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Evaluation of *Laurus nobilis* essential oil repellent effect on the date moth *Ectomyelois ceratoniae* under controlled conditions

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Abstract
Essential oils can be synthesized by all plant organs and they are among the most tested substances against insects under controlled conditions. In citrus orchards attacked by the date moth *Ectomyelois ceratoniae*, the role of bay laurel *Laurus nobilis* windbreak trees as a barrier to prevent the pest spread was noticed. To confirm this observation, a test was conducted under controlled conditions to evaluate the repellent effect of *L. nobilis* on *E. ceratoniae* last development stage (L5). Thanks to steam distillation technique, *L. nobilis* essential oil was extracted from bay laurel leaves and the preferred zone method was considered. Six essential oil doses were tested. After 24 hours of exposure on *E. ceratoniae* last larval instar a high repellency percentage (80%) of bay laurel essential was obtained for the 0.24 µl/cm^2 concentration. Consequently, bay laurel trees, could act as a barrier to prevent the spread the date moth in citrus orchards.

Keywords: *Ectomyelois ceratoniae*, *Laurus nobilis*, repellent effect

Introduction
Essential oils can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and can therefore be extracted from these parts. These substances are among the most tested treatments against insects, being able to act as fumigants and repellents (Park *et al.*, 2016; Wang *et al.*, 2006) [1-2], antioxidants (Bouzidi *et al.*, 2016) [3], unappetizing (Harwood *et al.*, 1990) [4] and may affect certain biological parameters such as growth rate, lifespan and reproduction.
Most of these substances have been tested against insect attacking stored products to establish new control practices with lower toxicity to mammals and low persistence in the environment.
Ectomyelois ceratoniae, commonly known as the date moth, is a micro-lepidopteran belonging to the Pyralidae family, whose larval development takes place in five stages. Particularly voracious and polyphagous, this insect attacks citrus cultivation particularly Thomson oranges fruits (Hached *et al.*, 2018) [5].
Several essential oils were tested to control the date moth in storage warehouses. Indeed, fumigant effect of *Pistacia lentiscus* essential oil was tested against *E. ceratoniae* with (LC50 = 3.29 µl/l, LC95 = 14.24 µl/l) as demonstrated by Bachrouch *et al*., (2010) [6]. They confirmed also that, thanks to its richness in monoterpenoids, this oil does not only act on the mortality of the date moth but also on the reduction of copulation rate, longevity, fertility and egg hatching. Besides, Mediouni *et al.*, (2012a) [7] reported the effectiveness of fumigation with essential oils of Eucalyptus (*E. camaldulensis, E. astringens, E. leucoxylon, E. lehmannii and E. rudis*) towards the date moth. Moreover, previous studies on essential oils extracted from *Thymus capitatus*, *Pinus halepensis* and *Rosmarinus officinalis* conducted by Amri *et al*., (2014) [8] proved the toxicity of these substances towards this pest.
Furthermore, in fields, the repellent effect of *Ferula assafoetida* essential oil, was tested by Peyrovi *et al*., (2001) [9] against adults of *E. ceratoniae* in a pomegranate orchard in Iran.
Following personal observations in citrus orchards where the bay laurel *Laurus nobilis* trees was planted as a windbreak and element of division between the elementary plots, the absence of the attack by the date moth was noticed although the neighboring plots were attacked by this pest. Thus, the barrier role of this plant to prevent *E. ceratoniae* spread was detected.
Laurus nobilis L. is an evergreen shrub, indigenous to the south of Europe and the Mediterranean area. This plant belonging to Lauraceae family which essential oil can be used

as antioxidant, antifungal, antibacterial and insecticidal agent (Bouzidi *et al*., 2019) [10].
In this context, this present work was carried out in the aim to evaluate the repellent effect of the bay laurel essential oil on the date moth fifth larval instar under controlled conditions.

Materials and Methods

Insect material

Larvae of the date moth last development stage (L5) were recovered from the mass rearing installed in the INAT entomology laboratory under optimal conditions: temperature (28 ± 1 °C), photoperiod 16: 8 (L: D) and relative humidity 65 ± 5% (RH).

Plant material and extraction of the essential oil

Leaves of the bay laurel used were recuperated in May 2016 from the region of "Ras Djbel".
The quantity of 1.5 kg of leaves was considered. The essential oil was extracted by steam distillation using a Clevenger-type apparatus for 3 hours. Essential oil was kept in tinted glass vials tightly closed at 4 ° C until used in the bioassays.

Repellent effect of the essential oil

To evaluate the repellent activity of the essential oil towards the date moth (L5), the method of the preferred zone at 25 ° C ± 1 ° C and 65% ± 5% RH described by McDonald *et al*.,1970 [11] was considered.
Six doses of *L. nobilis* (1; 1,5; 2; 2,5; 4 and 6 µl) were prepared by dissolution in 1 ml of aceton corresponding to 0,04; 0,06; 0,08; 0,1; 0,16 et 0,24 µl/cm^2 respectively. Solutions were homogeneously applied to half a filter paper disc using a micropipette, while the other half of the disk was treated only with 0.5 ml of acetone and was considered as a control. After complete evaporation of the solvent, ten (L5) *E. ceratoniae* larvae were placed in the center of each filter paper disc. Three replications were performed for each essential oil dose.

Percentage of repellency (PR) and determination of the median repellent concentration (CR50) of the bay laurel essential oil

Observations were done after 1.5; 3 and 24 hours from the beginning of the experiment. The number of L5 larvae present on the part treated with the essential oil noted by (Nt) and the number of those present on the control part treated with acetone note by (Nc) were counted.
Percentage repellency (PR) was calculated according to Cosimi *et al*., (2009) [12] and Nerio *et al*., (2009) [13] formula as follow: $PR = [(N_c-N_t) / (N_c+N_t)] \times 100$

The value of the repellency percentage allows identifying different repellant classes as described by McDonald *et al.*, (1970) [11].
The determination of the median repellant concentration (CR50) corresponds to the extract inducing 50% repellency in the populations of treated insects, expressed in (µl / cm^2).

Data analyses

Statistical analysis was performed using SPSS software (version 16.0). Means comparisons of the essential oil different doses effects on (L5) after 24 hours of exposure were made using the Duncan test at 5% level. The determination of the median repellant concentration (CR50) was carried out using Probit analysis software (Finney, 1971) [14]. Chi-square (χ^2) test was applied to test for homogeneity ratio (1:1) in order to assess the repellent activity of *L. nobilis* essential oil.

Results and Discussions

The essential oil repulsion percentages results on the last larval instar (L5) and the corresponding repellency classes are indicated in Table 1.

Table 1: Repulsion percentages (mean ± SE) of the bay laurel essential oil on *E. ceratoniae* (L5) larvae depending on the duration of exposure and the essential oil concentration on filter paper.

Concentration (µl/cm^2)	0, 04	0, 06	0, 08	0, 1	0, 16	0, 24
Exposure time (h): 1,5	13,33 ±1,54Aa	33,33 ±13,14Aa	6,66 ±1,8Aa	-33,3 ±6,1Aa	20 ±6,9Aa	-46,66 ±2,30Aa
Repellency class	I	II	I	0	I	0
Exposure time (h): 3	13,33 ±1,54Aa	6,66 ±2,3Aa	-6,66 ±1,6Aa	-20 ±4Aa	20 ±6,9Aa	-6,66± 2,30Aa
Repellency class	I	I	0	0	I	0
Exposure time (h): 24	0Aa	20 ±4Aab	6,66 ±2,3bAa	-33,33 ±4,6Aa	6,66 ±1,6Aa	80 ±20Bb
Repellency class	0	I	I	0	I	IV

Comparisons of means in the same column (uppercase letters) express the duration of exposure time effect on insect repellency. Comparisons of means on the same row (lowercase letters) express the effect of concentration on insect repellency. The values followed by the same letter are not significantly different according to Duncan's test with $p \leq 0.05$.
In fact, after 1,5 hours of exposure, the highest percentage repellency of 33.33% (Class II) was recorded for the concentration of 0.06 µl / cm^2 while the concentrations of 0.1 and 0.24 µl / cm^2 showed negative repulsion percentages recording -33.3 and -46.66% (Class 0) respectively.
After 3 hours of exposure, the highest repellency percentage of 20% (Class I) was obtained for the concentration of 0.16 µl / cm^2, while the concentrations of 0.08; 0.1 and 0.24 µl / cm^2 exhibited negative repulsion percentages (Class 0). After 24 hours of exposure, the greatest repellency effect of 80% (Class IV) followed by 20% (Class I) were recorded for the concentrations of 0.24µl / cm^2 and 0.06µl / cm^2 respectively. These results indicated that only the concentration of 0.24 µl / cm^2 showed significant repellency after 24 hours of exposure. This effect was confirmed by the variance analysis which showed a significant difference for the concentration of 0.24µl / cm^2 after 24 hours of exposure with ($P = 0.0065$, $F = 2.836$, $df = 17$).
Probit analyses indicated the sensitivity of *E. ceratoniae* towards the *Laurus nobilis* essential oil. The corresponding median repellency concentration (CR50) value is 0.062 µl / cm^2 (Table 2).

Table 2: Value of (CR50) of *Laurus nobilis* essential oil against the last larval stage (L5) of *E. ceratoniae* after 24 hours of exposure

Laurus nobilis essential oil	CR_{50}	Slope ± SEM	Degree of freedom	χ^2
	0,062	-0,94±0,75	5	16,377

In this study, the repellent effect of the bay laurel was confirmed towards the date moth last larval instar after 24 hours of exposure at the concentration of 0.24 µl/cm^2.

In fact, the repellent effect of *Laurus nobilis* essential oil was also proved on insects from stored foods such as *Sitophilus zeamais*, *Cryptolestes ferrugineus* and *Tenebrio molitor* (Cosimi *et al*., 2009) [12].

Besides, the study carried out by Mediouni *et al*., (2011) [15] on *Lasioderma serricorne* showed that the repellent action of *L. nobilis* essential oil was highly dependent upon oil concentration and exposure time. Moreover, against two major pests of stored grain *Rhyzopertha dominica* and *Tribolium castaneum L. nobilis* essential oil exhibited highly significant repellency effect (Mediouni *et al*., 2012b; Ncibi *et al*., 2019) [16-17]. Furthermore, against cabbage aphid, *Brevicoryne brassicae*, the repellency percentage was 86.67% for laurel essential oil in LC50 (Hosseini *et al*., 2013) [18].

Conclusion

A high repellency percentage (80%) of bay laurel after 24 hours of exposure on the last larval instar of *E. ceratoniae* was demonstrated. However, in storage warehouses, tests on the impact of this essential oil on the treated fruits must be carried out in order to determine the adequate doses allowing both the control of pests while preserving the quality of the fruits and ensuring consumer safety. We deduced, then, that this plant could act as a barrier that prevents the spread of this pest, hence the benefit of planting bay laurel trees in citrus orchards.

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