

Biopesticide Potential of *Laurus nobilis* and *Inula viscosa* Essential Oils for the Management of Tomato Leaf Miner (*Tuta absoluta*)

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Abstract

The tomato leaf miner, *Tuta absoluta*, is one of the most destructive tomato pests worldwide. The use of chemical pesticides can improve yield and fruit quality; however, it poses serious risks to human health and the environment. Consequently, research efforts are increasingly focusing on sustainable, eco-friendly pest management strategies, including the use of essential oils as biopesticides. This study evaluates the insecticidal activity of *Laurus nobilis* and *Inula viscosa* essential oils against the four larval instars of *T. absoluta*. Essential oils were assessed using a contact toxicity assay, with each dose tested across all larval instars and compared with an untreated control. Lethal doses (LD50 and LD90) were estimated using Probit regression analysis. The bioassays showed that both essential oils exhibited significant larvicidal activity with dose- and stage-dependent mortality. Early larval instars required lower doses to achieve 50% and 90% mortality (10, 20, and 30 µl for *I. viscosa*; 3, 5, and 10 µl for *L. nobilis*), whereas later instars were more tolerant and required higher doses. Complete mortality of all four larval instars was obtained at 100 µl of *I. viscosa* and 30 µl of *L. nobilis*. Overall, these findings demonstrate the larvicidal potential of these essential oils and support their use as biopesticides within integrated pest management programs in agriculture. To our knowledge, this is the first report of the larvicidal activity of the essential oils from *I. viscosa* and *L. nobilis* against *T. absoluta* in Algeria.

Keywords: biopesticides, crop pest, essential oils, pest management, tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is a globally cultivated crop that is adapted to worldwide mass consumption (Lecompte et al., 2014). This native tropical crop plays a significant role in the national and agricultural economy. It is the sixth-most-produced commodity in Algeria (FAOSTAT, 2025). However, it is one of the main crops that provides a propitious environment for several pests, such as nematodes, insects, and other arthropods. It is exposed to several viral, fungal, and/or bacterial diseases that severely reduce its yields (Simões et al., 2023).

The South American tomato leaf miner is considered to be one of the major tomato pests in many South American countries since the 1960s (Ponti et al., 2021). In 2008, this lepidopteran was observed for the first time in Algeria, in the Mostaganem area. Because its life cycle is synchronized with that of the tomato plant, it is recognized as a major obstacle within the tomato industry (Allache et al., 2012; Guenaoui, 2008). The larvae feed on entire tomato plants at all growth stages, creating mines and large galleries in their leaves and disrupting photosynthesis, thereby affecting plant development and severely reducing yields (Tesso, 2014).

In 2022, the Food and Agriculture Organization of the United Nations estimated that 3.70 million tons of pesticides were used in agriculture, a 4% increase from 2021 and a 13% increase over the previous decade (FAO,

2022). In Algeria, nearly 6,067,35 tons of pesticides are used in Agriculture (FAOSTAT, 2025). The application of chemical pest control for agricultural purposes plays a major role in improving productivity, quantity, and quality of yields. Although they guarantee food security and production, the control of pesticide residues is still not generalized, nor are the doses nor the frequency of pesticide use controlled by producers. The contamination of soil, ground, and surface water, and non-target organisms reduces biodiversity and directly impacts living beings (FAO, 2022), including humans, posing a real danger to health and the environment.

Within this context, research towards finding more sustainable and environmentally friendly pest management strategies has become critically important. Plant-based products, such as essential oils, are gaining more attention as biopesticides, and their application as insecticides, fungicides, or even bactericides has increased considerably within the integrated pest management approach in agriculture. For the most part, they belong to only a few families in the plant kingdom, primarily the Myrtaceae family, the Lauraceae family, the Lamiaceae family, and the Asteraceae family (Tanasă et al., 2024).

Therefore, in this study, we evaluated the larvicidal activity of the essential oils extracted from *L. nobilis* and *I. viscosa* against the four larval instars of *T. absoluta*. Both species are native Mediterranean plants (Khodja et al., 2023; Parolin et al., 2014) belonging to two different botanical families: the *Asteraceae* and the *Lauraceae*, known for their biological activities (Damasceno et al., 2019; Soković et al., 2019).

Materials and Methods

Plant Collection and Essential Oil Extraction

The aerial parts of *I. viscosa* and *L. nobilis* were both collected from the Mansourah region in Tlemcen province, Algeria, from July to October 2022. The plant material was dried naturally under shade at room temperature (25 - 26 °C) for 15 days before extraction. Each dried plant

material was hydrodistilled using a Clevenger-type apparatus for nearly 5 hr; the yields were 0.06% (w/w) for *I. viscosa* and 0.71% (w/w) for *L. nobilis*. The extracted essential oils were stored in sterilized, closed, amber-colored vials at 4 °C in a refrigerator until use.

Larvicidal Activity Bioassay

Tuta absoluta Larvae Collection

Tuta absoluta larval specimens were collected in April 2023 from infested tomato greenhouses in the Fellaoucen region (Tlemcen, Algeria). The simple random sampling method was applied to collect tomato leaves (about 10 kg). The collected leaf samples were placed in plastic bags and transferred to the laboratory for biological testing on the same day and the following day after collection.

Laboratory Bioassays

The *T. absoluta* larvae were carefully removed from the tomato leaves and then separated into their four larval instars. Larval instars were determined based on morphological characteristics described by the National Research Institute for Agriculture, Food, and the Environment and the Pest Distribution and Risk Atlas for Africa by Mujica et al. (2022). The early instar was colorless; the third instar gradually turned green, and in the last stage, they became orange or pink.

The larvicidal effectiveness of our essential oils was tested through the contact toxicity procedure reported by Slimane et al. (2014). The method relies on direct contact of the larvae with the treated surface, which parallels their direct contact in a treated field. The essential oils were applied in their pure, undiluted form to facilitate reproducibility under natural conditions and to ensure that the observed larvicidal activity is fully attributable to the essential oils' bioactivity.

Ten larvae of each instar were placed separately in petri dishes (90 mm) containing filter paper over fresh tomato leaves. Using a micropipette, the filter paper was soaked with the

different applied doses of each essential oil; the petri dishes were then secured using tulle and maintained in rearing laboratory conditions at 25 °C. Each experiment was conducted three times for each applied dose and larval instar, with a control group. The applied doses tested per petri dish for *I. viscosa* essential oil were: 10, 20, 30, 50, 80, 100, 200 µl; those tested for *L. nobilis* essential oil were: 1, 3, 5, 10, 20, 30, 50 µl. Counts were recorded 24 hr after birth as alive or dead. The applied doses were initially determined based on results from previous studies (Aissaoui et al., 2023; Belhoucine et al., 2016; Lo Pinto et al., 2019; Mssillou et al., 2022), and the doses reported in this study were selected following preliminary tests. Different doses were tested on a small batch of *T. absoluta* larvae to select the dose range that resulted in a dose-response effect.

Statistical Analysis

To evaluate the insecticidal effectiveness of the two essential oils across the four larval instars, a series of analyses was conducted using the Probit regression model in IBM SPSS Statistics version 20. This method was selected to determine lethal doses (LD50 and LD90) based on observed mortality rates at various dosage levels. The doses were log-transformed to better linearize the dose-response relationship.

To confirm the accuracy of the dose-response relationship (R^2), regression curves demonstrating the relation between log-transformed doses and probit-transformed mortality data were also produced. The

coefficient of determination (R^2) was calculated for each larval instar separately. Finally, the 50% (LD50) and 90% (LD90) lethal doses were obtained from the 95% confidence interval table, thus providing the doses required to achieve the expected mortality.

Results and Discussion

The essential oils of both *L. nobilis* and *I. viscosa* had larvicidal effects with variable mortality rates, compared to the control groups after 24 hr of exposure. The lethal dose (LD) levels of the *I. viscosa* and *L. nobilis* essential oils obtained using the probit regression model are presented in Tables 1 and 2, respectively. The results show significant differences between oils and larval instars.

Results from the bioassay showed that of the seven applied doses tested for *I. viscosa* (10, 20, 30, 50, 80, 100, 200 µl), only the two higher doses, 100 µl and 200 µl, effectively killed all larval instars. While effective knockdown doses varied across the larval instars. For the first three larval instars, lower doses of 10 µl, 20 µl, and 30 µl resulted in significant mortality, whereas the fourth instar required higher doses of 50 and 80 µl.

As for the seven doses tested for *L. nobilis* (1, 3, 5, 10, 20, 30, 50 µl), the bioassay results showed that the mortality doses also varied across larval instars. In the first larval instar, the lowest dose tested, 3 µl, resulted in a mortality rate >50%, preventing the observation of any sublethal effects. Lower effective doses for second and third larval instars (3, 5, 10 µl) were

Table 1

LD50 and LD90 Values of Inula viscosa Essential Oil Against the Four Larval Stages of Tuta absoluta, with the 95% Confidence Intervals

| Larvae instar | LD50 (µl) | 95% Confidence interval for LD50(µl) | LD90 (µl) | 95% Confidence interval for LD90(µl) |
|------------------------|-----------|--------------------------------------|-----------|--------------------------------------|
| 1 st instar | 11,729 | 6,366; 15,504 | 21,971 | 16,503; 51,519 |
| 2 st instar | 22,032 | 16,174; 27,878 | 40,144 | 31,159; 69,231 |
| 3 st instar | 21,458 | 15,609; 25,708 | 29,745 | 25,029; 61,995 |
| 4 st instar | 61,315 | 29,534; 175,214 | 169,584 | 87,103; 7193,102 |

Table 2

LD50 and LD90 Values of Laurus nobilis Essential Oil Against the Four Larval Stages of Tuta absoluta with the 95% Confidence Intervals

| Larvae instar | LD50 (µl) | 95% Confidence interval for LD50(µl) | LD90 (µl) | 95% Confidence interval for LD90(µl) |
|------------------------|-----------|--------------------------------------|-----------|--------------------------------------|
| 1 st instar | 2,094 | 1,281; 3,007 | 4,944 | 3,377; 11,616 |
| 2 st instar | 13,920 | 9,231; 21,518 | 53,963 | 31,763; 163,291 |
| 3 st instar | 12,346 | 8,806; 17,112 | 31,335 | 21,620; 64,213 |
| 4 st instar | 18,093 | 13,354; 23,767 | 36,169 | 26,849; 68,732 |

effective for the knockdown. As for the fourth larval instars, they required higher doses (10 and 20 µl). All the larvae instars subjected to the essential oils succumbed at the highest doses of 30 and 50 µl.

With the probability values (p values < 0.05) confirming the reliability of the measured responses, which refers to the larval mortality. The analysis shows a significant relationship between the increasing mortality in parallel with the increasing essential oil doses (Figure 1) and (Figure 2). The coefficient of determination (R^2) value indicates a valid relationship between log-transformed doses and probit-transformed mortality. The R^2 values ranged between 0.86 and 1 for the *I. viscosa* essential oil, and between 0.950 and 1 for the *L. nobilis* essential oil.

Essential oils are known for their insecticidal, antifungal, and antibacterial properties, which help protect plants in nature (Corrêa et al., 2023; Kesraoui et al., 2022). They are generally characterized by 20 to 60 components at different levels of concentrations; among them, the secondary metabolites, which represent a combination of terpenes, phenolic, and phenylpropanoid compounds, and that play a significant part in plant chemical defense (Bakkali et al., 2008; Kumar et al., 2022; Yu, 2025).

The insecticidal potential of various essential oils has been demonstrated in many studies. Essential oils of *Pistacia atlantica* and *Pistacia lentiscus* have shown insecticidal activity against the confused flour beetle *Tribolium confusum* (Tabti et al., 2020). The antiparasitic activity of the *Ammoides verticillata* essential oil

has proved its effectiveness against the olive fruit fly *Bactrocera oleae* (Senouci et al., 2020). Research by Saraiva et al. (2020) confirmed that *Pelargonium graveolens* geranium essential oil can be used as a biopesticide against the housefly, *Musca domestica*, and the Australian sheep blowfly, *Lucilia cuprina*.

Laurus nobilis essential oil has proved its efficiency against several insect species like the common house mosquito *Culex pipiens* (Aissaoui et al., 2023), the melon aphid *Aphis gossypii* Glover (Ebrahimi et al., 2013), and the yellow fever mosquito *Aedes aegypti* (Fernandez et al., 2018), including the pest targeted in this study, *T. absoluta*. A study by Lo Pinto et al. (2019) showed that *Laurus nobilis* essential oil has larvicidal activity against *T. absoluta* and demonstrated its potential as an alternative pest-control agent. Similarly, exposure to *I. viscosa* essential oil of the bean beetle *Callosobruchus maculatus* (Mssillou et al., 2022), the olive fruit fly *B. oleae* (Belhoucine et al., 2016), and the wheat weevil *Sitophilus granaries* (Polatoglu et al., 2015) has resulted in significant mortality. The larvicidal activity of *L. nobilis* and *I. viscosa* essential oils against *T. absoluta* observed in our results is consistent with previous reports demonstrating their insecticidal/larvicidal activity.

Furthermore, the favorable response of *T. absoluta* to various essential oils like *Thymus capitatus*, *Tetraclinis articulata* (Bouayad Alam et al., 2017), *Thymus vulgaris*, *Cymbopogon citratus* (Ngongang et al., 2022), and many more, as reported in the review article by Dervisoglou et al. (2023), has been documented in different studies. However, to our knowledge, this is the

Figure 1

Log-Transformed Dose-Response Mortality of the Four Larval Instars to Inula viscosa Essential Oil

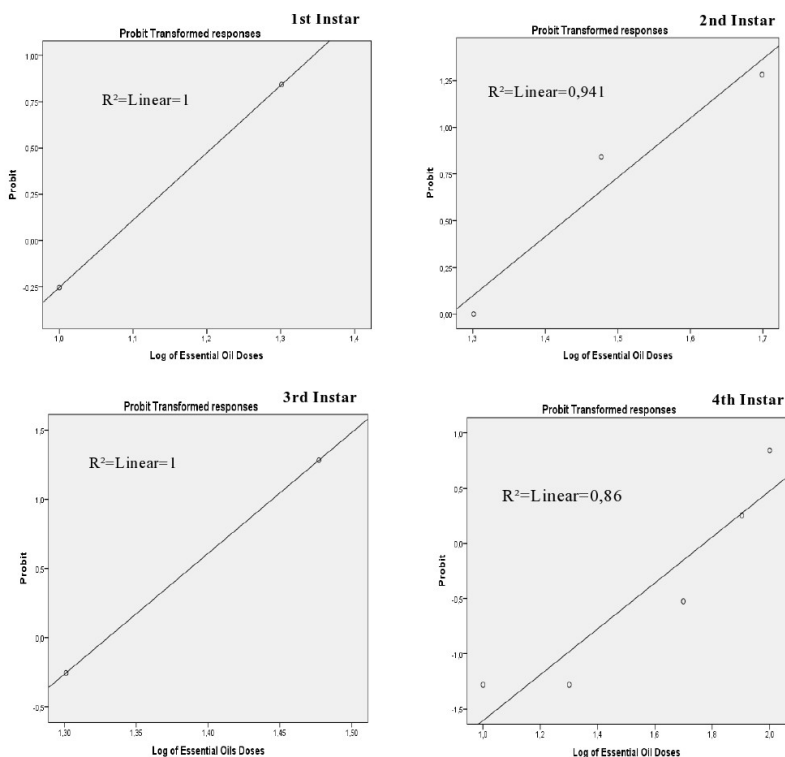
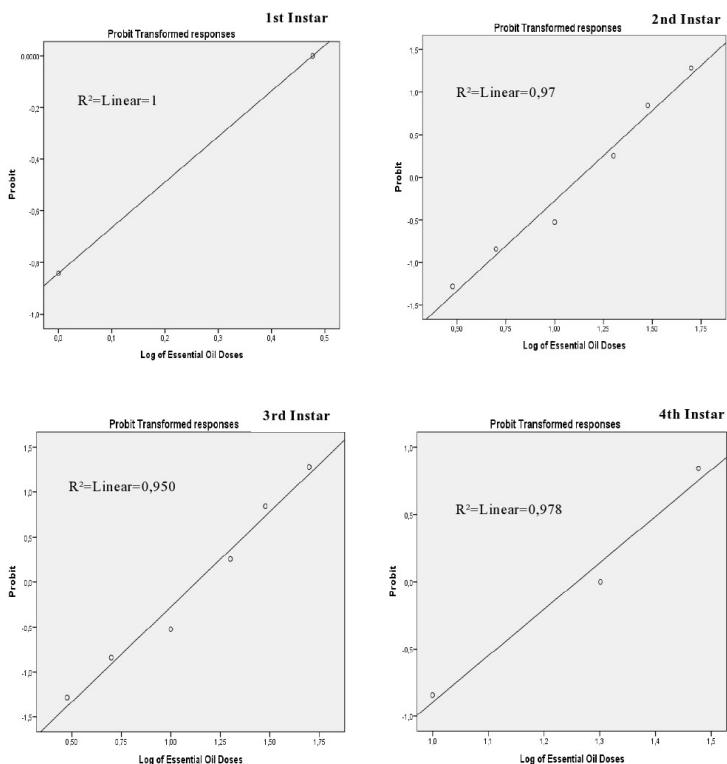


Figure 2

Log-Transformed Dose-Response Mortality of the Four Larval Instars to Laurus nobilis Essential Oil



first report of the larvicidal activity of both *I. viscosa* and *L. nobilis* essential oils in Algeria against one of the most significant agricultural pests, *T. absoluta*.

Chemical composition studies of the essential oil obtained from the Algerian *Laurus nobilis* done by Goudjil et al. (2015) and Jemâa et al. (2012) revealed that 1,8-cineole is the dominant terpene compound. More recent studies by Belasli et al. (2020) and Aissaoui et al. (2023) confirmed the chemical profile of this essential oil, reporting the same major compound 1,8-cineol. The latest research by Chibani et al. (2024) validated the presence of 1,8-cineole as the dominant compound in Algerian *Laurus nobilis* essential oil, which could be responsible for the insecticidal activity documented and observed in the present essential oil. Terpenes show insecticidal activity by either interacting with the insect's integument, digestive enzymes, or by interfering with their neurological system (Ayllón-Gutiérrez, 2024; Bakkali, 2008; Câmara et al., 2024; Isman et al., 2000). Therefore, the presence of secondary metabolites in the chemical composition of *L. nobilis* essential oil strongly supports the larvicidal activity observed against *T. absoluta* in this research.

The first report on the chemical composition of Algerian *I. viscosa* essential oils has identified it as a significant source of sesquiterpene acids (Haoui et al., 2015). A more recent study done in the same plant collection region as our study, by Ainseba et al. (2023), determined the chemical composition of the essential oil of *I. viscosa*. The result showed that the essential oil was primarily composed of hydrocarbon sesquiterpenes and oxygenated sesquiterpenes, with the major compounds being alloaromadendrene, α -bisabolol, α -cadinol, zingibrene, germacrene-D, bicyclogermacrene, τ -muurolol, β -copaene, and (E)- β -farnesene. In a study by Belhoucine et al. (2016), the chemical analysis of *I. viscosa* essential oil identified thymol and eugenol as the dominant compounds, which are considered active

compounds responsible for inhibiting *B. oleae*. In a recent study by Mssillou et al. (2022), the major active compounds identified were bornyl acetate and borneol, which caused mortality in *Callosobruchus maculatus*. The larvicidal activity of *I. viscosa* essential oil observed in this study may be linked to the previously mentioned terpene compounds.

A previous study by Umpiérrez et al. (2012) examined two essential oils from the same botanical family as *I. viscosa* as potential biocontrol tools against *T. absoluta* and demonstrated that these Asteraceae essential oils exhibit insecticidal activity against this pest. It is interesting to note that, for one of these essential oils, *Eupatorium buniifolium*, the most abundant components are hydrocarbons, sesquiterpenes, and monoterpenes, suggesting that sesquiterpenes may play a role in the insecticidal/larvicidal activity.

Conclusions

The evaluated essential oils in this study showed favorable activity against the four larval instars of *T. absoluta* at very low doses. These results confirm the high interest of the application of essential oils as biopesticides within the integrated pest management strategies in agriculture. Considering the continued spread of *T. absoluta* in warm climates, including tropical, subtropical, and Mediterranean regions, which severely affects tomato production, these findings contribute significantly to the exploration of sustainable, environmentally friendly management strategies to control one of the most invasive pests of tomato crops worldwide. Further tests and statistical analysis could be applied to better determine the mode of action and application of the essential oils. These results also open the possibility for further research on the efficacy of essential oils extracted from aromatic plants in the Mediterranean landscape for use against *T. absoluta* and other crop pests.

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