $\pm$ 0.04 d
F	-	46.9*
C.V. (%)	-	13.2

\*Means followed by the same letter in a column do not differ significantly according to Tukey's test at 5% probability. F = F-test: significance test. C.V. = Coefficient of variation.

The daily mortality of second-instar *T. absoluta* caterpillars over six days subjected to different concentrations of *C. bonariensis* essential oil showed that all caterpillars died on the first day of evaluation at concentrations of 0.10% and 0.07%, indicating high efficacy. At a concentration of 0.04%, caterpillar mortality persisted until the fourth day of the evaluation. Concentrations of 0.01% and 0.005% resulted in caterpillar mortality that extended to the last day of evaluation (sixth day) (Figure 4).

*Conyza bonariensis* essential oil was shown to cause mortality in *T. absoluta* caterpillars, with variations in the duration of action according to the concentration used, with higher concentrations resulting in faster and more effective action (Figure 4). These results suggest that *C. bonariensis* essential oil has significant potential as a control agent for *T. absoluta* eggs and caterpillars and provides an effective alternative for the integrated management of this pest.

Information regarding the effects of botanical essential oils on *T. absoluta* eggs and caterpillars is limited. *Conyza bonariensis* essential oil is a rich source of various bioactive secondary metabolites with promising insecticidal properties, particularly against leaf eating caterpillars (*Spodoptera littoralis*) (Lepidoptera: Noctuidae) [49]. Compounds such as  $\beta$ -amyrin-3-acetate,  $\beta$ -amirenona, and 5,4'-dihydroxy-6,7-dimethoxyflavone, first isolated from the *Conyza* genus, have demonstrated pronounced toxic effects on *S. littoralis* [49].

*Conyza dioscoridis* L. essential oil had significant adverse effects on *S. littoralis* survival, fecundity, and oviposition, and interfered with pupal and adult development [49]. This is due to the effects of these compounds on phenolics, terpenoids, and other bioactive metabolites [49]. The cumulative mortality rate reached 76.6% during the pupal stage and 83.3% during the adult stage, and after the fourth instar, *S. littoralis* larvae were fed *C. bonariensis* oil [49]. Furthermore, specific compounds such as  $\beta$ -amirenona, lupeol acetate, and 5,4'-dihydroxy-6,7-



dimethoxyflavone demonstrated a remarkable ability to suppress fourth-instar *S. littoralis* larvae, achieving significant reductions of 50%, 60%, and 73.3% at concentrations of 0.3%, 0.5%, and 0.5%, respectively [49]. These results corroborate the findings of the present study, indicating that these bioactive compounds are effective in controlling *T. absoluta* eggs and caterpillars and highlighting their potential as control agents for this pest.

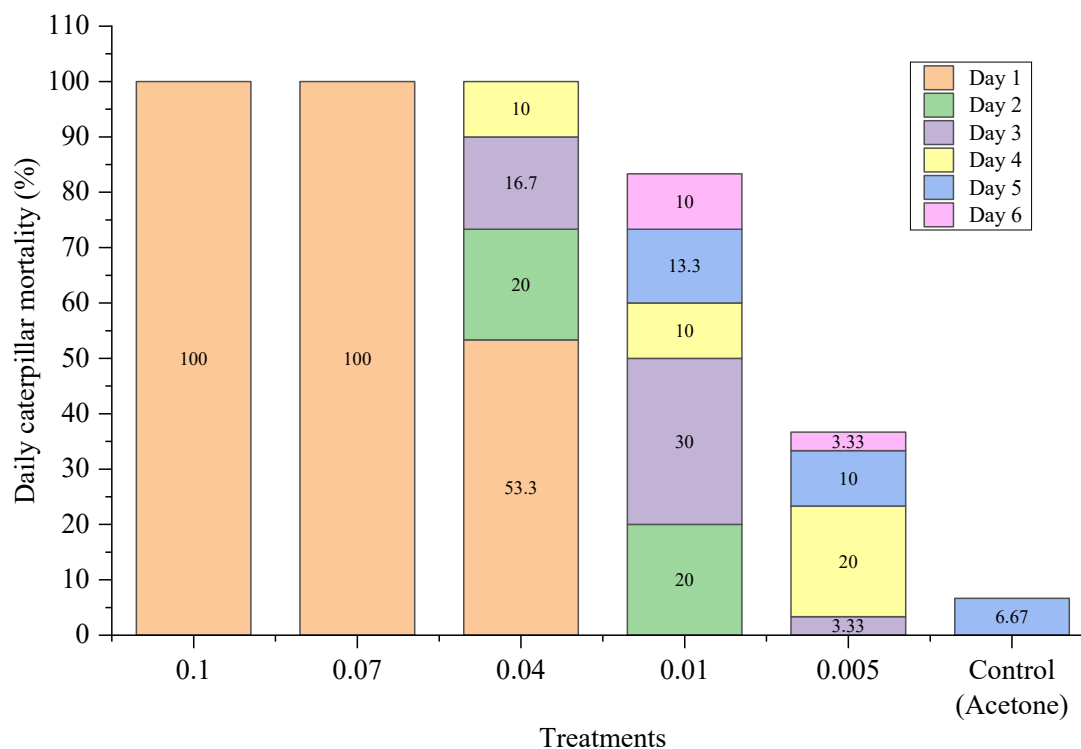


Figure 4: Daily mortality of second-instar *Tuta absoluta* caterpillars over six days. The caterpillars were subjected to different concentrations of hairy fleabane essential oil (*Conyza bonariensis* L.).

The insecticidal activity, including larvicidal and ovicidal effects, can be attributed to the presence of phenolic compounds, triterpenes, and sesquiterpenes [50]. Owing to their lipophilic properties, triterpenes and sesquiterpenes can facilitate permeability through the insect egg membrane or tissues, where they can damage the reproductive system tissues or inhibit vital enzymes [51]. Pentacyclic triterpenes play a role in plant defense, exert antifeedant effects on insects, and exert various other pharmacological activities [52].

Flavonoids are an important class of secondary metabolites found in plants that have beneficial effects on human health and adverse effects on insect pests. They have antifeedant properties and growth-inhibitory effects on insects, likely because they interfere with endocrine regulation [53].

Furthermore, leaves from plants of the *Conyza* genus demonstrated toxicity and antifeedant effects against first-instar larvae of Pink Bollworm (*Pectinophora gossypiella*) (Lepidoptera: Gelechiidae) due to the presence of  $\alpha$ -cadinol, caryophyllene oxide,  $\beta$ -eudesmol, and  $\alpha$ -selinene [54].

Sesquiterpenes with an elemene skeleton demonstrate moderate antifeedant activity against larvae of *S. littoralis* [55]. Furthermore, the insecticidal activity of  $\beta$ -phellandrene against the German Cockroach (*Blattella germanica*) is correlated with its ability to inhibit the enzyme acetylcholinesterase (AChE) [56].

#### 4. CONCLUSIONS

The spontaneous plant species *S. americanum*, *S. viarum*, and *I. purpurea* can be considered viable host plants for *T. absoluta* as the pest can complete its life cycle on them. Therefore, it is crucial to implement effective control measures for these plants to prevent an increase in *T. absoluta* populations in tomato crops.

*Commelina benghalensis*, *A. viridis*, *B. pilosa*, *R. brasiliensis*, *C. echinatus*, and *C. bonariensis* provide an unfavorable environment for the development of *T. absoluta*. The essential oil extracted from *C. bonariensis* was effective in controlling *T. absoluta* eggs and caterpillars, constituting as an effective option for managing this pest at both the egg and caterpillar stages.

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