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Hayette Bouzeraa
Department of Biology, Larbi Tebessi University, Tebessa, Algeria

Bessila-Bouzeraa M
Laboratory of Ecology, Environment and Vegetal Biology, Biology Department, Badji Mokhtar University, Annaba, Algeria

Labed N
Research Laboratory on Ecosystem pollution and Biodiversity, Chadli Bendjdid University, El Tarf, Algeria

Sedira F
Department of Biology, Larbi Tebessi University, Tebessa, Algeria

Ramdani L
Department of Biology, Larbi Tebessi University, Tebessa, Algeria

Correspondence
Hayette Bouzeraa
Department of Biology, Larbi Tebessi University, Tebessa, Algeria

Evaluation of the insecticidal activity of *Artemisia herba alba* essential oil against *Plodia interpunctella* and *Ephestia kuehniella* (Lepidoptera, Pyralidae)

Hayette Bouzeraa, Bessila-Bouzeraa M, Labed N, Sedira F and Ramdani L

Abstract
In the present study, the efficiency of the insecticidal activity of *Artemisia herba alba* essential oil was evaluated on two insect pests of stored food, *Plodia interpunctella* and *Ephestia kuehniella*. Different concentrations of the oil were tested on the last instar larvae for repellent activity (25, 75, 100, 120, 130 and 150µl/ml ethanol for both insect) and fumigant toxicity bioassay (50, 75, 100 and 120 µl/l air for *P. interpunctella* and 50, 150, 180, 200 and 600 µl/l air for *E. kuehniella*) under laboratory conditions. Results showed repellent activity of the oil against both insect with stronger effect on *P. interpunctella* larvae. The oil caused toxicity in both insect larvae. Median lethal concentrations of the oil (LC_{50}) recorded against *P. interpunctella* larvae were 162.1, 144.9 and 141.1 µl/l air at 24, 48 and 72h after exposure. LC_{50} values of this oil against the larvae of *E. kuehniella* were 901.1, 782.4 and 514.2 µl/l air after exposure at the same times, respectively. According to the results, the oil had a stronger activity on *P. interpunctella*, whereas, *E. kuehniella* was the most tolerant to the oil activity. These results would lead to a prior knowledge in the specificity of essential oils toward different insect orders and confirm the efficiency of essential oils in the control of insect pests of stored product.

Keywords: *Ephestia kuehniella, Plodia interpunctella, Artemisia herba alba,* essential oils, toxicity

1. Introduction

Insect pests can have negative impacts on agricultural production, crops or post-harvest, and cause nutritional quality alteration of grains leading to enormous economic losses. Stored product pests are divided into two groups: some species are considered as primary pests attacking the whole grain viz., the coleopteran grain weevils (*Sitophilus granarius, S. zeamais, S. oryzae*) [1], the lesser grain borer (*Rhyzopertha dominica*) [2] while others follow the initial damage as secondary pests viz., the coleopteran flour beetles (*Tribolium confusum*, *T. castaneum*) [3, 4], the Lepidopteran flour moth (*Plodia interpuntella*, *Ephestia kuehniella*) [5, 6] which feed regularly in processed foods, on grain that is damaged or is going out of condition, so are more common in domestic kitchens and larders.

Plodia interpunctella, Indian meal moth, is an important common household pest. A single female may lay up to 400 eggs at 25 °C individually or in groups [7]. Larvae have five instars as determined by head capsule width [8, 9]. They measured from 8 to 10 mm long at their last instar. They feed on dried fruits and nuts and developed, preferably, in durum wheat flour. The infestation and damage by *P. interpunctella* are present in warehouses and stored food commodities [10].

Ephestia kuehniella, Mediterranean flour moth, is a cosmopolitan pest of cereal. It feed and developed, preferably, in soft wheat flour that provides favourable environmental conditions. A single female may lay up to 350 eggs at a temperature of 25 to 27 °C. Caterpillars are very active spinners and pass by six instar larvae. They measured from 13 to 16 mm long at their last instar. Their outbreak leads to infest product and to accelerate growth of molds, including toxigenic species [11].

Due to the intensity of plant-insect interactions, plants have well developed defence mechanisms against pests and are excellent sources of new insecticidal substances [12]. Once in contact with insects, natural plant substances penetrate the organism and reach, more or less quickly, at the cellular level, target enzymes and proteins leading to an abnormal biological functioning of the organism [13] resulting in paralysis and death.

Essential oils are usually extracted from various parts of the plant [14, 15]. A total of 269 chemicals from plants that are active against stored-product insects are sorted plant species from which they were extracted [16]. These chemicals approved their effectiveness in bulk stored grains and seeds [17, 18] and have been reported as effective fumigants [19-21], attractants or repellents [12], antifeedents or deterrent of oviposition or population growth [22-24] against many stored product insects.

Artemisia herba alba (Asterales, Asteraceae) is an aromatic plant commonly used in traditional medicine as anti-diabetic, anti-bacterial, anti-viral, anti-oxidant, anti-haemorrhagic, anti-pyretic or anti- spasmodic [25]. Furthermore, the volatility of its essential oil due to monoterpene and camphor major compound confers their effectiveness against the pest stored product: *Sitophilus oryzae* as fumigant [21], *Tribolium castaneum* as very repellent [18] and their potential effect against *Callosobruchus maculatus* and *Rhyzopertha domonica* [26].

Essential oils active compounds could differ from plant family to another and within the same family, and the sensitivity could vary from an insect species from a stage to another [27]. No study has been reported concerning the activity of *A. herba alba* on the two stored food insect pests *P. interpunctella* and *E. kuehniella*. The present study discussed about the effectiveness of repellent and larvicidal effect of this essential oil on the two insects mentioned above. This would lead to a prior knowledge of the insecticidal activity of the essential oil against related insect species.

2. Materials and Methods

2.1 Insects

The larvae of *P. interpunctella* and *E. kuehniella* were collected from the infected durum and soft wheat flour stock, respectively, and reared in laboratory at 25°± 3 and 60±5% relative humidity. The last instar larvae were collected from stock colony and used for tests. All the experiments were carried out under the same environmental conditions.

2.2 Plant oil extraction

The essential oil of *A. herba alba* was extracted from the plant using a Clevenger's apparatus hydrodistillation method. 250g of dried aerial parts of the plant were boiled in 2.5L of distilled water for 4 hours to obtain the distilled volatiles (yellow oil). Anhydrous Sodium Sulfate (0.1 g/ml oil) was added to absorb water if necessary. The oil was stored in hermetic amber glass vials to reduce evaporative loss and to avoid reaction with any source of light, and kept in refrigerator at 4 °C until when needed for bioassays. Plant oil yield obtained was 1.9%.

2.3 Repellency test

Different concentrations (25, 75, 100, 120, 130 and 150 µl/ml) of the essential oil were prepared by dissolving the oil in absolute ethanol. A filter paper (9cm ø) was cut into two halves and one concentration, circa 300µl, was applied in one half of the filter paper as uniform as possible using a micropipette. The second half of the filter paper was impregnated with ethanol alone, circa 300µl. The two halves of the filter paper, treated and untreated, were dried to evaporate the solvent completely. After that, the two halves of the filter paper were attached lengthwise edge to edge with odourless adhesive tape and were put in Petri dish (9cm ø) (adhered side at the bottom). Ten last instar larvae of *P. interpunctella* were released at the centre of the filter paper and then Petri dish was sealed tightly and kept in dark in laboratory conditions. The same experiment was conducted for *E. kuehniella* larvae. Three replicates were set for each concentration of the oil. Number of larva present in both treated and untreated halves of the filter paper was recorded each 30mn for 2 h of the experiment.

The percent repellency (PR) of each oil concentration was calculated using the formula [28]:

PR (%) = Nu-Nt *100/ Nu+Nt

Nu: number of larva on untreated half
Nt: number of larva on treated half

2.4 Toxicity test

The toxicity of *A. herba alba* essential oil was evaluated on different concentrations (50, 75, 100, et 120 µl/l air) and (50, 150, 200, 300, 600 µl/l air) for *P. interpunctella* and *E. kuehniella*, respectively, using fumigant method. The assay was conducted in 125 ml glass vials. Each concentration was applied on a filter-paper (2cm ø) that was attached to the lower side of the vial's lid. Ten last instar larvae of *P. interpunctella* were put into the vial and were provided with 100g of semolina as food then the vials were sealed tightly. The same procedure was applied for the last instar larvae of *E. kuehniella* that were provided with 100g of flour. Three replicates were set for each concentration of essential oil with complete set of control. All the experiment was conducted at constant temperature (25 ± 3 °C), photoperiod (8L: 16D) and relative humidity (60% ± 5). The larvae were exposed to the different concentrations of *A. herba alba* essential oil for 24h, 48h and 72h. The number of dead larvae was counted each period of time. The percentage of mortality was calculated using the following formula of Abbott [29] to eliminate natural mortality and to determine the real toxicity of the oil:

Abbott's percent corrected mortality = Mt (%) –Mc (%) * 100/100-Mc (%)

Mt: mortality in treated
Mc: mortality in control

2.5 Statistical analysis

All the data were analyzed using one way ANOVA test using Minitab Software (version 16, PA State College, USA). Significant differences between means were determined using Tukey multiple range test at P ≤ 0.05. Probit analysis was done to calculate Median Lethal Concentration LC_{50} using Graph Pad Prism 7.

3. Results

The essential oil of *A. herba alba* was tested on *P. interpunctella* and *E. kuehniella* larvae at different concentrations (25, 50, 75, 100, 120, 130 et 150µl/ml) for repellent activity. Results showed the oil have repellent activity against both insect after 2h of exposure (Table 1). Significant differences in ANOVA test were recorded. Lower concentration of the oil (25µl/ml) showed lower repellency (35%) on *P. interpunctella* larvae. However, lowest repellency (13%) were recorded on *E. kuehniella* larvae at the same concentration. Furthermore, maximum repellent activity (50%) was recorded at the concentration of 100µl/ml for *P. interpunctella*; whereas, 43% of repellency were recorded at the concentration of 120µl/ml for *E. kuehniella*.

In general, repellency increases with increase in concentration in treatment. However, the results showed repellency decreases from the concentrations 100µl/ml and 120µl/ml to 150µl/ml on *P. interpunctella* and *E. kuehniella,* respectively.

Table 1: Repellent activity of *Artemisia herba alba* essential oil against *Plodia interpunctella* and *Ephestia kuehniella* larvae

		Repellency rate (%)					
Insectes	**Concentrations (µl/ml)**	**30mn**	**60mn**	**90mn**	**120mn**	**Mean repellency rate (%)**	**Repellency class**
P. interpunctella	25	40	46	33	20	35^{ABC}	2
	75	70	30	40	20	40^{ABC}	2
	100	40	40	60	60	50^{A}	3
	120	60	40	20	20	35^{ABC}	2
	130	-40	0	20	0	-5^{BC}	0
	150	-40	0	-20	-20	-20^{C}	0
E. kuehniella	25	0	0	14	40	13^{ABC}	1
	75	20	20	0	40	20^{ABC}	1
	100	34	0	46	0	20^{ABC}	1
	120	26	26	66	54	43^{AB}	3
	130	-20	20	40	60	25^{ABC}	2
	150	20	-20	0	0	0^{BC}	0

Repellency scale from the less to the most repellent (0 to 5), where <0.1= class 0, 0.1-20= class 1, 20.1-40= class 2, 40.1-60= class 3, 60.1-80= class 4 and 80.1-100%= class 5.

Numbers in the same column of mean repellency rate (%) followed by the same letters do not differ significantly (*P*>0.05) in ANOVA and Turkey's test.

Fumigant toxicity of *A. herba alba* essential oil was evaluated from mortality recorded in the larvae of *P. interpunctella* and *E. kuehniella* after 24, 48 and 72 hours of exposure at different oil concentrations (50, 75, 100, and 120 µl/l air) (50, 150, 180, 200 and 600 µl/l air), respectively. Percent mortality was presented in figure 1, 2. Results showed that the oil possessed toxic effect toward both insect and exhibited larvicidal activity at higher concentrations. The mortality increases with an increase in the oil concentration. Significant differences were observed for *E. kuehniella* larvae (F=7, 39; p=0,005) (F= 11,37; p=0,001) (F= 8,58 ; p=0,003) after 24h, 48h and 72h of exposure, respectively. A considerable variation in the oil toxicity among the insects was observed. Lower percent mortality (3.33%) was recorded at 75µl/l air and at 150 µl/l air against *P. interpunctella* and *E. kuehniella* after 24 hours of exposure, respectively. Higher percent mortality, 40% in *P. interpunctella* and 53.33% in *E. kuehniella*, was recorded at higher oil concentrations, 120µl/l air and 600µl/l air, respectively, after 72 hours of exposure.

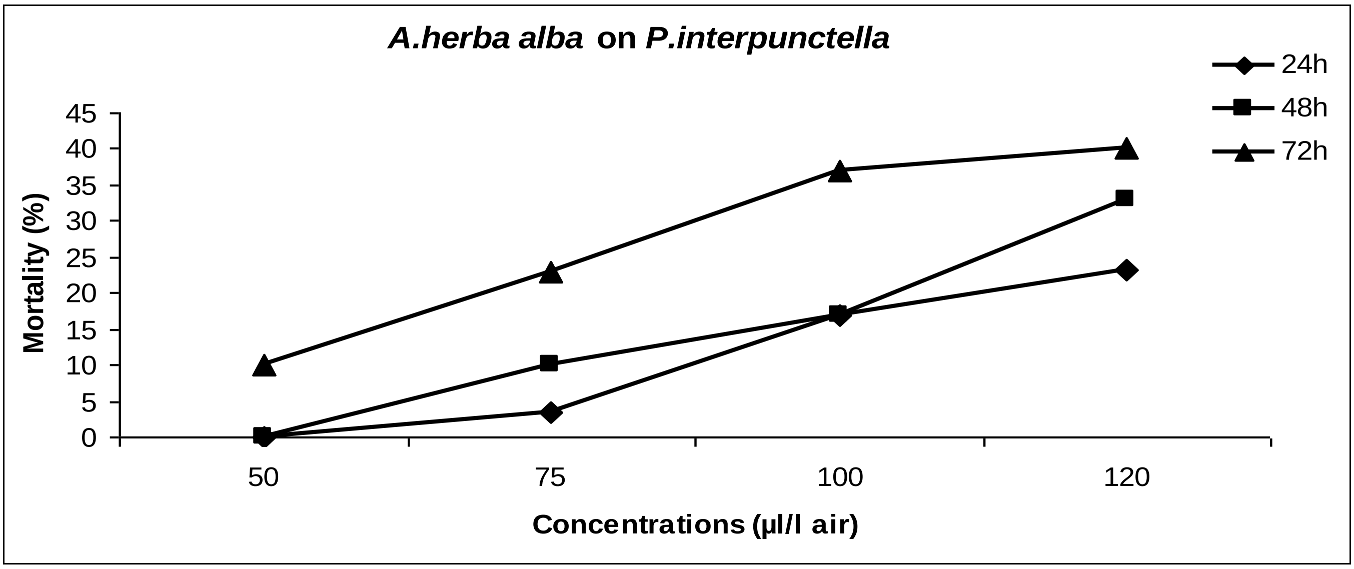

Fig 1: Percent mortality of *Plodia interpunctella* larvae exposed to *Artemisia herba alba* essential oil at different concentrations and times

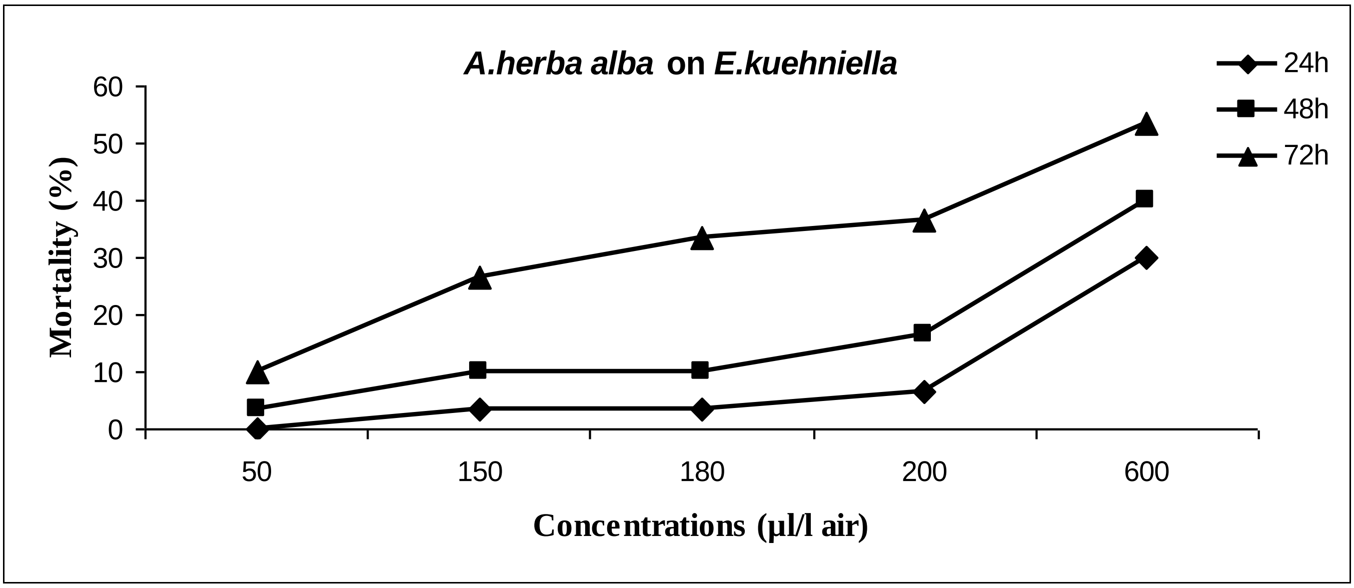

Fig 2: Percent mortality of *Ephestia kuehniella* larvae exposed to *Artemisia herba alba* essential oil at different concentrations and times

LC_{50} values of the essential oil of *A. herba alba* against *P. interpunctella* larvae were 162.1, 144.9 and 141.1 µl/l air; whereas, LC_{50} values of this oil on the larvae of *E. kuehniella* were recorded at 957.3, 826.9 and 463.2 µl/l air after 24, 48 and 72 hours of exposure, respectively (Table 2).

Table 2: LC_{50} values of *Artemisia herba alba* essential oil against *Plodia interpunctella* and *Ephestia kuehniella* after 24, 48 and 72 h of exposure

insects	times	LC 50(µl/l air) 95% confidence limits	P value	R^2	DF	Slope±SE
P. interpunctella	24h	162.1 (130.9-487.1)	0.429	0.96	2	3.77±0.93
	48h	144.9 (124.6-251.1)	0.429	0.97	2	3.92±0.74
	72h	141.1 (116-260.1)	0.429	0.97	2	1.95±0.29
E. kuehniella	24h	957.3 (836.8.7-1148)	0.003	0.99	3	1.82±0.12
	48h	826.9 (651.9-1228)	0.003	0.98	3	1.27±0.11
	72h	463.2 (333.6-825.5)	0.003	0.96	3	0.83±0.10

DF: Degree of Freedom; SE: Standard Error.
P: Values with $p<0,05$ are significantly different

4. Discussion

Insects have developed considerable genetic diversity and great potential for adaptation to environmental changes; thereby, they differently respond to their environment. Poorly selected the specific insecticide to target insect pests can lead to an inefficiency of the insect control. Plant chemical components vary from plant to another and the insecticidal activity change from an Insect order to another and within the same family. In the present study, *P. interpunctella* and *E. kuehniella* differently responded to the insecticidal activity of the oil of *A. herba alba.* According to the results, the larvae of *P. interpunctella* are the most sensitive to the oil repellent activity. The repellency is the result that the insects couldn't support odours which considered as disagreeable due to the potentiality of phytocompounds present in the plant oil. Davis (1985) [30] proposed some potential mode of action of repellents: an attractive signal inhibition, a reverse perception of the attractive signal to an irritant message or activation of a receptor that could disrupt or lead to an inappropriate behavioural response. Furthermore, it was reported that an interference of essential oil with behavioural functions of insects [31], result in an alteration in the behaviour and memory in the cholinergic system [32]. Several studies of plant volatile and their constituents were shown to effectively disrupt the recognition process of the host substrate and influence the walking behavior of insects [33, 34]. Moreover, essential oils can compromise insect respiration by impairing muscle activity, leading to paralysis [35, 36]. In this study, the repellency decreases at high concentrations of the oil may be due to an alteration of the locomotion activity that the repellency behaviour was disrupted and the larvae responded to the toxic effect of the oil.

The mortality observed in relation to the toxic effect of the oil is due to volatile oil substances which could block the tracheal respiration of the insects leading to their death [37]. Several studies showed that most essential oils comprise of monoterpenes compounds bind to proteins receptors and act by inhibiting acetylcholinesterase enzyme (AChE) [38, 39], interfering with the neuromodulator octopamine [40] or GABA-gated chloride channels [41] interrupting neurotransmission leading to physiological imbalance and, thereafter, death of the target insect. Another proposed mode of action of essential oils is interfering with the metabolism of juvenile hormones and ecdysones [42].

According to LC_{50} values, *E. kuehniella* larvae are more resistant to the oil fumigant than *P. interpunctella* larvae as the doses required to kill the larvae of *E. kuehniella* were much higher than those required for *P. interpunctella* larvae. Studies carried out on respiration rate of *Tenebrio castaneum* adults determined that the insect has a lower rate of air exchange and consequently a smaller diffusion of oil toxic compounds into the insect [43, 44]. The differences found between the susceptibility of *P. interpunctella* and *E. kuehniella* toward the toxicity of *A. herba alba* oil could be explained by differences in the respiration rates of insects that a study should be undertaken. On the other hand, a possible explanation can be based on the differences in the length of the insects body observed in both larvae. Larvae of *P. interpuntella* are smaller in length than larvae of *E. kuheniella*, so it could be that in *P. interpunctella* the concentration of the volatile oil spread into all organism cells compared to that in *E. kuehniella.*

5. Conclusion

Over several factors, the insecticidal activity of essential oils depends on insect's physiology that each insect species differently respond to their environment.

The results of this study are in accordance with earlier studies. They confirm widely the use of essential oils in insect pests management programme in particular the use of *A. herba alba* against *P. interpunctella* and *E. kuheniella.*

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