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In vitro study of the effectiveness of two essential oils: *Rosmarinus officinalis* L. and *Thymus vulgaris* L. against the potato tuber moth, *Phthorimaea operculella*, Zeller (1873) (Lepidoptera: Gelechiidae)

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In vitro study of the efficacy of two essential oils: Rosmarinus officinalis L .and *Thymus vulgaris* L. on the potato moth, *Phthorimaea operculella*, Zeller (1873) (Lepidoptera: Gelechiidae)

The potato moth is recognized as the most dreadful pest of these strategic crops in stock. This study is part of the search for alternatives to chemical control by using two biopesticides, namely the essential oil of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) obtained by the method of hydrodistillation and by steam distillation (Rdt 0.67%; Rdt 1.53% respectively). An initial work was carried on the study of the life cycle of *Phthorimeae operculella* under laboratory conditions (temperature 30±2°c; humidity 60% and a photoperiod of 12 h). The results show a relationship between the duration of the stages and the temperature at which they are reared (egg, 3-5 days; (L1) 3-4 days; (L2) 2-3 days; (L3) 3-4 days; (L4) 3-5 days; pupa, 5-7 days; adult, 11-15 days). The in vitro application of both biopesticides showed a real insecticidal action towards the tested individuals with an almost identical efficiency (3.3±0.30 and 3.68±0.13).

Keywords: *Phthorimeae operculella*, potato, hydrodistillation, steam distillation, *T.vulgaris*, *R. officinalis*, biopesticides, Rdt, life cycle.

Introduction

The potato, the fourth most important food crop in the world after wheat, rice, and maize, is cultivated in over a hundred countries and serves as a staple food for more than one billion people (Singh & Kaur, 2009; Singh & Kaur, 2016). In 2019, global production was estimated at 388,191,000 tons, with China being the leading producer (FAOSTAT, 2019). In Algeria, production reaches approximately 4,653,322 tons, with an average annual per capita consumption of 111.5 kg, placing the country among the world's top consumers (FAOSTAT, 2018; Bouzagui, 2018; APS, 2018).
Like other crops, potatoes are exposed to many pests, among which *Phthorimaea operculella* is the most damaging (Navarre et *al.*, 2009), responsible for severe losses, particularly during storage (Hanafi, 1999; Sileshi & Teriessa, 2001). In developing countries, farmers, constrained by the high costs of cold storage, often resort to chemical insecticides, which pose environmental concerns.
In this context, the use of biopesticides derived from plant extracts emerges as a sustainable and integrated alternative (Das, 1995). This study aims to evaluate the in vitro effectiveness of two essential oils extracted by hydrodistillation—*Rosmarinus officinalis* and *Thymus vulgaris*—on *P. operculella* populations, following a preliminary study of its biological cycle under controlled conditions.

2. Materials and Methods

The study was conducted at the Plant Biology Laboratory 2 of Abdelhamid Ibn Badis University (Mostaganem), between March and June 2021. Larvae and pupae of *Phthorimaea operculella* were collected from infested potato tubers originating from various regions of Algeria (Oued Souf, Mostaganem, Ain-Nouissy). Laboratory rearing was adapted from the method of Badegana and Ngameni (2001), under controlled conditions (26–30 °C, 60% RH, 12-hour photoperiod), using a nutrient medium based on potato powder, fungicide (mancozeb), antibacterial agent (aureomycin), vitamin C, and sugar water.

Fig. 1: Aromatic plant of *R. officinalis*.

Fig. 2: Aromatic plant of *T. vulgaris.*

The biological cycle of *P. operculella* was monitored in vitro using Petri dishes containing individuals at different larval stages (L1 to L4), pupae, and adults, with daily observations under a stereomicroscope.

Essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* were extracted between March 10 and 25, 2021, using two methods: hydrodistillation (Clevenger apparatus) and steam distillation (FAOSTAT, 2019). The plants were locally harvested and shade-dried. The extracted oil was purified using sodium sulfate (Na_2SO_4) and stored at 4 °C away from light.

Fig. 3: Preparation of *R. officinalis* dilutions.

The essential oil yield (Yield %) was calculated as follows:
Yield (%) = (EO / DM) × 100, where EO is the mass of essential oil obtained and DM is the mass of dry plant material used.
For toxicity tests, dilutions (20, 40, 80, and 100 µl) of each oil were prepared using DMSO, chosen for its relatively low toxicity compared to acetone. Bioassays were performed in Petri dishes on 480 larvae (various instars) and 192 adults. Each dish contained slices of healthy potato and four individuals of the same stage. The oils were applied using spray bottles, and mortality was recorded at 2, 4, 8, 24, 48, and 72 hours post-application.

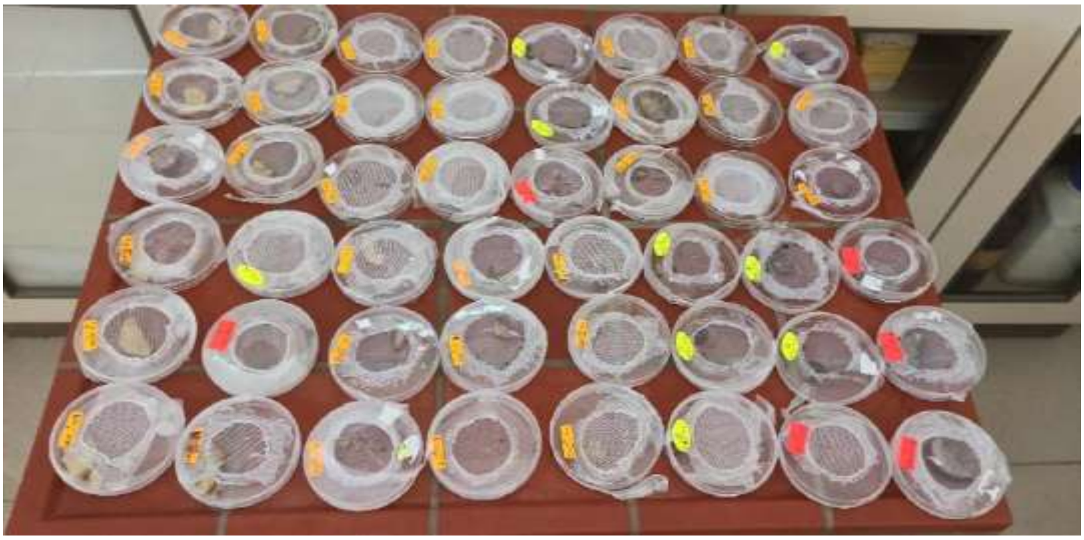

Fig. 4: Experimental setup for the mortality test of essential oils.

Insecticidal efficacy was evaluated by calculating the LD_{50} and LD_{90} based on corrected mortality rates (Abbott's formula) relative to dosage. Statistical analysis was performed using COSTAT software, applying ANOVA followed by the Newman-Keuls test ($\alpha < 0.05$).

3. Results and Discussion

3.1. Essential Oil Yields

The extractions revealed a higher yield for *Thymus vulgaris* (1.53%) compared to *Rosmarinus officinalis* (0.67%), despite differences in the mass of plant material used. The extraction techniques significantly influenced the yields: steam distillation resulted in a higher yield than hydrodistillation, particularly for *T. vulgaris* (1.53% vs. 0.99%). These findings are consistent with those of Imelouane et *al*. (2009) and Oulebsir et *al*. (2015), though they exceed the yields reported by Amarti et *al*. (2010) and Boutekedjiret et *al*. (2003).

The observed yield variations are attributed to multiple factors: extraction method, plant condition (fresh or dried), harvest period (Zantar et *al*., 2015), environmental conditions, cultivation practices, and plant age (Bourkhiss et *al*., 2011; Fadil et *al*., 2015). Sidali et *al*. (2014) showed that dried leaves yield more oil than fresh ones. Geographic origin also plays a significant role, as highlighted by Neffar and Benabdrrahmene (2013).

3.2. Bioecological Cycle of *Phthorimaea operculella*

The rearing of *P. operculella* under controlled conditions (30 ± 2 °C, 60% RH, 12-hour photoperiod) allowed for detailed monitoring of its biological cycle:

Eggs: Incubation period of 3 to 5 days, consistent with Youssef (2019), Broodryk (1971), Lopez (1988), and Salama et *al*. (1973). Development is temperature-sensitive (Rondon & Xue, 2010; Broodryk, 1971).

Larvae (L1 to L4): Complete development over 14 to 21 days. Stage durations are similar to those reported by Verma (1967), Rodriguez (1999), and Broodryk (1971), with minor variations due to temperature differences.

Pupa: Duration of 5 to 7 days, in line with the results of Singh and Charles (1977), Salas and Quiroga (1985), and Joy (2011). In cooler climates (12–13°C), development can take up to 36 days (Palacios et *al*., 1999).

Adult: Average lifespan of 11 days, with noticeable sexual dimorphism (females live longer). Mating occurs 19 to 24 hours after emergence and lasts about 2 to 2.5 hours. These data align with findings by Gamboa and Notz (1990), Haines (1977), and Palacios et *al*. (1999).

These results confirm the potential of *T. vulgaris* and *R. officinalis* essential oils as biological alternatives to chemical insecticides for integrated management of potato tuber moth. Understanding the biological cycle of *P. operculella* allows for optimized intervention timing. Climatic conditions and extraction methods significantly influence the performance of plant extracts.

3.3. Mortality Bioassay

A mortality bioassay was conducted to compare the effects of DMSO and acetone on *Phthorimaea operculella* before testing the essential oils (*Thymus vulgaris* and *Rosmarinus officinalis*). The cumulative mortality rates were calculated at different concentrations (20, 40, 80, and 100 µl).

3.3.1. Cumulative Mortality ("Global")

Cumulative mortality was assessed to summarize the temporal progression of *P. operculella* deaths in response to essential oil concentrations.
Mortality rates were calculated using the formula:
Mortality rate (%) = (Number of dead individuals / Total number of individuals) × 100
The data revealed significant differences in effectiveness between the four concentrations of each oil and between the two oils themselves. Two hours post-treatment, mortality at 20 µl was 13.33% for both oils. For *R. officinalis*, the mortality rates for 40, 80, and 100 µl were 20%, 26.66%, and 38.33%, respectively. In contrast, *T. vulgaris* produced higher rates: 30%, 41.66%, and 51.66%, respectively. No mortality was observed with DMSO, and only 1.66% with acetone.
From 8 hours post-treatment, *T. vulgaris* concentrations of 40, 80, and 100 µl exceeded 50% mortality (56.66%, 70%, and 86%). The highest mortality for *R. officinalis* at this point was 55% (100 µl). The acetone control reached 10%, and DMSO remained at 0%.
After 24 hours, mortality reached 90% for *T. vulgaris* and 70% for *R. officinalis* at 100 µl. Other concentrations of *R. officinalis* (20, 40, 80 µl) had lower mortality (43.33%, 46.33%, 66.66%) compared to *T. vulgaris* (58.33%, 71.66%, 80%). Mortality in controls increased to 1.66% (DMSO) and 18.33% (acetone).
At the end of the experiment (72 h), complete mortality (100%) was observed with *T. vulgaris* at 80 and 100 µl, while *R. officinalis* reached 91.66% and 98.33% for the same concentrations. For 20 and 40 µl, *T. vulgaris* showed 71.26% and 92.36% mortality, while *R. officinalis* showed 60% and 76.66%.

Control mortality peaked at 18.33% (DMSO) and 36.66% (acetone). These findings demonstrate that acetone has a stronger effect on *P. operculella* mortality than DMSO. Therefore, DMSO was selected as the solvent for essential oil dilutions in this study.

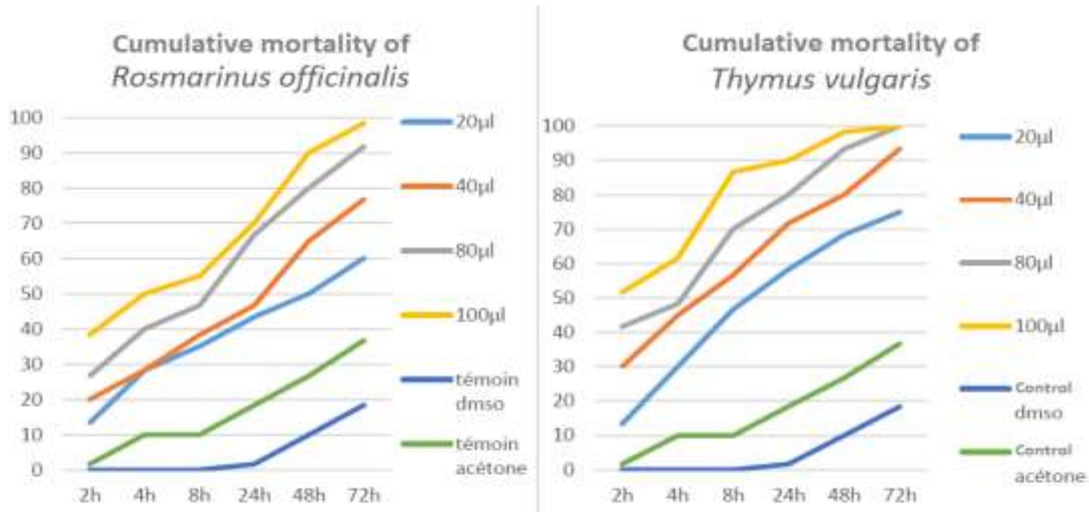

Fig. 5: Overall Cumulative Mortality.

3.3.2 Corrected Overall Mortality

The effectiveness of a product is assessed by the mortality it causes in the targeted pest species. However, the number of individuals found dead in a population treated with a toxic substance does not represent the exact number killed by the substance itself. This is because natural mortality always occurs in any population, and it must be accounted for in addition to mortality caused by the treatment. Therefore, mortality percentages must be corrected using Abbott's formula:

$CM = [(M2 - M1) / (100 - M1)] \times 100$

Where:

M1 = Mortality percentage in the control group

M2 = Mortality percentage in the treated group

CM = Corrected mortality percentage

Fig.6: Overall Corrected Mortality.

3.3.3 Sensitivity of Larval Stages of *P. operculella* to the Two Essential Oils

3.3.3.1 – Larval Stage (L1)

The recorded mortality of first-instar larvae showed a high sensitivity to both rosemary and thyme essential oils. Indeed, all concentrations exhibited larvicidal effects.

The 80 µl and 100 µl concentrations of rosemary oil caused total mortality after 48 hours of treatment, while the 40 µl and 20 µl concentrations resulted in mortality rates reaching up to 83.33% and 66.66%, respectively.

In contrast, the 80 µl concentration of thyme oil also caused total mortality after 48 hours of treatment, and total mortality was observed after 72 hours for the 40 µl and 100 µl concentrations. Moreover, the 20 µl concentration showed satisfactory effectiveness with a mortality rate of 91.66%.

Low mortality was recorded in the control groups, reaching a maximum of 16.66% after 48 hours of treatment.

This larval stage appears to be quite sensitive to lower concentrations (20 µl and 40 µl) of thyme oil compared to rosemary oil, as shown by the mortality rates for the same concentrations.

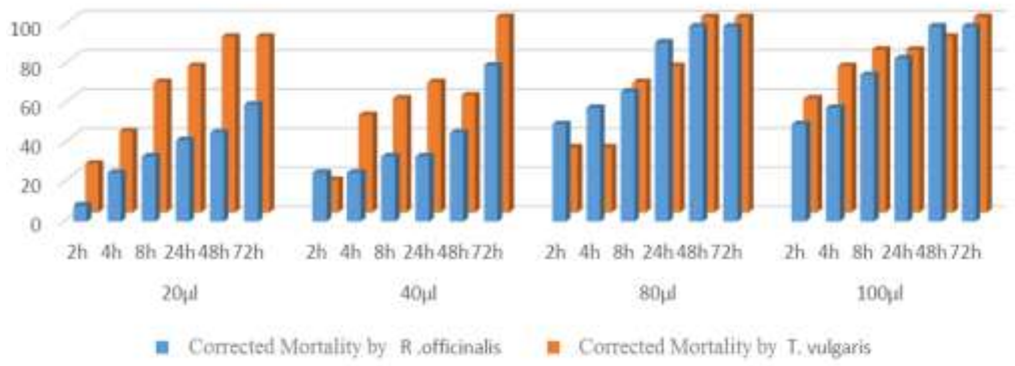

Fig.7: Cumulative Mortality of the L1 Larval Stage.

3.3.3.2 – L2 Larval Stage:

The results obtained for the second larval stage (L2) showed a certain degree of tolerance to the tested concentrations. Indeed, only the 100 µl concentration of rosemary oil achieved total mortality after 72 hours. A moderate mortality rate was observed with the 80 µl concentration (83.33%), and lower rates of 58.33% and 66.66% were recorded for the 20 µl and 40 µl concentrations, respectively.

In contrast to rosemary oil, thyme oil demonstrated a higher efficacy: the 100 µl concentration caused total mortality (100%) as early as 8 hours post-treatment, while the 80 µl and 40 µl concentrations achieved full mortality at 48 and 72 hours, respectively. The 20 µl concentration of thyme resulted in relatively low mortality (66.66%), which was lower compared to the results obtained for the L1 larval stage.

The control group showed a gradual increase in mortality, with a minimum of 8.33% at 48 hours and a maximum of 16.66% by the final observation.

When comparing the mortality rates between the two essential oils at the same concentrations, results at 20 µl were nearly identical. However, at 40 µl, 80 µl, and 100 µl, thyme oil showed significantly higher effectiveness.

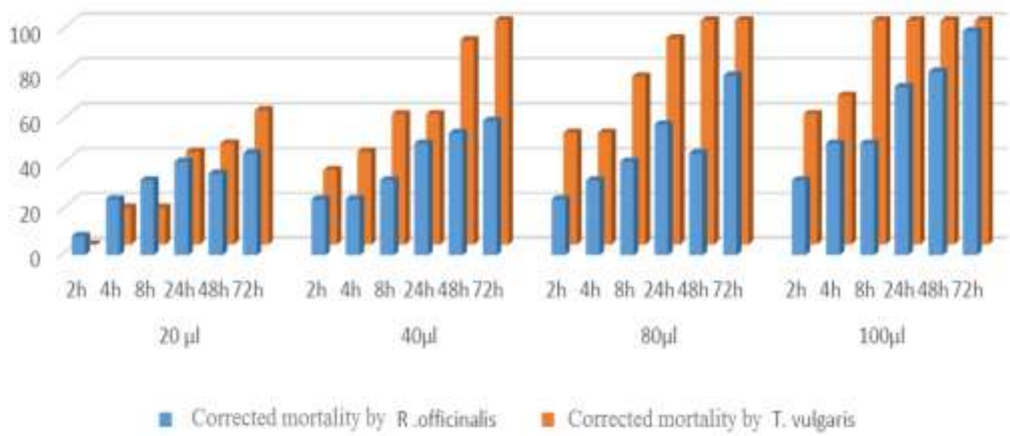

Fig.8: Cumulative Mortality of the L2 Larval Stage.

3.3.3.3 – L3 Larval Stage

For this stage, the results showed satisfactory efficacy for rosemary oil at concentrations of 40 µl and 80 µl, with mortality rates of 75% and 91.66% respectively by the final day. A lower mortality of 50% was observed for the 20 µl concentration. Only the 100 µl concentration of rosemary achieved total mortality (100%) after 48 hours of treatment.

On the other hand, thyme oil again showed high efficacy, with total mortality (100%) recorded 72 hours after treatment for both the 100 µl and 80 µl concentrations. The remaining concentrations led to maximum mortality rates of 91.66% and 75% for the 20 µl and 40 µl doses, respectively.

In the control groups, mortality was initially zero but began to appear on the final day, reaching 16.66% by the end of the experiment.

Unlike the L2 larval stage, the 40 µl, 80 µl, and 100 µl concentrations of both essential oils showed similar levels of effectiveness in L3. However, a notable difference in efficacy was observed at the 20 µl concentration.

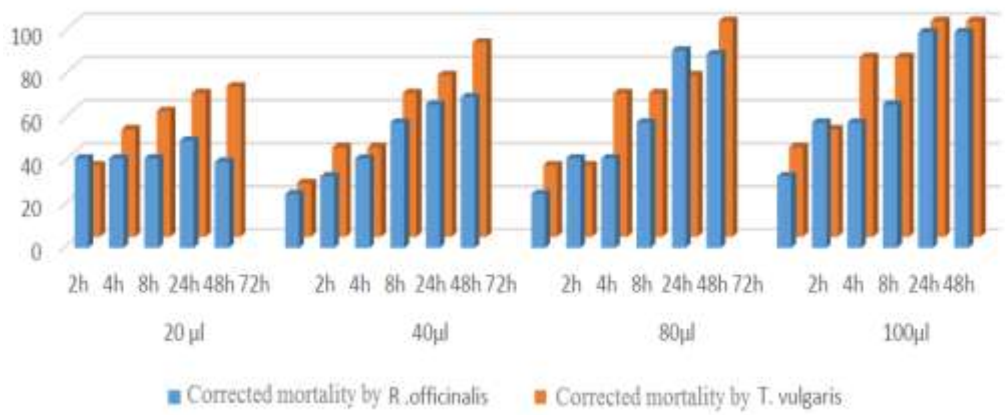

Fig.9: Cumulative Mortality of the L3 Larval Stage.

3.3.3.4 – L4 Larval Stage

The fourth larval stage showed a certain level of resistance to rosemary oil, with only the 100 µl concentration causing total mortality by the end of the third day, similar to the results observed for the previous stage. At the 80 µl concentration, a mortality rate of 91.66% was recorded 72 hours after treatment—comparable to the L3 stage, but with a slightly delayed action. For example, this same concentration caused 66.66% mortality at 48 hours, compared to 91.66% at the same time point in the L3 stage (see Figure 10).

The 20 µl and 40 µl concentrations of rosemary oil demonstrated lower effectiveness, with mortality rates of 58.33% and 66.66%, respectively.

Thyme oil, on the other hand, continued to display strong larvicidal activity, outperforming rosemary oil even against the more resistant L4 stage. Total mortality (100%) was achieved after 72 hours for the 100 µl and 80 µl concentrations. A substantial mortality rate of 91.66% was also recorded for the 40 µl concentration on the final day. Compared to rosemary, thyme showed a quicker onset of action—while rosemary's 40 µl dose caused 75% mortality in the L3 stage after 48 hours, thyme caused 83.33% at the same time point in the L4 stage.

The 20 µl concentration of thyme oil induced a mortality rate of 66.66%, which is similar to the rate observed for rosemary at 40 µl.

For the first time during the bioassay, the control groups recorded mortality within 24 hours after treatment with DMSO, showing a low mortality rate of 8.33%, which remained unchanged until the end of the test.

The larvicidal activity of thyme proved to be more effective than that of rosemary, especially in the final larval stage, which exhibited a degree of resistance to rosemary.

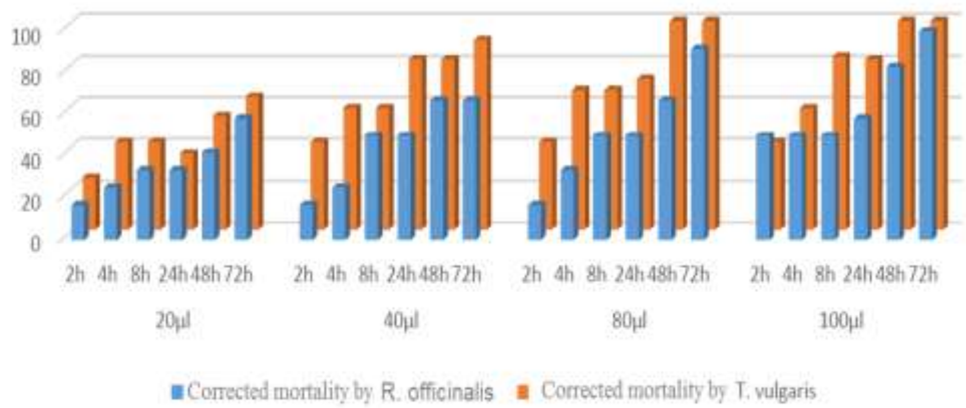

Fig.10: Cumulative Mortality of the L4 Larval Stage.

3.3.3.5 – Adult Stage

The adult stage proved to be the most resistant to the insecticidal effect of rosemary essential oil. The three concentrations (40 µl, 80 µl, and 100 µl) resulted in 91.66% mortality, while the 20 µl concentration caused a mortality rate of 66.66%. Despite the effectiveness of rosemary at earlier larval stages, a noticeable decline in efficacy was observed as the developmental stages progressed from L1 to L4. This is the first instance where none of the four concentrations achieved total mortality.

On the other hand, the results show that thyme was able to overcome the resistance of the adult stage, achieving 100% mortality with the 100 µl dose just 24 hours after treatment, followed by the 80 µl dose which reached total mortality by the end of the trial. For the 20 µl and 40 µl doses, maximum mortality rates of 75% and 83.33%, respectively, were recorded.

In the control groups, no mortality was recorded throughout the bioassay until the end of the test, when a mortality rate of 8.33% appeared.

While none of the four rosemary concentrations (20 µl, 40 µl, 80 µl, 100 µl) achieved total mortality, thyme oil showed complete mortality with two concentrations (80 µl and 100 µl) and notable mortality rates at the lower doses (20 µl and 40 µl). It is therefore clear that the insecticidal activity of *T. vulgaris* is superior to that of *R. officinalis*.

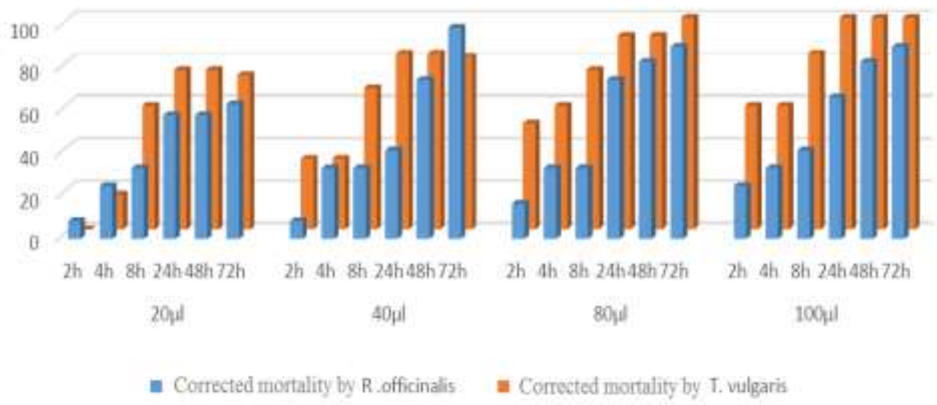

Fig.11: Cumulative Mortality of the Adult Stage.

3.3.4. Determination of LD_{50}, LD_{90} and LD_{100}:

LD_{50}, LD_{90}, and LD_{100} represent the lethal doses of a toxic substance that result in the death of 50%, 90%, and 100% of the exposed individuals, respectively.

To determine these doses, the corrected mortality rates are first converted into percentages, and the corresponding doses are transformed into their decimal logarithms. These transformations allow the establishment of a linear regression equation of the following form:

$Y = aX + b$

Where:

Y = Corrected mortality percentage

X = Logarithm (base 10) of the dose

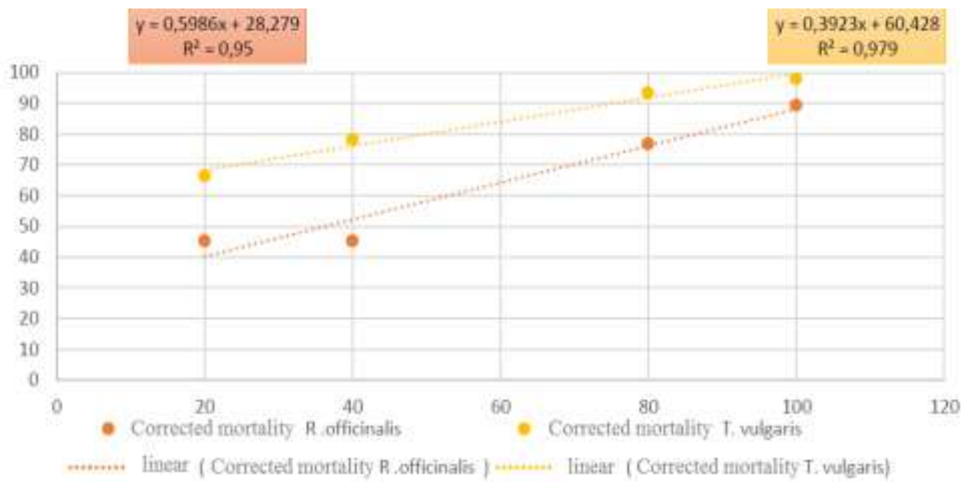

Fig.12: Linear regression curve for determining LD_{50}, LD_{90}, and LD_{100}.

For the essential oil of T. vulgaris, determining the LD50 was impossible due to its high efficacy against the potato tuber moth. Figure 12 shows that the projection of the 50% mortality rate is unrealistic using the linear curve. However, it does allow the projection of LD90 and LD100. In contrast, the linear curve for *R. officinalis* allows the projection of LD50 as well as LD90 and LD100.

We can deduce that the plant extracts studied possess significant toxic properties in vitro. However, the toxicity varies greatly depending on the plant species, the amount used, the treatment duration, and the pest studied.

Indeed, the essential oils of *R. officinalis* and *T. vulgaris* cause variable toxicity at increasing doses.

Furthermore, 90% of the *Phthorimaea operculella* population can be controlled with a dose of 75.38 µL/10 mL of Thymus vulgaris extract, a target that can only be reached with *Rosmarinus officinalis* extract at a dose of 100 µL/10 mL.

3.3.5 - Evaluation of insecticidal activity by variance analysis (ANOVA test)

3.3.5.1 - Mortality abundance according to developmental stages

The two-way statistical analysis of mortality in *P. operculella* individuals based on developmental stage and the two essential oils (*Rosmarinus officinalis* and *Thymus vulgaris*) showed a significant result.

Observations after treatment of different stages with the two oils reveal a regression of mean mortality rates that is slightly sensitive to the progression of developmental stages for thyme and gradual for rosemary.

The effect of *R. officinalis* essential oil shows high sensitivity at the L1 and L2 stages of *P. operculella*, with identical mean mortality values of 3.41±0.28 and 3.41±0.14 respectively, compared to other stages. On the other hand, the effect of T. vulgaris essential oil is very high at the L1 stage, with a mean mortality of 3.91±0.14.

At the L3 and L4 stages, *R. officinalis* shows a lower mean mortality of 3.16±0.80 and 3.08±0.14 respectively, while *T. vulgaris* stands out with superior effects, showing the same mean mortality of 3.66±0.14 at L2 and 3.66±0.38 at L3.

Strong resistance is observed at the adult stage to the effect of *R. officinalis* essential oil, with a mean mortality of 2.91±0.38, as well as to *T. vulgaris* oil with mean mortalities of 3.58±0.14 and 3.58±0.38 for L4 and adult stages respectively.

A mean mortality of 2±0.55 was recorded for stages L1, L2, L3, and a mean of 1±0.55 for stages L4 and adult.

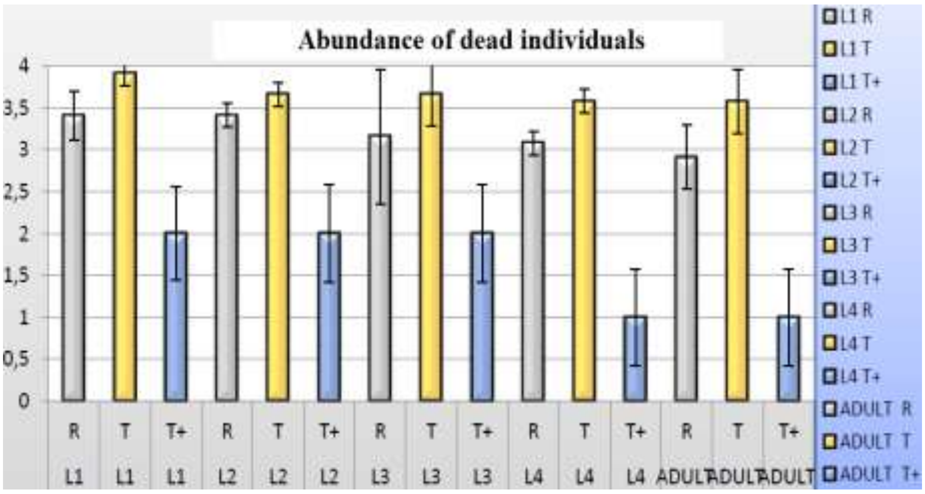

Fig.13: Average mortality according to developmental stages.

3.3.5.2 - Mortality abundance according to essential oils

The analysis of variance (Bekkara et *al*., 2007) conducted on the mortality of *Phthorimaea operculella* individuals following the application of the two essential oils—*Rosmarinus officinalis* and *Thymus vulgaris*—showed a significant result ("$p > 0.05$") with a degree of freedom value of "$df = 30$" and a coefficient of variation of about 15.07%.

The results obtained from evaluating the insecticidal efficacy of the two bio-insecticides showed no significant difference in mortality rates between the essential oils of the tested plants (*Rosmarinus officinalis* and *Thymus vulgaris*), with average mortality values of 3.3 ± 0.30 and 3.68 ± 0.13, respectively, and low mortality in the control groups with an average of 1.6 ± 0.54.

In light of these results, it can be concluded that the essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* demonstrated effectiveness under controlled conditions against *P. operculella* individuals.

Since the insecticidal action of both oils is nearly equivalent, the distinction becomes economic: rosemary has a lower market value than thyme (600 DA/kg for rosemary versus 1500 DA/kg for thyme).

3.4 - Efficacy of the essential oils of *Rosmarinus officinalis* and *Thymus vulgaris*

The most effective botanical oils are those offering a broad spectrum of activity against the different life stages of pests. The control agent must reduce the insect population at all stages and decrease the pest incidence (Lamiri et *al*., 2001).

The use of these plant substances as biopesticides in crop protection against insects has been the subject of many studies. Among them are works on our pest treated by different bio-pesticides applied through various treatments, allowing us to position our essential oils on the insecticidal efficacy scale.

In a study somewhat close to ours, Hannour et *al*. (2017) recorded mortalities of first larval stage ranging from 11.11% to 79.01% after using 1 µl to 8 µl of *R. officinalis* essential oil from Loukkos, and mortalities from 7.41% to 62.69% with *R. officinalis* essential oil from the Middle Atlas. In our case, however, a total mortality was obtained 48 hours after treatment with doses of 100 µl and 80 µl.

This difference may be due to the variety of rosemary used or the treatment method, knowing that the method used by those authors was fumigation.

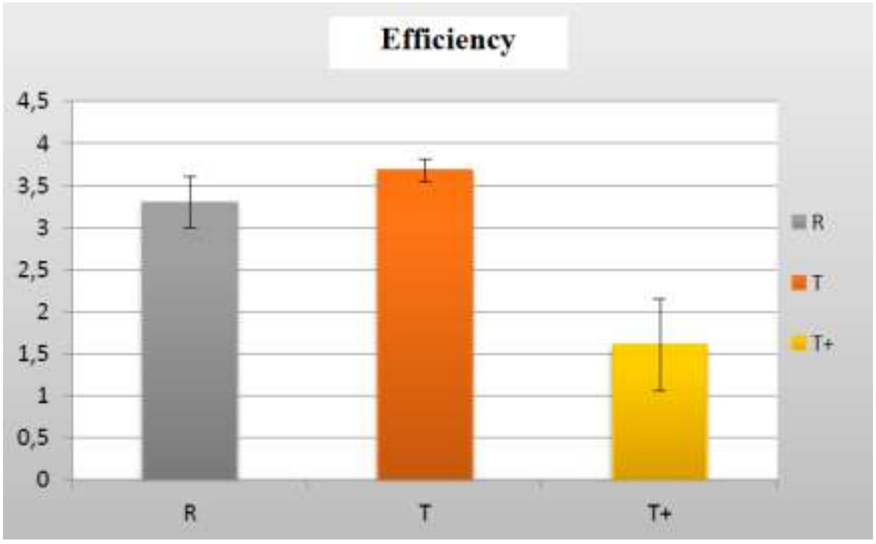

Fig.14: Average mortality according to essential oils.

Exposure of adults by fumigation to doses of essential oils of *Thymus syriacus* and *Myrtus communis* (2 µl/l air) resulted in 100% mortality after 24 hours of exposure, while a concentration of 0.05 µl/l air induced only 4% mortality for thyme and 2% for myrtle essential oil vapors (Tayoub et *al.*, 2016). In our case, doses of 100 µl and 80 µl of *Thymus vulgaris* were necessary to achieve total mortality at the adult stage, 24h and 72h after treatment, respectively.

Khatter (2010) used a 5% concentration of black pepper essential oil (*Piper nigrum*) diluted in acetone, which caused total mortality at all larval stages (L1, L2, L3, L4). This same mortality was established in our work with a much lower concentration of the essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* of about 1%, 0.8%, and even 0.4% at the larval stages L1 and L2, after converting our tested doses (µl/10 ml) to percentages (%).

Rafiee-Dastjerdi et *al.* (2013) reported that for lavender essential oil, the dose 1.23 µl/l air was the concentration required to kill 90% of the *P. operculella* population (DL90). In a very recent study, Jovanović et *al*. (2020) set the DL100 of citronella essential oil (*Cymbopogon citratus*) at approximately 0.883 µl/l air. Morey & Khandagle (2020) revealed that *Cinnamomum verum* oil presented promising toxic action (larvicide LC50 = 110.10 pg/cm², adulticide LC50 = 49.78 pg/cm²).

It has been reported that the insecticidal activity of plant essential oils depends on the type and constituents of the oils, the exposure period, and the method used in the bioassay (Abd El-Aziz, 2011; Moawad et *al*., 2007). These concentrations cannot be compared to ours since their treatments were applied by fumigation (insecticidal effect by inhalation), whereas our study evaluated insecticidal effect by contact.

Using the same method as ours, Zergui and Berkane (2020) found that L1 and L2 stages are the most sensitive to *Eucalyptus globulus* essential oil with average mortalities of 2.58 ± 0.9 and 2.47 ± 1, respectively. In our study, these averages were higher: 3.41 ± 0.28 and 3.41 ± 0.14 for *R. officinalis* essential oil, and 3.91 ± 0.14 for the L1 stage treated with *T. vulgaris* essential oil.

For other stages, Zergui and Berkane (2020) recorded mortality averages of 1.81 ± 0.75 for the third larval stage and 1.57 ± 0.77 for the fourth. The last developmental stage showed resistance to *Eucalyptus globulus* essential oil with an average of 1.22 ± 0.69—similar to what we obtained in our test—although our averages were higher: 2.91 ± 0.38 for rosemary and 3.58 ± 0.38 for thyme. These results are lower than ours despite their DL90 = 70.72 µl/10 ml and DL100 = 100 µl/10 ml being close to ours.

With further studies, the essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* could find a prominent place in control programs against the potato tuber moth (*Phthorimaea operculella*). This approach is likely to be advantageous as it is environmentally safe and socially acceptable.

Conclusion

The potato tuber moth, *Phthorimaea operculella*, is one of the most devastating pests both in the fields and in storage. This insect goes through four developmental stages, but only the larval stage is considered harmful. The damage caused by the larvae is generally more severe under storage conditions than in the field.

P. operculella larvae cause serious damage to stored potatoes by mining the tubers, which facilitates the penetration of fungal and bacterial pathogens leading to rot. Infested tubers thus become completely unsellable.

Control of this pest mainly relies on synthetic insecticides, which despite their effectiveness, pose significant risks due to their high intrinsic toxicity and persistence, resulting in ongoing environmental pollution and health hazards for consumers. Additionally, controlling this moth is difficult because of its high reproductive capacity and adaptability to various climatic conditions.

The life cycle, behavior, and population dynamics of *Phthorimaea operculella* were studied in vitro under laboratory conditions (temperature 30 ± 2 °C; humidity 60%; 12-hour photoperiod). A relationship between the duration of the developmental stages and temperature was observed when insects were reared on potato slices. The average durations were: egg (3-5 days), larva L1 (3-4 days), larva L2 (2-3 days), larva L3 (3-4 days), larva L4 (3-5 days), pupa (5-7 days), and adult (11-15 days).

Given the high cost and harmful effects of chemical insecticides, research is turning towards safer alternatives such as essential oils (EOs), which are volatile natural compounds characterized by strong odors and produced as secondary metabolites in plants.

This modest work focused on valorizing rosemary and thyme essential oils as biopesticides against the potato tuber moth.

The results show that both essential oils exhibited significant insecticidal activity against *P. operculella*, causing 100% mortality of all developmental stages at doses of 100 µl/10 ml and 80 µl/10 ml for thyme, and 100 µl/10 ml for rosemary. Acceptable mortality rates were also observed at lower concentrations.

It was noted that *P. operculella* individuals develop a gradual tolerance to *Rosmarinus officinalis* essential oil with the progression of developmental stages, while tolerance to *Thymus vulgaris* essential oil was less pronounced.

The results indicate that all tested oils are promising biocontrol agents against *P. operculella*. Rosemary oil showed promising toxicity (LD50 = 35.28 µl/10 ml; LD90 = 100 µl/10 ml; LD100 = 119 µl/10 ml), as did thyme oil (LD90 = 75.38 µl/10 ml; LD100 = 100 µl/10 ml). Both plant oils showed nearly identical efficacy and could be integrated into an ecological and environmentally friendly pest management strategy.

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