RESEARCH ARTICLE

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Insecticidal activity of the essential oil from seven accessions of *Artemisia herba-alba asso* domesticated in Errachidia (south-east of Morocco) against *Tribolium castaneum*.

Moumni Mohammed*, Pr Elwatik Lahcen*, Kassimi Abderahmane*, Homrani Bakali Abdelmonaim**

*(Laboratory natural substances, synthesis and molecular dynamics, Department of Chemistry, Faculty of Science and Technology of Errachidia, University My Ismail, Errachidia, Morocco).

** (National Institute of Agronomic Research - NIAR Regional Centre of Errachidia)

ABSTRACT

The present work conducted to determine the insecticidal activity of essential oils from seven accessions of Wormwood (*Artemisia herba-alba*) against *Tribolium castaneum* (larva, nymph and adult). These accessions have been collected from different regions in Morocco (Midelt, Boumeriem, Taznakt, Missour, Zawiat Sidi Hamza, Boudnib and Idelsane) and domesticated in the experimental station of Errachidia (Southeast of Morocco).

The insecticidal activity was assessed by determining the LC_{50} and LC_{90} , after seven days of treatment by fumigation of the essential oils. We found that Artemiseole chemotype represented by the accession of Boudnib and Idelsane has the highest toxicity compared to other chemotypes,. The adult stage of *Tribolium castaneum* is much stronger compared to the larval and chrysalis stages (pupae).

Keywords - Essential oil, Artemisia herba-alba asso, Tribolium castaneum, chémotype, Insecticidal activity.

I. INTRODUCTION

Tribolium castaneum (Herbst) is a common insect pest belonging to the order Coleoptera. This is one of two most dangerous insect pests (with *Silvanide Oryzaephi Jus surinamensis*) of stored cereals and their products [1, 2]., this is a very serious pest of food processing installations, such as mills, processing plants, warehouses and retail stores [3].

Chemical pest control is still the most widely used methods to control insect pests of food. These methods are very effective, but harmful to the environment and human health. Indeed, in 1995 has recommended the elimination of classic fumigants in 2005 for the developed countries and 2015 for the developing ones [4], which opens the way to the research for natural alternatives to chemical insecticides. Several research studies have demonstrated the effectiveness of aromatic plants and essential oils against the pest insects [5;6].

In this context, the aim of this study is to compare and evaluate the insecticidal effect of the essential oil of the aerial part of seven accessions of white wormwood cultivated in Errachidia (South-east of Morocco) against *Tribolium castaneum*

II. MATERIALS AND METHODS

1. Plant material:

The visible parts of *A. herba-alba* used in this study have been cultivated by the transplantation of wild individuals in the experimental station of Errachidia (Morocco).These individuals were collected from seven regions of Morocco: Midelt, Boumeriem, Taznakt, Missour, Zawiat Sidi Hamza, Boudnib and Idelsane (Ouarzazate). The experiment was conducted on twenty individuals (plants) for each accession.

2. Extraction and analysis of essential oil:

The dried aerial part (stems, leaves and flowers) of *Artemisia herba-alba* were collected in June 2010. The extraction of essential oils was performed by hydrodistillation in a modified Clevenger-type apparatus for 4h [7] in the laboratory of natural substances synthesis and molecular dynamics of the Faculty of Science and Technology of Errachidia. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4-5 °C prior to analysis. The major compound identified in each essential oil accession is indicated in the following table [8]

Accession	major compound	
Boudnib	Artemiseole (70,41%)	
Idelsane	Artemiseole (54.41%)	
Taznakt	α-Campholène aldéhyde (48,20%)	
Zaouiat sidi hamza	α-Campholene aldehyde (46,77%)	
Missour	α-Campholene aldehyde (41,09%)	
Boumeriem	α-Campholene aldehyde (32,77%)	
Midelt	Davanone (37,71%)	

 Table 1: Major compound of the essential oils (OE)

 tested [8]

3. Materiel animal:

The individuals of *Tribolium castaneum* are taken from a stock of *wheat* already infested by the pest. To use the progeny (larvae, nymphs and adults) in our study. The *Tribolium castaneum* adults were reared in sterilized glass Petri dishes (150mm) containing damaged wheat grains (slightly comminuted) and incubated in an oven under controlled conditions promoting their growth and accelerating their life cycle (at a temperature of $33 \pm 2 \degree C$)

4. Study of the effect of the insecticide of the essential oil vapor

This This test was performed on various stages (adult, larval and pupal) of the insect growth. Test solutions were prepared by dissolving 5; 10; 15; 20 et 30 μ l of *A. herbe-alba* essential oil in 1 ml acetone (corresponds respectively to the doses 14; 28; 57; 113 et 170 μ l/l). Whatman filter papers no. 1 are soaked with 1 ml by micropipette of each dose. After the evaporation of the acetone (15 min in air at room temperature), the paper disks are attached to the lids of Petri dishes. Then the dishes were then covered after the introduction of 20 insects (adult, larva or nymph) and 30 grams of wheat seeds damaged (slightly comminuted). Three replications were used for each dose. Filter paper was treated with 1ml of acetone alone as a control.

Observations on the number of insects present on both the treated and untreated dishes were recorded every 24 hours until the stabilization of mortality in the population. The corrected mortality was calculated using Abbott's formula [9].

Abbott's formula:	$MC = \frac{NIM - NIMT}{100 - NIMT} \times 100$
, and the second s	$\mathrm{MC} = \frac{100 - \mathrm{NIMT}}{100 - \mathrm{NIMT}} \times 100$

Mc: adjusted mortality rateMo: mortality rate in the treated dishesMt: mortality in the controlled dishes

1. Data Analysis

To compare the results, One-way analysis of variance (ANOVA) was performed using Excel 2007 software at the 0.05 percent level. Median lethal concentrations (LC_{50} , dose that kill 50% of the exposed insects) and the lethal concentrations (LC_{90} , dose that kill 90% of the exposed insects) were determined by the achievement of trendlines and obtaining of the regression equations. Mortalities were corrected by Abbott's formula [9].

III. RESULTS AND DISCUSSION

From the regression equations trendlines above, we got the LC50 and LC90 (table 2) of adults *Tribolium castaneum* induced by inhalation of EO of different accessions.

	Table 2: Classification of the different EO toxicity
$(LC_{50} \text{ and } LC_{90})$ tested on the adult stage of <i>T</i> .	$(LC_{50} \text{ and } LC_{90})$ tested on the adult stage of <i>T</i> .

<i>castaneum</i> by inhalation			
Accession	Regression equation	LC ₅₀ (µl/l)	LC ₉₀ (µl/l)
Boudnib	y = 0,008x + 0,154 (R ² = 0,838)	43,3	93,3
Idelsane	y = 0,009x - 0,020 ($R^2 = 0,938$)	57,8	102,2
Zaouiat sidi hamza	y = 0,005x - 0,007 (R ² = 0,991)	101,4	181,4
Missour	y = 0,004x - 0,049 (R ² = 0,976)	137, 3	237,2
Midelt	y = 0,005x - 0,106 (R ² = 0,967)	121,2	201,2
Boumeriem	y = 0,004x - 0,109 (R ² = 0,951)	152,3	252,3
Taznakt	y = 0,003x - 0,102 (R ² = 0,863)	200,7	334

 \mathbf{R}^2 : coefficient of determination

From the previous results, we decided that the assessment of the larvicidal effect should include the

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most toxic OE obtained from the accessions of: Idelsane, Boudnib and Zaouiat Sidi Hamza

Table 3: Classification of the different EO toxicity
$(LC_{50} \text{ and } LC_{90})$ tested on larval stage of T.
castaneum by inhalation

Accession	Regression equation	LC ₅₀	LC ₉₀
		(µl/l)	(µl/l)
Boudnib	y = 0,008x + 0,087 (R ² = 0,951)	26,94	50,47
Idelsane	y = 0.013x + 0.007 (R ² = 0.954)	37,92	68,69
Zaouiat sidi hamza	y = 0,012x - 0,026 (R ² = 0,978)	43,83	77,17

 \mathbf{R}^2 : coefficient of determination

The pupation lasts an average of 4.5 days, is an intermediate stage or metamorphic stage between the larva and the adult. During this growth phase (pupation), the insects are inactive (not able to move about) and without a cocoon. It is therefore very interesting to evaluate the toxicity of our EO on nymphs of this insect. For this we decided to test the effect of EO's accession basically of Boudnib and Idelsane which they demonstrate to have the highest effect of toxicity on adults and larva of *Tribolium castaneum*. After seven days of incubation the results obtained are summarized below.

Table 4: LC_{50} and LC_{90} of EO of two accessions tested by inhalation on nymphs of *T. castaneum*

Accession	Regression equation	CL ₅₀ (µl/l)	CL ₉₀ (µl/l)
Boudnib	y = 0.015x + 0.068 (R ² = 0.852)	28,8	55,5
Idelsane	y = 0,014x + 0,025 (R ² = 0,922)	33,9	62,5

R²: coefficient of determination

In general, the results of this study showed that the toxicity of the EO of white wormwood cultivated in Errachidia depends on the provenance (or chemotype), dose, of exposure to the essential oil and the growth stage of the insect studied (adult, larva and pupa). Indeed, according to this study, the highest toxicity is recorded by OE obtained from Boudnib accession followed by that of Idelsane and Zaouiat Sidi Hamza ecotype. It appears very clear that the Artemiseole chemotype represented by the accession of Boudnib and Idelsane presented the strongest toxicity compared to other chemotypes. We also noted that toxicity increases with the concentration of Artemiseole in the OE (which may explain the relatively high toxicity of the OE obtained from Boudnib accession compared to Idelsane accession).

However, the toxicity of the other two chemotypes (alpha-Campholene aldehyde and Davanon) does not follow the major compound concentration. It is therefore more likely that the toxicity of these oils is due to another compound or a synergy of several compounds.

Indeed, Seri-kouassi *et al.*, [10] showed that the toxicity of essential oils on insects is induced by the action of their major compounds, this is also confirmed by the work Ngamo & Hance [11] The insecticidal properties of the monoterpenes (all oxygenated monoterpenes) as 1,8-cineole, linalool, eugenol, cymene, carvacrol, safrole, thymol and terpineol have been demonstrated by several researchers [5;11;12;13;] This confirm our results obtained for the EO of Boudnib and Idelsane accessions very rich in Artemiseole

However, other researchers like Asawalam *et al* [14] showed that the combined toxic actions of the major components are more remarkable than individual action of these components.

From the results that we found in this study, it is also noted that adults are much more resistant compared to larvae and pupae, in fact, according Bostanian *et al* [15], essential oils have an effect directly on the cuticle of insects and mites, softbodied, but they are less effective against insects with hard shell such as adult of beetles. This may explain the resistance of adults and the fragility of larvae and pupae of the beetle covered in our study.

IV. CONCLUSIONS

The toxicity of the EO accession of white wormwood cultivated at Errachidia station depends on the accession (or chemotype), dose, time of exposure to the essential oil and the growth stages of the insect studied (adult, larva and pupa). The accession of Boudnib present the highest toxicity compared to other accessions. The larvae and pupae of *tribolium castaneum* are more sensitive to fumigation by the essential oil tested.

Despite the results encouraging, the effectiveness of different essential oils remains to be demonstrated in real situations (in warehouses, for example).

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