Efficacy of Essential Oils to Control the Carob Moth, *Ectomyelois Ceratoniae* Zeller (Lepidoptera: Pyralidae)

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Abstract – Measures of stored pest control are mainly based on application of synthetic insecticides and fumigants. Recently, natural pest control methods including essential oils have been increasingly explored. In this work, the fumigant activity of essential oil vapours distilled from *Citrus sinensis* and *Artemisia herba-alba* were tested against adults, larvae and eggs of the carob moth *Ectomyelois ceratoniae*. Results showed that fumigant toxicity depends on oil species, concentrations and exposure time. Aged larvae L5 were more susceptible than young larvae (L4 and L3). Mortality rate of Larvae (L5) reached 100% and 64% respectively for *Artemisia herba-alba* and *Citrus sinensis* at the concentration of 150 μl/l air after 24 hours exposure. The corresponding LC₅₀ for adults values were respectively 2.6 and 0.31 μl/l air for *Citrus sinensis* and *herba-alba*. The hatching rate decreased with increases in concentration or exposure time for the two oils. The exposure to vapours of essential oils from *Artemisia herba-alba* caused 0% of hatching rate at the concentration of 150 μl/l air after 48 hours exposure. With the same concentration and time exposure, hatching rate was 31.7% when eggs were exposed to *Citrus sinensis* oil. Results suggested that the two oils mainly *Artemisia herba-alba* essential oils could be used as an alternative to the synthetic fumigant in postharvest treatment program for the control of *E. ceratoniae*.

Keywords – *Ectomyelois Ceratoniae*, Fumigation, Essential Oil, Biopesticides, *Citrus Sinensis*, *Artemisia Herba-Alba*.

I. INTRODUCTION

The date palm (*Phoenix dactylifera* L.) has always played an important part of the economic and social life of the people from arid and semi-arid regions. Tunisia is one of the major date producing countries, the number of palm trees being estimated to be over 4 millions. In 2016, the national production was around 240,000 tonnes including 10315 tonnes of Deglet Noor organic variety dates for export [1].

However, dates are subjected to many diseases and pests that decrease their yield and deteriorate their quality. The date moth, *E. ceratoniae* is the major insect pest of dates, pomegranate and several other host plants in Tunisia in both field and storage [2], [3]. In fact, the presence of larvae and their excrements in date making theme unfit for human consumption, conditioning so, severe measurement in the marketing including exportation [4]. The post - harvest control of this pest is exclusively based on use of fumigants. Currently, phosphine and methyl bromide is the product most widely used [5], [6], [7]. Nevertheless, this chemical had numerous harmful side effects on human and environment [8], [9]. Thus the search of efficient and ecofriendly alternatives is required. In this respect, the use of plant essential oils [10], [11], [12], [13], [14] have been described as postharvest control alternatives against various stored Lepidopteran species including the date moth *E. ceratoniae*.

The aim of this study was to evaluate fumigant toxicity of *Citrus sinensis* and *Artemisia herba-alba* essential oils against adults, larvae and eggs of *E. ceratoniae*, in order to find new bioactive natural products.

II. MATERIALS AND METHODS

1. Insect Rearing

A laboratory rearing colony of *Ectomyelois ceratoniae* was established in the Laboratory of Plant Protection at the Regional Research Centre of Oasis Agriculture of Degache from infested field-collected dates.

2. Plant Material

*Artemisia herba-alba* leaves were collected locally in the locality of Gafsa (southern Tunisia) where it grows spontaneously during February 2016. *Citrus sinensis* (var. Thomson Navel) fruit peel was obtained from fruit gardens in the locality of Nabeul (northern Tunisia) in March 2016. The harvested material was air-dried at room temperature (20-25 °C) for one week and then stored in cloth bags.

3. Essential Oils Extraction

The essential oils were extracted by hydro distillation of the dried plant leaves and fruit peel (100 g of each sample in 500 ml of distilled water) using a modified Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4°C prior to analysis.

4. Fumigant Toxicity

To determine the fumigant toxicity of *Citrus sinensis* and *Artemisia herba-alba* essential oil, 2 cm diameter filter papers were impregnated with the different oil doses1, 3 and 6μl. Doses were converted to give equivalent fumigant concentrations of respectively 25, 75, 150 μl/l air. The impregnated filter paper was then attached to the screw caps of a 40 ml plexiglas bottle. Caps were screwed tightly on the vials, each of which contained 10 against adults and larvae (third, fourth and fifth instar larvae). Control insects for both stages (larvae or adults) were kept under the same conditions without any essential oils treatment. Each concentration and control was replicated four times. Mortality was recorded hourly until death of all insects.
To determine the fumigant toxicity of *C. sinensis* and *A. herba-alba* essential oil on *E. ceratoniae* eggs, fifty-one-day-old eggs for each concentration were strewn on egg cards that were glued. These egg cards were placed on 40 ml Plexiglas bottle. The eggs were exposed to essential oil vapour (1, 3, 6 and 10 µl) converted to give equivalent fumigant concentrations of respectively 25, 75, 150 and 250 µl/l air) for 24 and 48 hours. Each concentration and control was replicated four times. After 24 or 48 h, egg cards were placed in Petri dishes and the number of hatched eggs was scored. Egg hatchability was compared with the control and recorded.

Abbott correction formula [15] (Abbott, 1925) was applied to assess insect mortality. Results from all replicates were submitted to probit analysis [16] (Finney, 1971) to determine lethal concentrations (LC50).

### III. RESULTS AND DISCUSSIONS

Results were presented as percentage of mortality of *E. ceratoniae* adults, larvae and eggs exposed for various periods of time to essential oils from *Citrus sinensis* and *Artemisia herba-alba* (Fig. 1, 2, 3, 4, 5). At the lowest concentration (25 µl/l air), *C. sinensis* and *A. herba-alba* achieved more than 40% of larvae (L5, L4 and L3) mortality after 24 h of exposure.

At the highest concentration (150 µl/l air), *C. sinensis* oil led to 64, 72, 70 and 94% mortality respectively for L5, L4, L3 and adults after 24 h of exposure. Moreover, *A. herba-alba* oil achieved respectively oil 74, 70 and 84% mortality against L5, L4 and L3 and adults 96% at this concentration after 24 h of exposure. To kill the adults of this insect, higher concentrations and exposure times are required for *C. sinensis* essential oil. Probit analysis (Table 1.) showed that *E. ceratoniae* fifth stage larvae (L5) were more susceptible to *Citrus sinensis* and *A. herba-alba*.

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**Fig. 1.** Percentage of mortality of *E. ceratoniae* larvae (L5) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba* (b).

**Fig. 2.** Percentage of mortality of *E. Ceratoniae* larvae (L4) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba* (b).

**Fig. 3.** Percentage of mortality of *E. ceratoniae* larvae (L3) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba* (b).
The hatching rate of insect decreased with increases in concentration or exposure time to the two oils. The exposure to vapours of essential oils from Artemisia herba - alba caused 0% of the hatching rate at the concentration of 150 µl/l air after 48 hours exposure. With the same concentration and time exposition hatching rates was 31.2 % when eggs were exposed to Citrus sinensis oil.

In the current study, the essential oils obtained from Citrus sinensis and A. herba-alba showed insecticidal activity against the adults larvae and eggs of the date moth, Ectomyelois ceratoniae Zeller.

These results, and those reported earlier, indicate that the insecticidal activity of the essential oils varies depending on the developmental stage of the insect, plant species and the plant collection origin [17], [18], [19], [14]. Susceptibility of E ceratoniae varied between stages. Larvae L5 were more sensitive to the Citrus sinensis essential oil. Adults exhibited more susceptibility than larvae to the A. herba-alba essential oil.

Essential oils of many plants have fumigant activity due to their high volatility that might be of importance for controlling stored-product insects. The Artemisia genus, small herbs and shrubs, is one of the largest and most widely distributed aromatic plants. Previous studies demonstrated that Artemisia species essential oils have very important insecticidal activities [20]. Delimi et al. (2013) [21], revealed an insecticidal effect of essential oils extracted from A. herba-alba on Ephestia kuehniella populations. The bio pesticide is a dual mechanism of action. Administered to adults, the essential oil causes significant mortality compared with controls. While his administration to pupae, it extending their pupal development and disrupts reproductive adults by extending the preoviposition period and reducing the period for depositing eggs as fertilized females, who can’t live more than one or two days, which reduces the number of eggs deposited. Earlier authors reported that the effect Citrus sinensis essential oils have good fumigant toxicity on stored-product pests [22], [23]. Our study shows that eggs are less sensitive than adults and larvae when fumigated with essential oils from Citrus sinensis and A. herba-alba. In general, eggs are the most resistant stage and adults are the most susceptible stage to fumigation [5]. This is true for the commercial fumigants sulfonyl fluoride [24] and phosphine [25], as well as the essential oils [26].

Many studies have shown the toxicity of essential oils from various aromatic plants against the carob moth, Ectomyelois ceratoniae Zeller. Mansouri [27], investigat-ing the fumigant toxicity of L. officinalis essential oil against E. ceratoniae obtained 100% mortality after 18 h of exposure at 91 µl/l air. Pistacia lentiscus essential oil significantly reduced the copulation rate, the fecundity and the hatching rate of E. ceratoniae at concentrations ranging from 91 to 136 µl/l air [19]. Additionally, Mediouni Ben

Table 1. LC50 values (µl/l air) of C. sinensis and A. herba-alba essential oils against Ectomyelois ceratoniae larvae (L3, L4, L5) and adults.

<table>
<thead>
<tr>
<th></th>
<th>Citrus sinensis</th>
<th>A. herba-alba</th>
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<tbody>
<tr>
<td>larvae (L3)</td>
<td>3.23 (2.29 -4.981)</td>
<td>0.82 (0.07 - 1.50)</td>
</tr>
<tr>
<td>larvae (L4)</td>
<td>1.181 (0.0 - 2.329)</td>
<td>1.46(0.00-2.94)</td>
</tr>
<tr>
<td>larvae (L5)</td>
<td>0.501</td>
<td>0.89</td>
</tr>
<tr>
<td>Adults</td>
<td>2.6 (2.21 -3.01)</td>
<td>0.31 (0.004 -0.76)</td>
</tr>
</tbody>
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![Fig. 4. Percentage of mortality of E. ceratoniae adults exposed to various doses and periods of time of essential oil from C. sinensis (a) and A. herba-alba (b).](image1)

![Fig. 5. Percentage of E. ceratoniae egg hatchability exposed to various doses and exposure time of essential oil from C. sinensis and A. herba-alba.](image2)

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Jemâa et al., [14] indicated that essential oils from five Eucalyptus essential oils namely E. astringens, E. lehmannii, E. camaldulensis and E. leucocorydon E. rudis were toxic to E. ceratoniae. E. ceratoniae larvae were sensitive to the fumigant toxicity against of E. camaldulensis and E. occidentalis essential oils against [28].

IV. CONCLUSION

The present study has shown encouraging results of fumigant activity of Citrus sinensis and A. herba-alba essential oil against adults larvae and eggs of the carob moth. Eggs are less sensitive than adults and larvae.

The essential oil of Citrus sinensis and A. herba-alba can play an important role in pest control and reduce the need for synthetic insecticides. Larger scale and longer term studies would be required to determine the effects on end-use and risk to humans would need to be determined before commercialization.

REFERENCES

[27] E. Camaldulensis and E. Leucoxylon E. Rudis were toxic to E. Ceratonia and E. rudis were toxic to E. Ceratonia. E. ceratoniae larvae were sensitive to the fumigant toxicity against of E. Camaldulensis and E. Occidentalis essential oils against [28].


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