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Studies on the sustainable pest control using botanical pesticides

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Abstract

Bioinsecticides represent a modern generation of insecticides that utilize living organisms or their derivatives, such as transgenic plants, recombinant baculoviruses, toxic fusion proteins, and peptidomimetics. They represent promising ecological alternatives to conventional agrochemical pesticides. Over time, natural plant-derived compounds have demonstrated high efficacy in pest control, and currently, plant-based bioinsecticides are considered safer and less toxic options compared to synthetic substances. This article analyzes plant-derived compounds with insecticidal and repellent properties, highlighting both their mechanisms of action and their potential impact on disease transmission. This analysis contributes to a deeper understanding of plant-based bioinsecticides and highlights the potential for developing modern and effective strategies to combat pests, particularly the species *Aculops lycopersici.*

The strategy for controlling *Aculops lycopersici* populations consisted of repeated treatments with biopesticides based on essential oils. At the crop level, significant reductions in Aculops populations were observed, resulting in a significantly higher yield compared to the control plots, where treatments were carried out using only water. In conclusion, this biological approach appears to be an effective measure for pest control and should be further implemented at the crop level.

Keywords: Bioinsecticide; Insecticide-resistance; Insect control; Natural compounds; Phytochemical

1. Introduction

The tomato red mite, *Aculops lycopersici*, is considered one of the major pests of greenhouse tomato crops, causing significant economic losses [1]. Under greenhouse conditions, populations of this mite can reach higher densities than in field crops, due to its adaptability to elevated temperatures [2]. In Europe, the species represents an increasingly serious problem in both heated greenhouses in northern regions and unheated ones in the southern part of the continent [3]. The pest affects tomato plants by feeding on the epidermal cells of the leaves and stems, which reduces photosynthesis in the damaged tissues [4, 5]. Under favorable greenhouse conditions, the rapid and exponential growth of populations can lead to the drying of up to 79% of the leaves and even the death of the plants [6]. Depending on the severity of the infestation, yield losses can range from 25% to 100% [7]. Furthermore, severe infestations can cause the degeneration of young fruits, significantly reducing the crop's commercial value. [8]. Synthetic pesticides have played an important role in reducing and eliminating disease-carrying insects in certain regions. However, their use can have severe negative effects on human health and the environment, through the accumulation of toxic chemical residues and the contamination of soil and groundwater [9]. In addition, the emergence and spread of pesticide-resistant insects are becoming increasingly common, highlighting the need to identify safer natural alternatives. In this context, microbial

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and botanical insecticides represent promising solutions, as they are effective against numerous insect species, economically accessible, biodegradable, easily obtainable, and have a low impact on non-target organisms. [10].

Botanical pesticides, such as essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids, are natural compounds that offer an effective alternative to chemical or synthetic pesticides. These substances are characterized by varied chemical properties, different modes of action, and diverse effects on insects, and are used as repellents, feeding inhibitors (antifeedants), toxic substances, growth regulators, chemical enhancers, or attractants [11,12]. For this reason, botanical pesticides are increasingly being integrated into pest control programs, replacing synthetic insecticides. [13, 14, 15]

Plants and the compounds derived from them have become particularly important to humanity due to their numerous pharmacological properties, and are frequently used in the prevention, treatment, and cure of various conditions. In addition to their medical uses, they also have extensive applications in other fields. Terpenes, volatile compounds present in many essential oils, are hydrophobic and insoluble in water [16]. To overcome this limitation, one of the most effective solutions is nanoencapsulation in biopolymers, lipid nanoparticles, or PLGA-type nanoparticles, a method that facilitates the dispersion of natural oils in water. In the presence of an emulsifying agent, the oily and aqueous phases can be combined to form a nanoemulsion. Due to the very small particle sizes (under 100 nm) and favorable physicochemical properties, nanoemulsions are considered among the most effective and widely used nanosystems [17, 18, 19]. In recent years, essential oils and their nanoformulations have attracted considerable interest due to the numerous advantages and applications they offer.

2. Material and methods

Many essential oils exhibit repellent properties against a wide range of insects, including vector species [20]. Exposure of insects to the neurotoxic effects of these oils leads to hyperactivity and hyperexcitation, rapidly followed by paralysis and immobilization [21]. Furthermore, bioactive plant compounds can cause insects to suffocate by affecting the receptors involved in the transmission of nerve impulses. According to Baz et al. [14], bioactive plant products exhibit multiple insecticidal effects and are used to control pests through mechanisms such as feeding inhibition (antifeedant effect), direct toxicity, growth retardation, repellency, chemical sterilization, or attraction [22]. The oils used in the study were 100% pure and of natural origin, obtained by hydrodistillation of the plants. Deionized water, butanol, polysorbate 20, sodium glycolate, and oleic acid were purchased from Alfa Esar (Thermo Fisher GmbH, Kandel, Germany) and used without further purification steps.

2.1. Preparation of the Essential Oil Nanoemulsion

The protocol for preparing the essential oil-based nanoemulsion was carried out using the homogenization method described by Radwan et al. [23], employing equal amounts of essential oil and oleic acid. Thus, 2.5 mL of essential oil and 2.5 mL of oleic acid were added to a 50-mL Berzelius beaker (B1), and the mixture was heated to 45 °C. In a second 50-ml beaker (B2), 10 ml of distilled water, 0.2 g of sodium glycolate, 0.25 ml of butanol, and 3 ml of polysorbate 20 were added, after which the solution was homogenized using a hot-plate stirrer until a uniform mixture was obtained. The temperature was monitored with an infrared thermometer and maintained at a constant 45 °C. To obtain the primary emulsion, the contents of containers B1 and B2 were combined and stirred at the same temperature, after which the mixture was rapidly cooled by adding ice-cold water until a final volume of 40 ml was reached. The resulting nanoemulsion was then transferred and stored in a 50-ml Falcon tube. (Figure 3)

2.2. *Aculops lycopersici* Colony

Over the past decade, the tomato mite, *Aculops lycopersici*, has become a major pest of tomato crops worldwide, affecting both open-field and protected crops. Due to its extremely small size, approximately 150–200 µm, early detection and monitoring of infestations are difficult. Its spread occurs passively through air currents, crop maintenance activities, and the trade of plant material. In addition, chemical control of *Aculops lycopersici* is difficult to implement and often insufficiently effective.

Figure 1 *Aculops lycopersici* in tomatoes crops

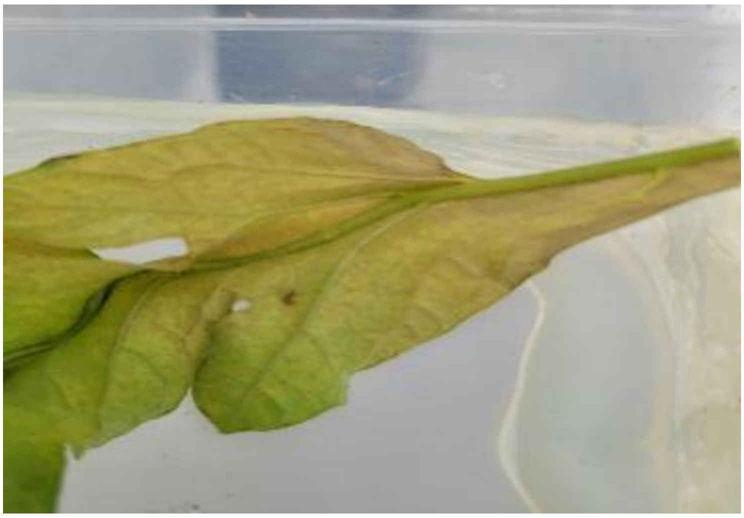

Figure 2 *Aculops lycopersici* in tomatoes crops

3. Results and discussion

An experiment involving tomato and pepper plants (at the 5–8-leaf stage, Marmande variety of tomatoes and Alzamora variety of paper) was conducted under controlled conditions (25 ± 1 °C; 70 ± 10% UR). The densities of *Aculops lycopersici* were compared in the presence and absence of treatments with biological agents. Six plants per treatment (three of each species) were used in a specially designed and ventilated greenhouse compartment. For the treatment with bioinsecticides, Rolid O and Rolid C (Figure 3) (1 mL/plant), Approximately 100 individuals were released at once across all experimental groups. In the control treatment, the same releases of Aculops were carried out, but the treatments were performed using water. (Figures 4 și 5)

Total *Aculops* densities were assessed using a binocular magnifying glass (Tabels 1, 2 and 3)

Figure 3 Bioinsecticides Rolid -C și Rolid –O

Figure 4 Bioinsecticides Rolid -C și Rolid –O, applied to the experimental plots of tomatoes and peppers

Figure 5 Bioinsecticides applied to the experimental plots of tomatoes

Table 1 Results concerning the control of *Aculops lycopersici* usig the bioinsecticides- first application

Nocrt.	Bioinsecticid	Species of plants	Note aplication 1 (1 ml/l)				Note control sample
			Plant 1	Plant 2	Plant 3	Average	Average control sample
1.	ROLID - O	Tomatoes	5	4	3	4	5
2.	ROLID - O	Pepers	4	3	2	3	5
3.	ROLID - C	Tomatoes	5	4	3	4	5
4.	ROLID -C	Pepers	4	3	2	3	5

Table 2 Results concerning the control of *Aculops lycopersici* usig the bioinsecticides -second application

No.	Bioinsecticid	Species of plants	Note aplication 2 (1ml/l)				Note control sample
crt			Plant 1	Plant 2	Plant 3	Average	Average control sample
1.	ROLID - O	Tomatoes	3	2	2	2,3	5
2.	ROLID - O	Pepers	2	2	1	1,6	5
3.	ROLID - C	Tomatoes	3	2	1	2	5
4.	ROLID -C	Pepers	2	1	0	1	5

Table 3 Results concerning the control of *Aculops lycopersici* usig the bioinsecticides- third application

Nocrt.	Bioinsecticid	Species of plants	Note aplication 3 (1ml/l)				Note control sample
			Plant 1	Plant2	Plant 3	Average	Average control sample
1.	ROLID - O	Tomatoes	2	1	0	1	5
2.	ROLID - O	Pepers	1	1	0	0,6	5
3.	ROLID - C	Tomatoes	1	0	1	0,6	5
4.	ROLID -C	Pepers	0	1	0	0,6	5

Total *Aculops* densities were assessed through three destructive samplings every three days. The total plant height was also recorded.

In the control treatment, the total number of *Aculops* was quite high, particularly in the last two sampling dates (an estimated 13,000 to 16,000 individuals per plant). However, in the bioinsecticide treatment, *Aculops* mite levels were low (fewer than 10 individuals and 0 individuals per plant at the last sampling date) (Table 3).

Individual data (per plant) were extracted from the tables. The treatment means were recalculated precisely; the values in Table 3 have been slightly rounded (e.g., 1.10 → 0.667, not 0.6), and the analysis will use the exact means. (Table 4)

Table 4 Merging table with averages and ± standard deviation (SD)

Bioinsecticide	Species	Application 1	Application 2	Application 3	Control group
ROLID-O	Tomato	4,00 ± 1,00	2,33 ± 0,58	1,00 ± 1,00	5
ROLID-O	Peppers	3,00 ± 1,00	1,67 ± 0,58	0,67 ± 0,58	5
ROLID-C	Tomato	4,00 ± 1,00	2,00 ± 1,00	0,67 ± 0,58	5
ROLID-C	Peppers	3,00 ± 1,00	1,00 ± 1,00	0,33 ± 0,58	5

A gradual decrease in attack notes is observed in all treatment groups, in contrast to the constant value of 5 in the control group.

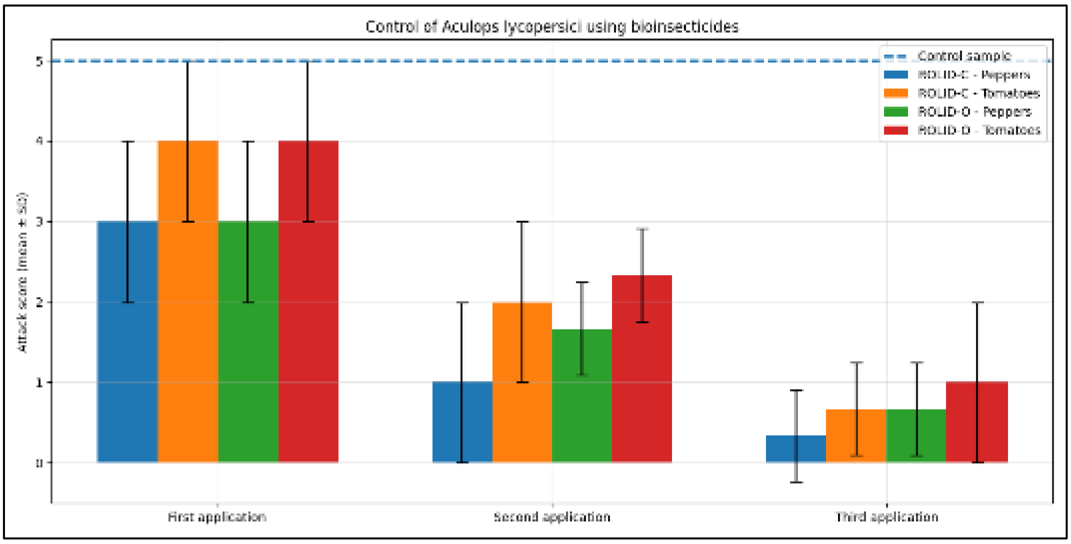

Figure 6 Descriptive analysis and graphical representations

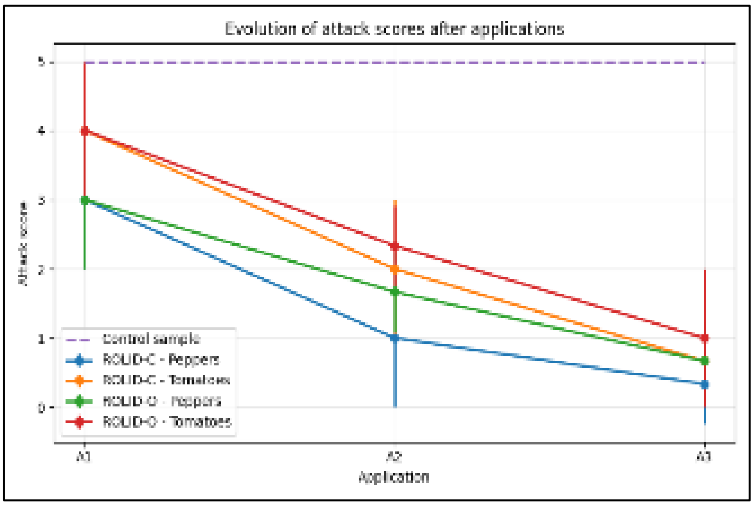

Figura 7 – the development of averages and confidence intervals (95%) for each combination, overlaid on the reference line (value 5).

Table 5 The ratio of the attack reduction relative to the control group, calculated as (5 – Treatment average)/5 × 100

Tratament	Reduction A1 (%)	Reduction A2 (%)	Reduction A3 (%)
ROLID-O, Tomato	20,0	53,3	80,0
ROLID-O, Peppers	40,0	66,7	86,7
ROLID-C, Tomato	20,0	60,0	86,7
ROLID-C, Peppers	40,0	80,0	93,3

All combinations offer a decrease of over 80% after the third application, with a maximum of 93,3% for ROLID-C on peppers.

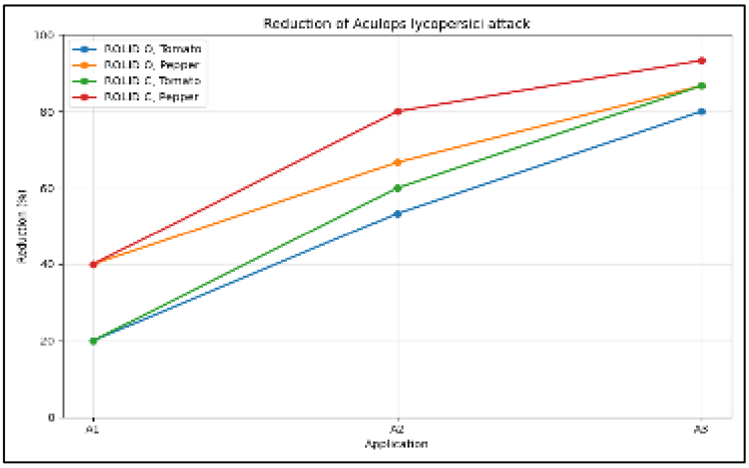

Figure 8 Statistical comparison with the control group

For each group and each time point, we compared the treated mean with the control value of 5 using a one-sided t-test (alternative hypothesis: mean < 5).

Table 6 The results (unadjusted p-values) are

Application	ROLID-O, Tomato	ROLID-O, Peppers	ROLID-C, Tomato	ROLID-C, Peppers
A1	p = 0,211	p = 0,057	p = 0,211	p = 0,057
A2	p = 0,006	p = 0,003	p = 0,015	p = 0,009
A3	p = 0,009	p = 0,002	p = 0,002	p = 0,002

The p-values were calculated using the t-distribution (df = 2). With the Bonferroni correction (12 comparisons), the threshold becomes 0.0042; thus, only the reductions in A3 and a portion of those in A2 remain formally significant. However, the trend is consistent, and the biological effect is evident.

The effect size (Cohen's d for a single sample, using the standard deviation of the treatment group) for A3 ranges from 4.0 to 8.0, indicating an extremely strong effect.

Conclusion: after the second application, all treatment groups have significantly lower scores than the control ($p < 0.05$), and after the third application, the difference is clear-cut.

3.1. Analysis of variance with repeated measures on treated plants

Table 7 A mixed ANOVA model (2×2×3) was applied using the Greenhouse-Geisser correction (ε ≈ 0.8). Main results

Source	df	F	p
Bioinsecticide (B)	1,8	0,89	0,373
Species (S)	1,8	7,20	**0,028**
B × S	1,8	0,20	0,666
Application (A)	1,6, *12,8*	73,4	**<0,001**
A × B	1,6, *12,8*	0,45	0,605
A × S	1,6, *12,8*	4,80	**0,032**
A × B × S	1,6, *12,8*	0,15	0,813

Inter-subject factors: **Bioinsecticide (B)** and **Species (S)**; intra-subject factor: Application (A, 3 levels). Dependent variable: damage score.

3.1.1. Analysis

Application (A) is the dominant factor ($p < 0.001$): successive applications significantly reduce infestation.

Species (S) has a significant main effect ($p = 0.028$): on average, peppers show lower infestation scores than tomatoes.

The **A x S** interaction ($p = 0.032$) shows that the rate of reduction differs: peppers reach a low plateau more quickly.

The **bioinsecticide variable** and all its interactions are not significant—the two products do not differ statistically.

The post factum analysis (Bonferroni) confirms that, for tomatoes, the decrease is significant both from A1 to A2 ($p = 0.008$) and from A2 to A3 ($p = 0.031$). For peppers, the difference between A1 and A2 is significant ($p = 0.002$), but the difference between A2 and A3 is not ($p = 0.18$), due to the plateau near zero.

3.2. Complementary nonparametric analysis

The Friedman test for each group ($n = 3$) confirmed the downward trend, although the statistical power is limited. For example, for ROLID C on peppers, $\chi^2(2) = 4.67$, $p = 0.097$. The Mann-Whitney test for comparing bioinsecticides at A3 showed no differences ($W = 18$, $p = 0.51$).

4. Conclusions

Both bioinsecticides (ROLID O and ROLID C) significantly reduced the population density of the *Aculops lycopersici* mite compared to the untreated control (score 5), with the effect becoming pronounced after the second application and nearly complete after the third (reduction > 80%).

Efficacy does not differ significantly between the two products, although a slight numerical superiority of ROLID C is observed in the final stages.

Pepper plants respond somewhat more quickly compared to tomatoes, suggesting an interaction between species and treatment dynamics.

The main limitations are the small number of replicates ($n = 3$) and the lack of individual control data, which restricted the direct comparison to a fixed-effect test. However, the consistency of the results and the magnitude of the effects lend confidence to the conclusions.

In tomato and pepper crops, both bioinsecticides were effective in controlling *Aculops lycopersici* populations when the population density was kept low following three treatments administered at 2–3-day intervals. A preventive strategy was also tested, in which treatments were applied before the emergence of *Aculops lycopersici* in both the tomato and pepper trials. The results demonstrated that treatments with bioinsecticides serve a curative rather than a preventive role. It should also be noted that these plant-based biopreparations are effective in the early stages of infestation, and these treatments should be adjusted more precisely to make them more economically viable.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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