Potential of *Rosmarinus officinalis* aqueous extract in managing *Tuta absoluta* Meyrick infestations

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ABSTRACT

Tuta absoluta is the primary pest of tomatoes in its native Latin America. It was first detected in Spain in 2006 and quickly spread to Mediterranean countries, including Algeria by 2008. Medicinal plants, known for their bioactive compounds, offer a natural alternative to chemical pesticides. To promote the use of medicinal plants from the Batna region and combat tomato leaf miner, a laboratory study evaluated the efficacy of aqueous rosemary (Rosmarinus officinalis) leaf extract. Four doses (0.5%, 0.75%, 1%, and 1.5%) were tested against an untreated control and laboratory sucrose (100 ppm). The foliar treatment was applied every 15 days from 6 May to 1 July 2023 in the morning (8:00–10:00 am) using a hand sprayer for uniform coverage. Efficacy was assessed based on infestation levels, mine counts, and overall effectiveness. Mean values with standard errors were calculated for each parameter. Normality was verified using Shapiro-Wilk tests (α = 0.05); for non-normal distributions, non-parametric Kruskal-Wallis and Dunn's tests were applied. Results showed the control group had the highest infestation (50.10 ± 5.76%) and mine count (1.72 ± 0.22). The 0.5% and 1.5% rosemary extracts significantly reduced infestation by 35.15% and 37.20%, respectively ($p < 0.05$). The study confirms that rosemary leaf extract has toxic effects on T. absoluta, making it a viable alternative to chemical pesticides, capable of replacing conventional treatments without yield loss.

Keywords: Foliar spraying, infestation rate, Rosemary, SAADA variety, tomato leaf miner.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop, with annual production surpassing 189 million tonnes. In Algeria, production grew notably after the 2000 National Plan for Agricultural Development (PNDA), reaching 1.64 million tonnes in 2021. However, regional disparities exist, with Batna province declining to 58,057 quintals in 2020-2021 (DSA, 2024), underscoring cultivation challenges.

The tomato leafminer (*Tuta absoluta* Meyrick) is a highly destructive pest, first identified in South America in 1935 and now globally widespread, causing 80-100% crop losses. Its invasive success stems from high reproductive rates, multiple annual generations, and effective dispersal (Biondi *et al.*, 2018). Larvae damage all plant growth stages and attack other solanaceous crops like potatoes and eggplants (Mansour *et al.*, 2018; Ponti *et al.*, 2021). Recognized as a quarantine pest by EPPO (2008), its threat to agriculture remains severe.

While effective short-term, traditional chemical methods present significant drawbacks, synthetic pesticides cause environmental pollution, harm non-target organisms, disrupt beneficial microbes, and leave food chain residues (Campos *et al.*, 2021; Kumar *et al.*, 2023). Of particular concern is *T. absoluta* resistance development from overuse (Guedes and Siqueira, 2012), creating a cycle of increasing pesticide dependence with reduced efficacy. These issues have spurred interest in sustainable pest management alternatives.

Botanical pesticides derived from medicinal plants present an effective and eco-friendly pest management solution (Chama *et al.*, 2022). Studies confirm their efficacy against *T. absoluta*, including *Zygophyllum album* extract (Abderrahmene *et al.*, 2019), as well as rosemary essential oils exhibiting significant insecticidal activity (Pavela and Benelli, 2016). These alternatives combat pest resistance while meeting demands for sustainable agriculture.

Rosemary (*Rosmarinus officinalis* L.), a Lamiaceae family member, shows significant biopesticidal potential due to its bioactive compounds (phenolic acids, flavonoids, essential oils) with antioxidant, antimicrobial, and insecticidal properties (Lesnik *et al.*, 2021; Chama *et al.*, 2022). While most research focused on essential oils (Pavela and Benelli, 2016; Campos *et al.*, 2021), aqueous extracts offer practical advantages: lower cost, simpler preparation, and reduced non-target toxicity (Isman, 2020).

Despite their potential, key questions remain about rosemary aqueous extracts' practical use against *T. absoluta*. Optimal concentrations, application frequency, and mode of action require further study (Pavela *et al.*, 2019). Additionally, rosemary's phytochemical variability due to environmental factors, a phenomenon observed in other medicinal plants used for green pesticides (Benelli *et al.*, 2019). This variability demands region-specific research for consistent efficacy. Addressing these gaps could provide farmers with effective, accessible alternatives to synthetic pesticides.

The objective of this study is to evaluate the insecticidal efficacy of aqueous rosemary extracts at four concentrations (0.5%, 0.75%, 1%, and 1.5%) against *T. absoluta* larvae under controlled conditions. The research will assess the impact of foliar application on both pest mortality and tomato plant health, while comparing the performance of the extract to that of a laboratory sucrose-based biopesticide (100 ppm) and untreated controls.

MATERIALS AND METHODS

The rosemary aerial parts (leaves and stems) were collected during the pre-flowering phase (14 October 2022), in the morning between 10.00 and 11.00, in Batna Province, a semi-arid high-altitude region (>1000 m) with calcareous soils that enhance secondary metabolite production (Batna Forestry Department, 2023). These environmental conditions yield rosemary with significant phytochemical potential, ensuring authentic plant material aligned with regional ethnopharmacological practices.

The widespread adoption of pre-flowering harvesting (González-Minero *et al.*,2020) in medicinal and aromatic plant production ensures dual benefits: (i) optimization of bioactive compound (rosmarinic acid, carnosic acid, and essential oils) profiles to meet pharmacopoeial quality thresholds, and (ii) stabilization of secondary metabolite accumulation (e.g., tanshinones in *Salvia miltiorrhiza*; rosmarinic acid in *Rosmarinus officinalis*). Autumn harvesting in Batna's Mediterranean climate maintained moderate temperatures and solar exposure, preserving volatile compounds and antioxidant capacity (Chetia *et al.,* 2025).

The study employed tomato seedlings (Saada F1 hybrid) grown from seeds in potting soil. After germination, seedlings with two true leaves were transplanted into pots and maintained under laboratory conditions. Natural infestation by tomato leaf miner occurred spontaneously, confirmed on 19 March 2023 through observation of characteristic translucent leaf spots.

Fresh rosemary leaves were shade-dried to preserve heat-sensitive compounds and ground into a fine powder to maximize extraction efficiency. Different concentrations were prepared by suspending specific weights of powder in 100 ml of distilled water, followed by 2.5 hours of agitation (with covered beakers to prevent evaporation). After 24 hours of settling, supernatants underwent triple filtration to remove residual material. Final extracts were stored in airtight, foil-wrapped amber bottles at 4°C for up to 72 hours to prevent microbial growth and degradation of active compounds (Sharma *et al.,* 2020; Lesnik *et al*., 2021). The laboratory sucrose solution (1.6 g/L) was freshly prepared before each application to avoid contamination risks (Brahim and Lombarkia., 2020). Sucrose is approved in France for use against *Cydia pomonella* (codling moth) and *Ostrinia nubilalis* (European corn borer) under biocontrol regulations (ANSES/EC 1107/2009). Studies by Brahim and Lombarkia (2020) demonstrate that sucrose enhances plant resistance by disrupting oviposition behavior, likely through alterations to leaf surface metabolites that deter egg-laying. Notably, higher doses do not improve efficacy; optimal results are achieved at low concentrations (e.g., 0.01% for *C. pomonella*), as excessive sucrose may lose its deterrent effect or even attract pests

Foliar sprays were applied from 6 May to 3 July 2023 at 15-day intervals (five total applications). Treatments were conducted between 8:00 and 10:00 am to coincide with peak stomatal opening and minimal evaporation, optimizing absorption (Sharma *et al.*, 2020). Applications were made using calibrated sprayers maintaining a 15-20 cm distance from plants, with volumes adjusted according to growth stage (50 ml-100 ml). Treated pots were physically isolated to prevent cross-contamination. This protocol specifically targeted vulnerable *Tuta absoluta* developmental stages while minimizing potential phytotoxic effects.

A completely randomised one-factor design was employed to evaluate the insecticidal effects of aqueous rosemary extracts at four concentrations (0.5%, 0.75%, 1%, and 1.5%) and a sucrose solution (100 ppm) against *Tuta absoluta*, with an untreated control. The experiment comprised six treatments, each of which was replicated three times across 18 experimental units that were arranged in a standardised six-column × three-row grid. The sucrose treatment was used as a biopesticide reference rather than as a control, while the untreated group provided baseline mortality data.

The study evaluated treatment effects on *Tuta absoluta* infestation in tomato plants through systematic weekly sampling. Three quantitative parameters were measured: (1) infestation levels, determined by monitoring larval presence throughout the experimental period; (2) average mines per leaf, calculated as the ratio of total mines to total leaves examined; and (3) treatment efficacy, assessed using Abbott's formula. All measurements were conducted through careful visual inspection of experimental plants to ensure accurate data collection.

$$\text{Efficiency} = 100 \times ((T0 - Tt)/T0)$$

T0: % total of leaflets affected in the control treatment;
Tt: % total of leaflets affected in the treated treatment.

All data analyses were performed using Statistica 8. For each measured parameter, including overall infestation rates, leaf-level infestation patterns, and larval population dynamics, mean values were calculated with corresponding standard errors. Prior to conducting comparative analyses, the assumptions of parametric testing were verified through the implementation of Shapiro-Wilk normality tests ($\alpha = 0.05$) on all datasets. In the case of significant deviations from normality. Non-parametric tests Kruskal-Wallis and Dunn's test was used to compare between groups. This conservative approach ensured robust detection of statistically meaningful differences while maintaining an overall type I error rate of 5%.

RESULTS AND DISCUSSION

Effect of modalities concentration on *Tuta absoluta* infestation rate in tomato leaves

The findings indicated that the most effective method of reducing the infestation rate was attributed to rosemary extract at a concentration of 1.5%, with a result of 37.25 ± 4.89, in comparison to the rate obtained by the control group (50.10 ± 5.76). The infestation rates resulting from the treatment with 0.75% sucrose and sucrose are 44.10 ± 5.92 and 45.32 ± 6.63, respectively. A statistical analysis of *Tuta absoluta* infestation data was conducted, which yielded several significant findings. The Kruskal-Wallis test revealed significant differences among the groups ($H = 15.72$, $p = 0.01$). This finding indicates that rosemary extract exerts a globally significant effect on potato infestation. The Dunn's Post-Hoc Test demonstrated that the doses (0.5%, 1%, and 1.5%) exhibited significantly lower values in comparison to the Control group. Conversely, the doses 0.75% and sucrose 100 ppm versus the Control group did not demonstrate a significant outcome (Fig. 1).

These results align with the growing resistance of *Tuta absoluta* to conventional pesticides, necessitating sustainable alternatives. The dose-dependent efficacy of rosemary extract (RE) supports findings by Benelli *et al.* (2019) on neurotoxic and antifeedant mechanisms of plant-derived compounds. The lack of efficacy from sucrose reinforces that RE's pesticidal properties are mediated by specific bioactive compounds (Pavela and Benelli, 2016).

Efficacy of treatments of rosemary extracts and sucrose

The findings indicated that the most efficacious agent in significantly reducing the infestation rate was rosemary extract at a concentration of 1.5% (37.20 ± 4.14), in comparison to the control group (0.00 ± 0.00). This outcome was in contrast to the lower concentrations of 0.75% and sucrose 100 ppm, which exhibited (16.95 ± 6.43) and (14.44 ± 3.97), respectively, in relation to the control group. A statistical analysis of *Tuta absoluta* infestation data was conducted, which yielded several significant findings. The Kruskal-Wallis test revealed significant differences among the groups ($X2 = 27.43$, $p \leq 0.001$). This finding indicates that rosemary extract has a globally significant efficacy on potato infestation. The Dunn's post-hoc test demonstrated that the doses (0.5%, 1%, and 1.5%) exhibited significantly elevated efficacy values in comparison to the control group, with p-values of ≤ 0.001, ≤ 0.05, and ≤ 0.001, respectively. Conversely, the efficacy values of the dose (0.75%) and sucrose 100 ppm, in comparison to the control group did not reach statistical significance (Fig. 2).

The 25.7% reduction in infestation with 1.5% RE (vs. control) supports the threshold effect hypothesis for botanical insecticides (Isman, 2020). Divergent results at 0.75% may reflect temporal larval mortality or spray coverage variability (Campos *et al.,* 2021). These findings highlight RE's potential for integrated pest management, particularly in organic systems (Biondi *et al.,* 2018).

Dose effects of rosemary extracts and sucrose on tomato leaf mines

The data presented in Figure 3 shows the variability in the average number of leaf mines per leaf according to the treatment modalities. This study revealed a significant decrease in the average number of leaf mines in the group sprayed with 1.5% rosemary extract, with the lowest value recorded as (1.0 ± 0.1), compared to the control group, which had an average of 1.72 ± 0.22. Conversely, the 100 ppm dose showed a non-significant increase in value to (1.39 ± 0.14) compared to the control. In fact, Kruskal-Wallis Test (H-statistic = 28.6, $p < 0.001$) revealed significant differences in average number of leaf mines among potatoes groups. Dunn's Post-Hoc Test indicated that the doses (0.5%, 0.75%, 1%, and 1.5%) had significantly reduced the average number of mines per leaf, contrary to sucrose that had no significant effect on the number of mines per leaf.

The reduction in leaf mines aligns with documented antifeedant mechanisms of botanical extracts (Benelli *et al.*, 2019). Sucrose's lack of effect suggests RE's activity is mediated by specific bioactive compounds (Pavela and Benelli, 2016). These results could guide concentration optimization under field conditions.

Abbott treatment efficacy

Analysis of the results showed the occurrence of wide differences in efficacy between the compared treatments against *Tuta absoluta*. The aqueous extracts of rosemary registered great performances as far as efficacy was concerned. Indeed, Abbott's formula efficacy application resulted in highlighting following evidences: the most efficacy concentration was 1.5% with 26.4% (Table 1).

These findings reinforce botanical extracts as viable alternatives to synthetic pesticides (Isman, 2020). However, study limitations (controlled conditions, unassessed long-term effects) warrant further field validation and mechanistic research (Kumar *et al*., 2023).

CONCLUSION

The present study demonstrates the efficacy of rosemary extract as a natural pesticide against *Tuta absoluta* in tomato crops. It was demonstrated that higher concentrations were more efficacious in terms of pest control, thus confirming a clear dose-response relationship. The findings provide a robust rationale for the incorporation of rosemary extract into sustainable farming practices, serving as a viable alternative to synthetic pesticides. It is recommended that future research endeavours concentrate on the execution of field testing and the identification of active compounds. This work contributes to the development of eco-friendly solutions for the management of this destructive pest.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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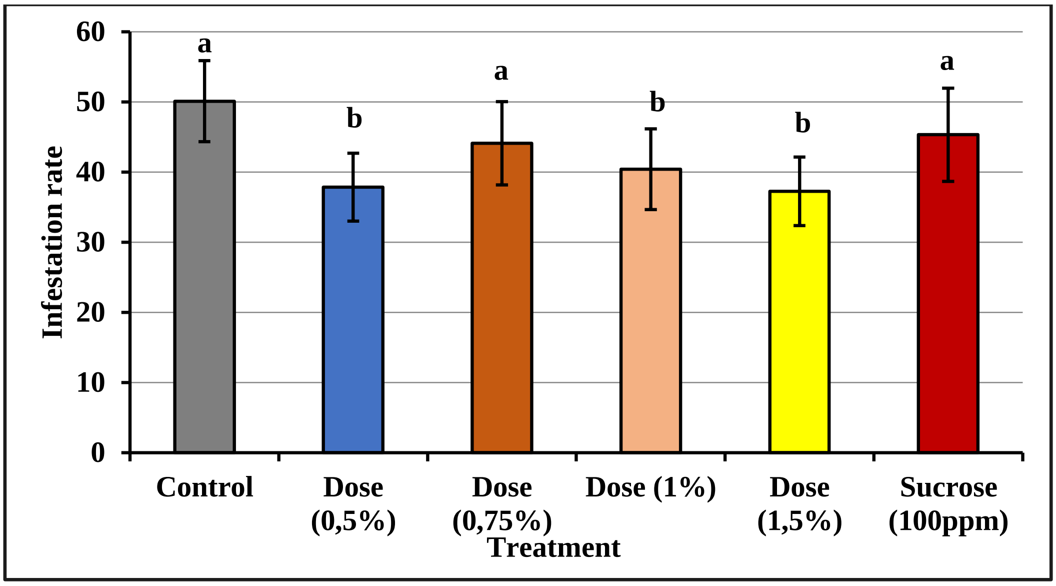

Fig.1. Variation of *Tuta absoluta* infestation rate on tomato leaves, depending different concentrations of rosemary extracts and sucrose. *Values are means ± SD, (n= 10); Dunn's Post-Hoc comparisons test was used to compare different modalities of treatments with control; letters (a, b) : design homogeneous groups.*

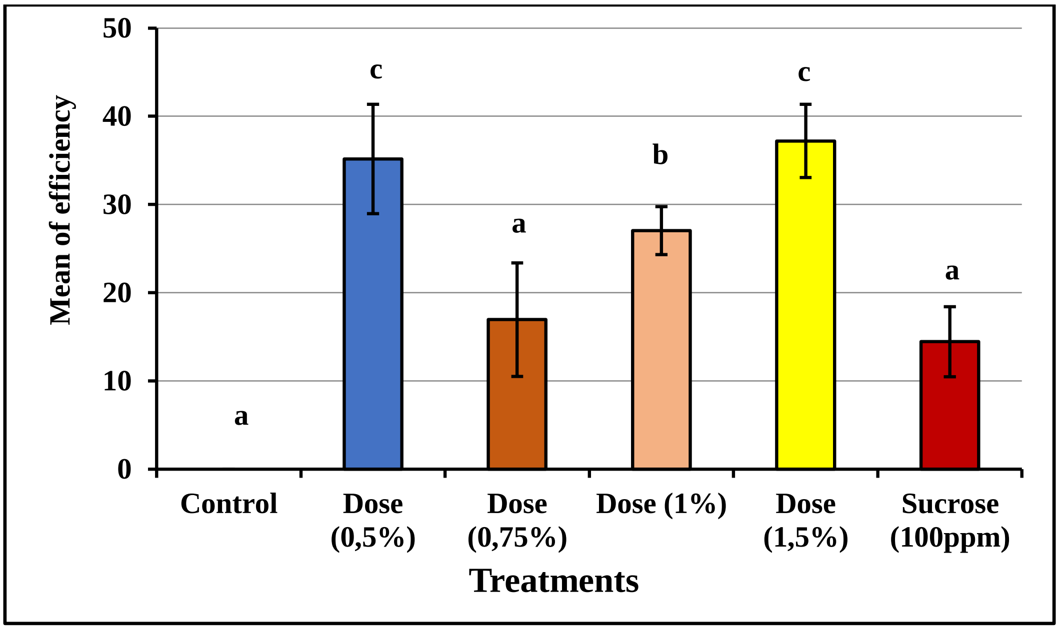

Fig.2. Variation of different treatments efficacy of rosemary extracts and sucrose against *Tuta absoluta* infestation rate in tomato leaves. *Values are means ± SD, (n= 10); Dunn's Post-Hoc used to compare different modalities of treatments efficacy with control; letters (a,b,c) design homogeneous groups.*

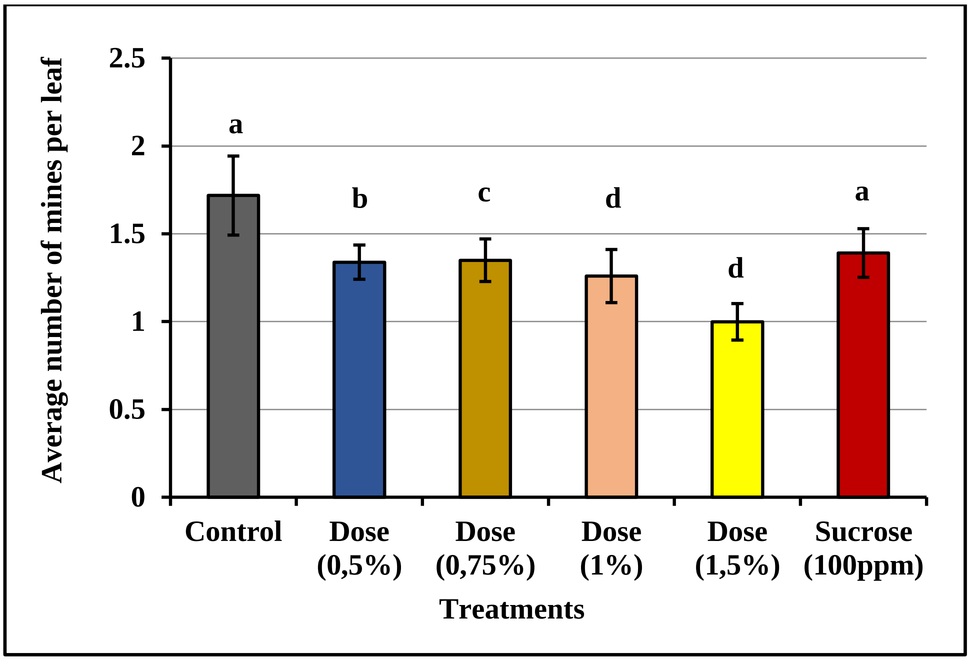

Fig.3. Variation of different treatments of rosemary extracts and sucrose on the average number of *Tuta absoluta* leafs. Values are means ± SD, (n= 10); *Dunn's Post-Hoc* used to compare different modalities of treatments on average number of leaf mines with control; letters (a, b, c, d) design homogeneous groups.

Table 1. Calculated treatment efficacy rates using Abbott's formula:

Treatment		**Average Efficacy**
Rosemary aqueous extract	**0.5% Dose**	24.5%
	0.75% Dose	12.5%
	1% Dose	20.9%
	1.5% Dose	26.4%
Sucrose (100ppm)		11.3%