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Comparative Study of the Insecticidal Activity of Essential Oils of Four Spontaneous and Cultivated Plants on Wheat Weevils (*Sitophilus granarius* L.)

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ABSTRACT

Synthetic insecticides are widely used to preserve foodstuffs; however, their extensive application poses significant risk to both human health and the environment. This underscores the urgent need for natural and less hazardous alternatives. In this context, the present study aimed to evaluate the insecticidal effect of essential oils extracted from four spontaneous and cultivated Moroccan aromatic plants against wheat weevils (*Sitophilus granarius* L.), a major pest of stored grains. The plants *Origanum compactum*, *Origanum elongatum*, *Satureja calamintha*, and *Thymus zygis* were collected from different regions in Morocco. The plants material both wild and cultivated were dried and pulverized. The essential oils from the dried powdered plants were extracted by hydrodistillation using a Clevenger-type apparatus. The adult wheat weevils (*Sitophilus oryzae*) were collected from storage facilities in the Taounate region of northern Morocco. The insects were treated with the essential oils at concentrations ranging from 0.1 to 1.0% for 24 hours. The essential oils demonstrated significant insecticidal effect, with all the oils achieving 100% mortality at 1% concentration within the first 24 hours of exposure. A comparative analysis of oils derived from the wild (spontaneous) and cultivated plants revealed variations in efficacy. Notably, essential oils from wild *Thymus zygis* achieved complete insect mortality in less than 24-hours. These findings underscore the potential of essential oils as effective tools for protecting stored foodstuffs. The findings also highlight the prospects for use of essential oils as bio-insecticides, offering a natural and environmentally friendly alternative for pest control.

Keywords: *Origanum compactum*, *Origanum elongatum*, *Thymus zygis*, *Satureja calamintha*, Essential oil, Insecticidal effect.

Introduction

Due to its geographical location, Morocco is endowed with a rich and diverse flora. There are about 4,200 plant species, of which only 100 are currently exploited. Most of the plants used worldwide are mainly for traditional medicine, as source of molecules for allopathic medicines, as well as for the production of essential oils and flavours for perfumery.[1] Weeds and insect pests are among the main factors affecting agricultural production. According to literature, pest control methods primarily rely on the use of synthetic pesticides.[1] Under normal conditions, synthetic pesticides are very effective in the control of agricultural pests. However, they have many drawbacks, including insect habituation, the selection of resistant strains,[2] intoxication, environmental pollution, and ecological disruption.[3] With this in mind, researchers and policymakers are seeking inexpensive, efficient, safe, and readily available alternative control methods, such as the use of plant extracts (essential oils, organic extracts, etc.). Farmers have long used plants to enhance food flavour or protect harvested produce.[4]

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Additionally, essential oils extracted from plants are widely used by farmers for pest control.[4-5] The insecticidal, larvicidal, ovicidal, sterilizing, anti-feedant, and repellent properties of essential oils have been the subject of several studies.[6-8]

In the search for alternative pest control methods, the plant kingdom offers a wide range of possibilities. Numerous studies are currently underway to isolate or identify secondary metabolites extracted from plants that exhibit insecticidal, repellent, or anti-feedant properties against insects. It is therefore necessary to understand the pharmacology of these plant species in order to combat pests effectively. This knowledge is crucial for developing any pest control strategy, whether preventive or curative, and provides the information needed to make informed decisions regarding pest management system.

Against this backdrop, the present study is aimed at assessing the *in vitro* insecticidal activity of *Origanum compactum*, *Origanum elongatum*, *Thymus zygis*, and *Satureja calamintha* essential oils on a cereal insect pest (*Sitophilus granarius* L.), In this research, we compared the effect of essential oils obtained from cultivated and spontaneously growing plants. The outcomes of this study are expected to facilitate and optimize crop production by reducing the damage caused by this devastating pest and reduce the over reliance on synthetic insecticides. This approach will help limit the harmful effects of chemical insecticides on the environment, human and animal health.

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Materials and Methods

Insects

The insects used in this study were young *Sitophilus granarius L*, 3 - 4 weeks old, obtained from rotting wheat grains on a farm in the province of Taounate. After harvesting, the insects were reared on healthy wheat grains under controlled laboratory condition (temperature of 28 ± 2.2°C and relative humidity of 65 ± 5.7%). Adult insects were fasted for 24 hours prior to the experiment.

Collection and identification of plant materials

Spontaneous plants

Spontaneous plants were collected in 2018 from two regions: Taza and Ksar El Kebir regions, Morocco (Figure 1).

Origanum compactum, *Origanum elongatum*, and *Thymus zygis* were collected from the Taza region at the following coordinates: 34°05'27.03"N; 03°56'32.20"W; Altitude, 1122 m and 34°01'22.3"N 04°04'02.2"W; Altitude, 1309 m.

Saturja calamitha was collected from the Ksar El Kebir region at the following coordinates: 34° 06'03.11" N 03° 56'35.94" W; Altitude 137 m. The plants were identified at the National Institute for Agronomic Research, Rabat, Morocco. Voucher numbers RAB114271, RAB114273, RAB11427, and RAB114240 for *Satureja Calamintha*, *Origanum compactum*, *Origanum elongatum*, and *Thymus zygis*, respectively were issued.

The plant materials were shade-dried at room temperature (24 ± 2°C) for two weeks, ground to a fine powder, and carefully stored until ready for use.

Figure 1: Location of the spontaneous plants collection. (a) Ksar El kébir region (b) Taza region

Cultivated plants

The plants were cultivated at the garden of the Institut Technique Agricole de Sahel Boutaher in Taounate province, Morocco (Figure 2). Pesticides and herbicides were not used throughout the cultivation period.

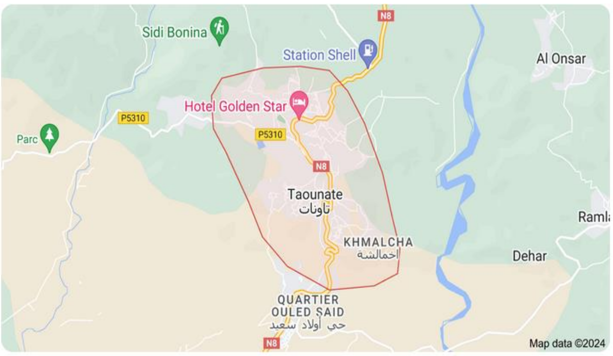

Figure 2: Location of the cultivated plants collection (Taounate province)

Extraction of Essential oils

Essential oils were extracted by hydrodistillation on a Clevenger-type apparatus. The essential oils were stored in a dark place at a temperature of 4°C. The extraction yield (R) of the essential oils was determined according to the equation (1):

$$R\ (\%) = \frac{MHE}{MMV} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where;

M_{HE} is the weight of essential oil obtained after distillation, and M_{MV} is the weight of dried plant material used.

Evaluation of insecticidal activity

The insecticidal activity of the essential oils (EO) was evaluated according to the method described by Kouninki *et al* (2007).[7] This method aims to determine the minimum dose of essential oil (EO) capable of inducing total mortality (100%) in wheat weevils *Sitophilus granarius* L. The most toxic essential oil is defined as the one requiring the lowest dose to achieve 100% mortality.

Essential oil solutions were prepared at five different concentrations: 0.1%, 0.15%, 0.25%, 0.5% and 1%, diluted in 1 mL of 3% Tween 80. Specific discs were treated with the essential oil solutions. Each treated disc was placed in a box, where ten adult weevils were deposited.

After 15 minutes, the number of dead weevils in the treated boxes was counted. At the same time, a similar assessment was carried out in untreated boxes to serve as control. Finally, control boxes were kept without any exposure to essential oils, in order to compare the results obtained with the treatment. The pest mortality rate (Tr) was calculated using the following formula (2):

$$Tr = \frac{\text{number of dead insects}}{\text{total number of insects in the box}}\ x\ 100$$

The efficacy of essential oils from the spontaneous plants were compared with that from cultivated plants at the same concentrations (0.1%, 0.15%, 0.25%, 0.5%, and 1%) over a 24-hour period.

Statistical analysis

Data were presented as mean ± standard deviations (SD) of triplicate determination. Data were analyzed by one-way analysis of variance (ANOVA) using Graph pad Prism 8.0.2 software. Comparison between means were done using Post hoc Turkey's HSD test. Differences between means were considered significant at $p < 0.05$.

Results and Discussion

Essential oil extraction yields

The essential oil yields of the cultivated and spontaneous plants are presented in Figure 3. Generally, the essential oil yields were higher in the cultivated plants than in the spontaneous plants. For example, cultivated *Thymus zygis* yielded 0.37% more essential oil than spontaneous *Thymus zygis.* It was also noted that cultivated *Origanum compactum* yielded 1.93% essential oil while spontaneous *Origanum compactum* yielded 1.17% essential oil.

Insecticidal activity of the essential oils

Satureja calamintha essential oil

The insecticidal activity of *Satureja calamintha* essential oil, both for the spontaneous and the cultivated plants is presented in Figure 4. The results showed that the plant essential oil demonstrated a concentration-dependent insecticidal effect against wheat weevils Sitophilus granarius L., with the higher concentrations (1% and 0.5%) being the most effective, and the lower concentration (0.1%) being the least effective. For the spontaneous *Satureja calamintha* essential oil, the highest concentration (1%) resulted in the highest insect mortality rate of 100.00 ± 0.00% after 24 hours. It was observed that the 0.5% concentration was as effective as the 1% concentration, which indicated that no additional increase in insect mortality rate is expected beyond that observed with 0.5% concentration of the essential oil. On the other hand, lower concentrations, 0.25% and 0.15% of *S. calamintha* EO resulted in significantly lower insect mortality of 73.25 ± 1.16% and 66.70 ± 0.58%, respectively, while the lowest concentration of 0.1% *S. calamintha* EO produced the lowest insect mortality rate (46.70 ± 0.58%) (Figure 4a). Similarly, the EO extracted from the cultivated *S. calamintha* resulted in a concentration-dependent insecticidal activity. The higher concentrations (1% and 0.5%) gave the same percentage mortality of 100.00 ± 0.00% after 24 hours, this was followed by the 0.25% concentration with 66.70 ± 0.58% insect mortality, and lastly the 0.15 and 0.1% concentrations, which displayed the lowest percentage mortality of 33.30 ± 0.58% for both concentrations (Figure 4b). The similarity in the results obtained for both the 0.5% and 1% *S. calamintha* suggests that the use of the 0.5% EO concentration would be more economical compared to the 1% EO concentration without sacrificing efficacy. These results showed that *Satureja calamintha* essential oil have potent insecticidal activity against *Sitophilus granarius* L. This finding is consistent with the work of Abbad *et al*. (2023),[9] who reported that EO obtained from wild *Satureja calamintha* was significantly more toxic, with LD_{50} of 1.99 µL/L and LD_{90} of 4.11 µL/L than that obtained from the cultivated plants (LD_{50} = 64.53 µL/L and LD_{90} = 17.63 µL/L). The insecticidal activity of *Satureja calamintha essential oil* may be attributed to the high content of the cyclic monoterpene pulegone which has been found in the essential oil of the wild plant.[10]

It has been reported that essential oil does not necessarily exert the same activity at different stages of an insect's life cycle, as there is significant variation in the sensitivity of insect species to the same essential oil.[6] Essential oils act directly on the cuticles of soft-bodied insects and mites. They are less effective on hard-shelled insects such as adult Coleoptera and Hymenoptera. Insecticide effectiveness may also depend on the sex of the insect. Indeed, several authors have demonstrated that male *Acanthoscelides obtectus* are more sensitive to essential oils than the females.[11,12]

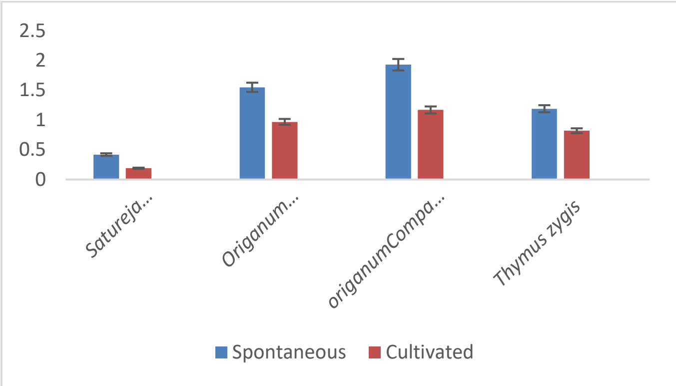

Figure 3: Extraction yields of essential oils from spontaneous and cultivated plants.

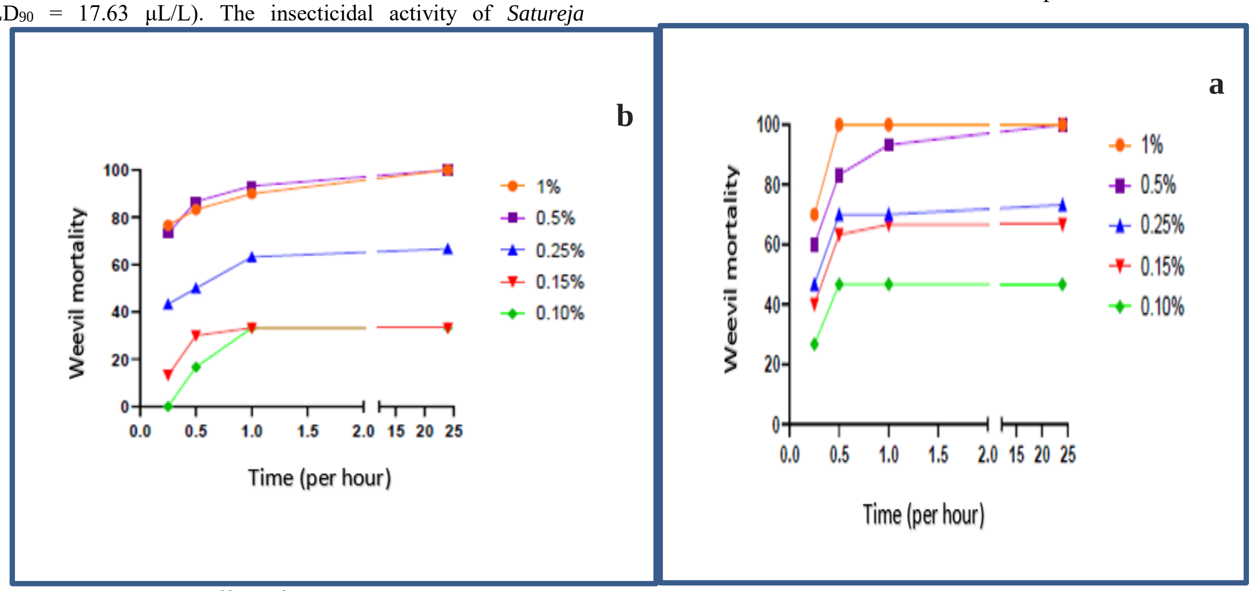

Figure 4: Insecticidal effect of *Satureja calamintha* essential oil on *Sitophilus granarius* L. (a) Spontaneous plant (b) Cultivated plant

Origanum elongatum essential oil

The results as presented in Figure 5 highlight the concentration-dependent impact of *O. elongatum* EO on insect mortality. For the spontaneous species, the highest three concentrations (1%, 0.5%, and 0.25%) showed identical and maximum insecticidal effect, with percentage insect mortality of 100.00 ± 0.00%. This indicates that increasing the essential oil concentration beyond 0.25% did not result in additional increase in the insect mortality rate, thus optimizing the use of *O. elongatum* EO. The 0.15% concentration achieved a mortality rate of over 50% (66.70 ± 0.58%), while the lowest concentration (0.1%) had percentage mortality of 33.30 ± 0.58%, demonstrating significant insecticidal activity (Figure 5a). This suggests that *O. elongatum* EO is highly potent and that even a concentration of 0.15% can be a viable and cost-effective option for pest control. In contrast, the impact of *O. elongatum* EO from the cultivated plants on insect mortality shows that the highest concentration (1%) was the most effective, exhibiting percentage insect mortality of 70.00 ± 0.00%, while lower concentrations exhibited variable but significant insecticidal effects, with percentage insect mortality of 50.00 ± 0.00%, 26.70 ± 0.58, 30.00 ± 0.00%, and 20.00 ± 0.00% at 0.5, 0.25, 0.15, and 0.1% concentrations, respectively (Figure 5b). Alkan (2020) investigated the insecticidal activity of the essential oils of different *Origanum species* against four stored product pests, it was reported that the essential oils of all species except *O. vulgare* and *O. onites* resulted in >90% mortality. Moreover, *Sitophilus oryzae* was found to be highly sensitive to the applied essential oils, with >90% mortality recorded with the essential oils of all species except *O. vulgare* and *O. onites*.[13]

Origanum compactum essential oil

Figure 6 presents the insecticidal activity of *Origanum compactum* essential oil against *Sitophilus oryzae*. The findings illustrated an apparent concentration-dependent effect of *O. compactum* EO on insect mortality. For the spontaneous plants, all the EO concentrations resulted in a significantly high insect mortality. The highest concentrations (1%, 0.5%) exhibited the highest insect mortality of 100.00 ± 0.00%, close followed by 0.25% with percentage mortality of 96.70 ± 0.58%, and 0.15% with percentage mortality of 86.70 ± 0.58%, while the lowest concentration (0.1%) displayed a percentage mortality of 53.30 ± 1.16% (Figure 6a). The significantly high insecticidal activity of spontaneous *Origanum compactum* EO even at low concentrations gives an insight into the minimum effective dose for practical applications. Kordali *et al*. (2008) tested the insecticidal properties of essential oil isolated from *Origanum acutidens* on *Sitophilus granarius* and *Tribolium confusum*, and they found out that *O. acutidens* EO resulted in 68.30% and 36.70% mortality in *S. granarius* and *T. confusum* adults, respectively after 96 hours of exposure.[14] For the *cultivated Origanum compactum* EO, a significant decrease in the percentage mortality of *Sitophilus oryzae* was observed as the EO concentration decreases. Thus, at 1% EO concentration, percentage mortality was 100.00 ± 0.00%, which decreased to 70.00 ± 0.00%, 33.30 ± 0.58%, 23.30 ± 0.58%, and 10.00 ± 0.00% at 0.5, 0.25, 0.15, and 0.1%, respectively (Figure 6b). Therefore, the EO obtained from wild *Origanum compactum* is significantly more toxic (LD_{50} = 1.988 µL/L and LD_{90} = 4.106 µL/L) than the cultivated plants (LD_{50} = 6.453 µL/L and LD_{90} = 17.625 µL/L). The higher insecticidal activity of the wild *Origanum acutidens* EO compared to the cultivated *Origanum acutidens* EO may also be attributed to the high monoterpene content of wild *Origanum acutidens* essential oil.[15] The insecticidal effect of major components of *Ocimum gratissimum* essential oils including; 1, 8-cineole, *β*-pinene, *α*-pinene, myrtenal, pinanol, L-carveol, transpinocarveol, and linalool has been tested against *Sitophilus zeamais* adults (Coleoptera: Curculionidae), and it was observed that at essential oil concentration that caused 100% mortality, 1,8-cineole resulted in 63.0 ± 2.1% mortality, and *β*-pinene caused 37.0 ± 2.4% mortality, while the other components had no insecticidal effect. However, the combination of all eight compounds resulted in a 100% insect mortality.[16]

These observations show that the combined action of essential oil major components is more remarkable than the action of the individual component. Indeed, recent study has shown that monoterpenes have neurotoxic effects, and act on different targets depending on their chemical structure.[17] Due to their volatility, and high lipophilicity, monoterpenes have a rapid inhalation-type toxic action on adult insects. They have fumigant effect that could be important in controlling insects in stored foodstuffs.[10]

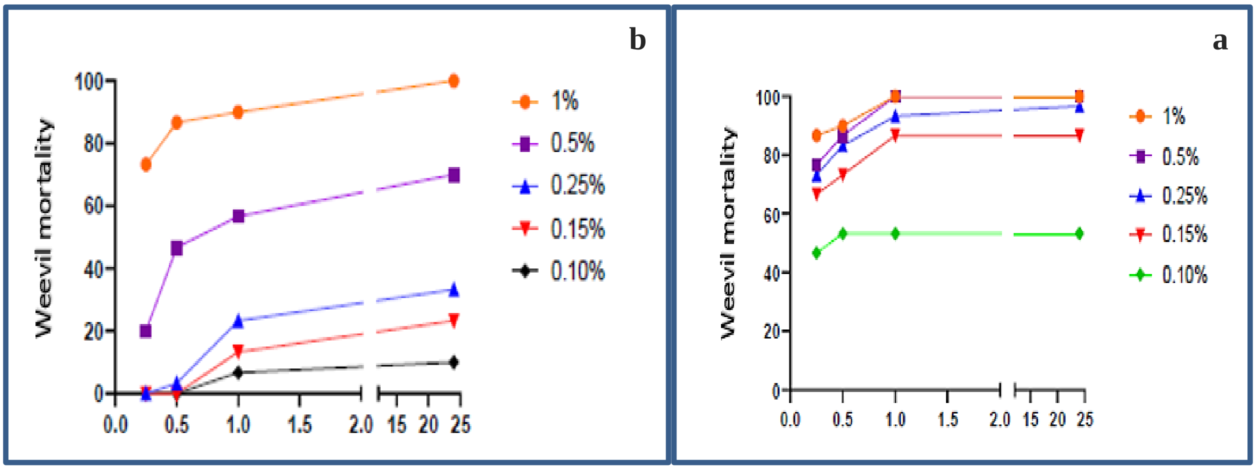

Figure 5: Insecticidal effect of *Origanum elongatum* essential oil on *Sitophilus granarius* L. (a) Spontaneous plant (b) Cultivated plant

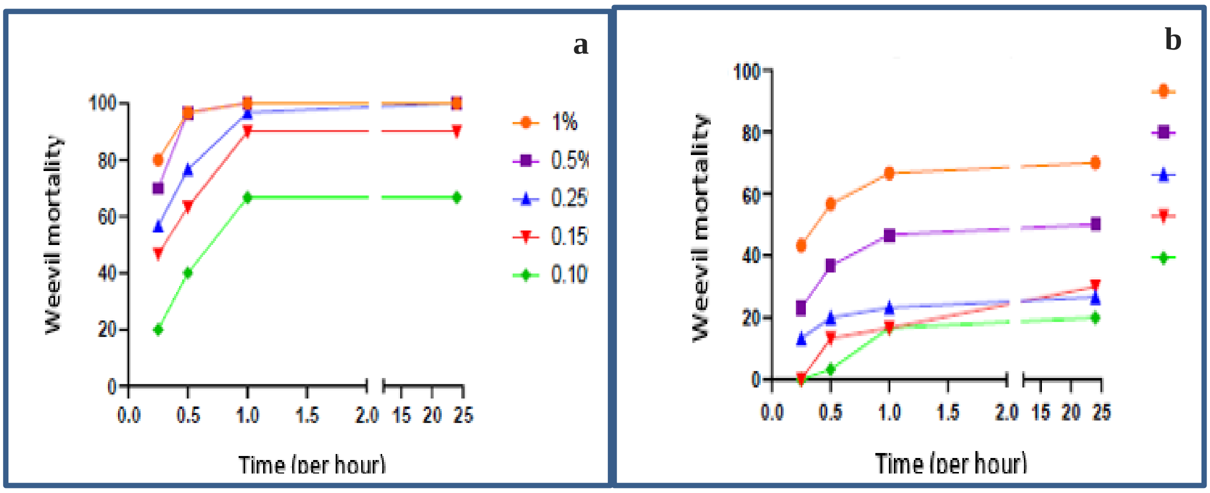

Figure 6: Insecticidal effect of *Origanum compactum* essential oil on *Sitophilus granarius* L. (a) Spontaneous plant (b) Cultivated plant

Thymus zygis essential oil

The insecticidal effect of *Thymus zygis* essential oil on *Sitophilus granarius* L. is highlighted in Figure 7. For the spontaneous *Thymus zygis* essential oil, all the concentrations except the lowest concentration resulted in a 100% insect mortality, while the lowest concentration (0.1%) resulted in 83.30 ± 0.58% mortality (Figure 7a). This finding suggests that increasing the concentration beyond 0.15% does not enhance insecticidal efficacy, allowing for cost-effective use without compromising effectiveness. The present results highlight the powerful insecticidal properties of spontaneous *T. zygis* EO. On the other hand, the cultivated *Thymus zygis* essential oil demonstrated a concentration-dependent increase in insecticidal activity, with the percentage insect

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mortality gradually increasing from 36.70 ± 0.58% at 0.1% concentration to 60.00 ± 1.00% at 0.15% concentration, then to 86.70 ± 0.58% at 0.25% concentration, and finally to 100% mortality at 0.5% and 1% concentrations (Figure 7b).

It is important to note that the four essential oils examined in this study exhibited exceptional insecticidal effect against *Sitophilus granarius* L. adults. The results varied depending on whether the plants were wild (spontaneous) or cultivated. Researchers have extensively studied the insecticidal action of essential oils derived from aromatic plants with the aim of reducing the losses caused by insect pests on stored grains. For example, the essential oils of *Artemisia campestris* and *Thymus algeriensis* have been shown to exhibit 100% mortality on *Sitophilus oryzae* after 72 h exposure.[18]

Similarly, the essential oils of rosemary, spearmint, and thyme have been shown to cause 100% mortality of *Sitophilus oryzae* after exposure for 48 h, 96 h, and 6 days, respectively.[19] Del Carmen *et al*. (2002) tested the efficacy of essential oils extracted from twenty-four aromatic plants of several families against the bruchid beetle (*Acanthoscelides obtectus*). The results showed that oils from seven plants of the Lamiaceae family (*Thymus serpyllum, Origanum vulgare, Satureia hortensis, Lavandula angustifolia, Rosmarinus officinalis, Origanum majorana, and Ocimum basilicum*) and *Petroselinum sativum* of the Apiaceae family were the most toxic, causing 100% mortality after 1 to 4 days of exposure.[3]

The biological efficacy of essential oils from *Laurus nobilis* (Lauraceae), *Citrus bergamia* (Rutaceae), and *Lavandula hybrida* (Lamiaceae) has been demonstrated against adult corn weevils *Sitophilus zeamais* (Coleoptera: Curculionidae), wheat weevils *Cryptolestes ferruginous* (Coleoptera: Cucujidae), and rice weevil larvae *Tenebrio molitor* (Coleoptera: Tenebrionidae).[20] The different susceptibility of *Sitophilus oryzae* to essential oils may be attributed to several factors, including the species' resistance to certain essential oil components, the nature of the oil, the concentration used, and the exposure time.[7,10,20,21]

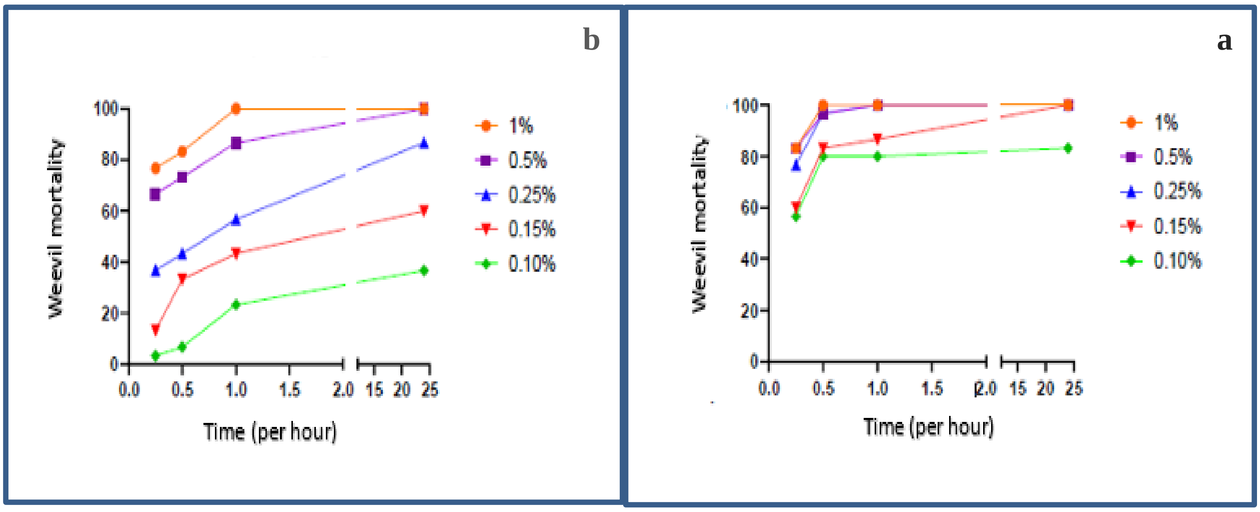

Figure 7: Insecticidal effect of *Thymus zygis* essential oil on *Sitophilus granarius* L. (a) Spontaneous plant (b) Cultivated plant

Conclusion

Plants synthesize numerous secondary metabolites that have different effects on insects: as repellents, attractants, developmental inhibitors, reproductive inhibitors, etc.The present study investigated the insecticidal activity of the essential oils of four spontaneous and cultivated aromatic plants; *Satureja Calamintha*, *Origanum compactum*, *Origanum elongatum*, and *Thymus zygis*. The results showed that the essential oils were highly effective, exhibiting mortality rates up to 100% after 24 hours of exposure. These results demonstrate the remarkable insecticidal effect of these oils (with variability between spontaneous and cultivated plants). The findings from this study signal the prospects for the use of these aromatic plants as natural insecticides for the control of stored grain pests.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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