



# Activity Evaluation of *Thymus Munbyanus* Essential Oil on Reproduction and Transgenerational Proprieties of the Pest Store *Ephestia Kuehniella* Zeller.

Bendjedid Hadjira\*, Yezli Amina, Yezli-Touiker Samira, Boudjelida Hamid

Laboratory of Excellence Applied Animal Biology, biology department, University Badji- Mokhtar, Annaba, Algeria

(Received: 11 June 2024

Revised: 16 July 2024

Accepted: 10 August 2024)

## KEYWORDS

*Ephestia kuehniella*,  
*Thymus munbyanus*,  
essential oil,  
vitellogenins  
and vitellins,  
progeny,  
Reproduction.

## ABSTRACT:

**Introduction:** The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the most important pests of stored products.

**Objectives:** The reprotoxicity and the transgenerational property of *Thymus munbyanus* subsp. *coloratus* essential oil (EO) were evaluated against *E. kuehniella*.

**Methods:** The EO was applied in vivo, by topical application with its inhibition doses (ID) on newly molted pupae in day 0 of the pupae stage (ID25: 15.38 and ID50: 25.22  $\mu$ l/ml of acetone). In a follow-up experiment, the adults that survived from treated pupae were investigated for different reproductive event and ovarian parameters for the two successive generations.

**Results:** The EO effects showed a significant decrease in the vitellogenins and the ovarian vitellin amounts, for ID25 and ID50, compared to control series. Moreover, the treatment reduced the duration of pre-oviposition and the oviposition periods, in addition, a lower fecundity and egg hatchability were noted for each generation. Furthermore, the EO reduced the number of basal oocytes per paired ovaries and the fresh weight of ovaries for the two generations, but also a reduction of the progeny was recorded for ID25 and ID50.

**Conclusions:** Thus, this study has highlighted the reprotoxicity and persistence effects of *T. munbyanus* essential oil throughout the interference with endocrine functions of *E. kuehniella*.

## 1. INTRODUCTION

Insects are the more diverse group of animals on Earth, and only 0.5% are considered pests, they destroy every year one fifth of the world's crop production [1]. Indeed, Insects in stored can contaminate foods, bran and grains directly via their surfaces and fragments of old cuticle parts and also indirectly by saprophytic microorganisms and all of these factors can affect quality products [2]. The need, for the prevention and control of insect pests, is a crucial issue facing crop protection. To date, the most common strategy for controlling these pests has depended on the use of conventional pesticides, most of them are synthetic pesticides [3].

Natural chemicals are receiving more attention since the synthetic pesticides provide undesirable effects to human health, environment and resistance development of new pest genera [4]. Biopesticides, such as the use of plant essential oils (EOs) may be a good alternative, for

insect pest control, since they are biodegradable and show no effects on non target organisms and environment [5]. Hydrophobic liquids with volatile compounds derived from plants are called essential or volatile oils. At room temperature, they have an oily look, and the strength of their scent varies depending on the volatile contain components. There are many uses, for the essential oils (EOs), in the culinary, cosmetic and pharmaceutical industries because they are non-toxic to vertebrates when present in low amounts [6]. Thus, as it was indicated the essential oils are becoming acknowledged as a viable substitute for chemical pesticides in the management of insect pests, at least in small-scale farming systems [7]. The nature of these chemicals, which include their quick degradation, poor residual capacity and different mechanisms of action, are partly responsible for these attitude [8]. Comparing synthetic insecticides to plant-derived compounds or extracts, the former often exhibits lesser acute toxicity



toward insects. However, its subacute toxicity was often seen and is significant because it can prevent crop loss due to its repellent, suppressant or deterrent effect and limit insect spreading, due the effects on reproduction (lowered fertility, fecundity) and longevity reduction. These effects, which are commonly referred to as anti feedings, cause to insects a lose in weight and body size, become less fertile and exhibit behavioural changes [9]. With their insecticidal qualities, EOs are produced by a number of plant families, including the Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae. These indicate their industrial values against a range of harmful insects, including Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera [10]. We can identify the genus *Thymus* as one of the Lamiaceae family genera with the greatest biological interest that grows in the Mediterranean region [11]. Among them, *Thymus munbyanus* species which exhibits a variety of biological properties, including insecticidal [12], antioxidant [13] and anti-microbial effects [14]. The primary cause of insect's expansion continues to be their reproduction capacity. Therefore, any rational strategy to reduce drastically the harm caused by insects must include an examination of reproduction [15]. According to previous work [16], the individual's reproductive success is determined by their capacity to generate viable progeny, or offspring, who are able to reproduce themselves. Additionally, a popular fitness indicator is the quantity of eggs laid, which indicates the fertility of the female [17]. It is true that oviposition, or spawning, is a crucial part of a female's life and that fertility is impacted by all factors that influence oviposition [15]. Hence, the use of essential oils to suppress oviposition becomes a pertinent standard for managing pest infestations and managing stored products [18].

One of the most significant pests of stored products is the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), which mostly damages cereals and other products that look like flour [19]. Despite being native to the Mediterranean basin, this pest is present throughout the world [20]. Because it can seriously harm both flour and whole grains, it is regarded as an economically significant pest [1]. For both scientific and commercial purposes, *E. kuehniella* is one of the most popular laboratory hosts for entomophagous insect because of its short lifespan, high

fecundity rate (200–300 eggs per female) and ability to develop and reproduce on an easy-to-make factitious diet [21].

## 2. OBJECTIVES

This present work aimed to assess the reproduction perturbation, as well to determinate the persistence or transgenerational proprieties of the *T. munbyanus* essential oil (EO) from Algeria. In order to develop additional information on its mode of action, the amount of vitellogenins and vitellins were measured on both generations G0 also G1. The duration periods of pre-oviposition and oviposition, fecundity, hatching rate, ovary weight, number of basal oocytes were determined on the 2 successive generations after the treatment on newly molted pupae in G0 and number of descendants from parents that survived to the treatment of the EO in G0 were evaluated (progeny assay).

## 3. METHODS

### Insects rearing

*Ephestia kuehniella* (Zeller, 1879) is a Lepidoptera of the Pyralidae family. This insect is a frequent host of mills and silos it also can be found in many stored foods such as flour, seeds, cereal grains (wheat, corn rice), semolina, oatmeal, muesli and pasta [22]. This flour moth was bred and maintained in rearing under the laboratory-controlled condition (temperature of 27 °C, relative humidity of 80%) to assure their development [23].

### Plant collection

The plant material consists of aerial parts samples subspecies of Algerian *T. munbyanus subsp. coloratus* (Boiss & Reut) Greuter & Burdet. The harvest was carried out in June 2022 when the plant was flowering stage, from the Chetaibi region (Northeast Algeria, Annaba district; 36°56'47.4"N 7°23'43.9"E). The essential oil (EO) of this plant was extracted by hydrodistillation and since the chemical composition of the essential oil was known and described in a previous study [12].

### Quantification of vitellogenins and vitellins

*T. munbyanus* EO was administrated by topical application at different doses; ID<sub>25</sub> (15.38 µl/ml) and ID<sub>50</sub> (25.22 µl/ml) already determined in a previous study [12], on newly molted female pupae (<8h old) of *E. kuehniella*. Th biochemical analyzes of the



vitellogenins and the fatty substances were carried out on the female pupae, at different ages (0, 1, 3, 5 and 7 days). For the yolk analysis, the ovaries of newly emerged female adults (0 days old) from the control and treated series for the generation 0 (G0) and generation 1 (G1) of *E. kuehniella*, also were dissected and then removed. The biological samples are weighed and then stored in a Tris-HCL-NaCl buffer (pH 7.4). The extraction of vitellogenins and vitellins is carried out according to Postlethwait [24] and Fabre [25]. Then the amount of vitellin and vitellogenins were made to the protocol of Bradford. [26].

### Reproductive parameters

Newly emerged adult male and female from pupae of *E. kuehniella*, controls and treated separately at the two doses (ID<sub>25</sub> and ID<sub>50</sub>) with *T. munbyanus subsp coloratus* EO, are mated. The different series of pairs of G0 and G1 are maintained under controlled conditions and regular monitoring has made it possible to determine the following different parameters, duration of the preovipository period, duration of ovipository period or duration (in days) of laying, female fertility, egg viability or hatch percentage and the fresh weight of ovaries. The number of basal oocytes per pair of ovaries.

### Effects on the number of descendants from surviving adults (Progeny assays)

The newly exuviated adults, males and females, from the control and treated series whose individuals survived the treatment of the pupae (DI<sub>25</sub> and DI<sub>50</sub>) were placed in petri dishes containing a nutrient (flour). Six repetitions of different types of pairs are performed (see below) and after 48 hours the adults are removed from the petri dishes. The monitoring of generation 1 or G1 is then carried out from the egg stage until the exuviation of adults; the delayed effect of the EO of *T. munbyanus subsp coloratus* will be assessed, by counting the eggs, last instar larvae (L5), pupae and adults from each series of pairs. The different couples carried out are as follows:

- Pairs 1: MT x FT: Control Males x Control Females  
 Couples 2: MDI<sub>25</sub> x FID<sub>25</sub>: Males DI<sub>25</sub> x Females ID<sub>25</sub>  
 Couples 3: MDI<sub>50</sub> x FID<sub>50</sub>: Males DI<sub>50</sub> x Females ID<sub>50</sub>

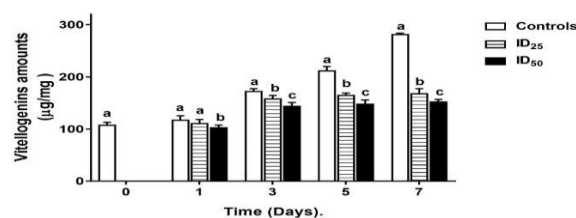
### Data analysis

Statistical analyses were performed using Prism version 7 for Windows (GraphPad software, La Jolla, CA,

USA, www Graphpad.com) also data are presented as the mean  $\pm$  SD. Repetitions and numbers of individuals were also cited. One way and two ways analyzes of variance (ANOVA at  $P \leq 0.05$ ) followed by a post-hoc honestly significant difference (HSD) Tukey's test was used to compare between the different series.

## 4. RESULTS

The treatment with the *Thymus munbyanus* EO was used *in vivo*, topically on newly molted pupae in the day of pupal exuviation of *E. kuehniella* with the two inhibition doses (ID<sub>25</sub>: 15.38 and ID<sub>50</sub>: 25.22  $\mu\text{l} / \text{ml}$ ). Then, the vitellogenesis content was evaluated in females in fatty bodies, at different times during the pupal stage (0, 1, 3, 5 and 7 days after exuviation). For the control pupae, the mean values of the vitellogenin (yolk-genin) content of the fatty body at 0 and 1 days are similar ( $p > 0.99$ ), then it increased significantly at 3 days ( $172.41 \pm 15.17 \mu\text{g} / \text{mg}$ ;  $p = 0.0002$ ) and 5 days ( $211.41 \pm 38.53 \mu\text{g} / \text{mg}$ ;  $p = 0.209$ ). Vitellogenins content show a maximum value at day 7 which was around  $280.75 \pm 23.22 \mu\text{g} / \text{mg}$  of fat (Fig. 1). The comparison between the control and treated series indicates that the vitellogenin content decreases on days 3, 5 and 7 with the two tested doses ( $p < 0.001$ ); on day 5, a dose-response effect is noted ( $p = 0.0040$ ). The statistical results, relating to the analysis of variance with two classifications, revealed effects in the treatment ( $F(2, 67) = 34.39$ ;  $p < 0.001$ ), age ( $F(4, 67) = 59.26$ ;  $p < 0.001$ ) and the treatment-age interaction are very highly significant ( $F(8, 67) = 5.051$ ;  $p < 0.001$ ).

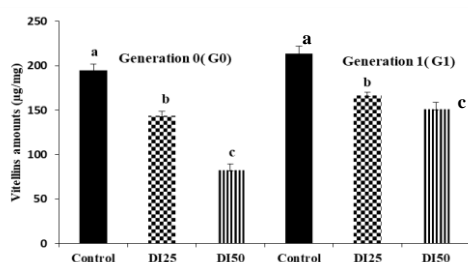


**Figure 1:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephesia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on the content of vitellogenins ( $\mu\text{g}/\text{mg}$  of fatty body) at the pupal stage, over time (days) ( $m \pm \text{SD}$ ,  $n = 12$ ). Different lowercase letters above the same exposure time indicate a significant difference ( $p < 0.05$ ).

Vitellins amounts (ovarian yolk), in females of *E. kuehniella*, at emergence, are of the order of  $194.5 \pm$



7.03 in the control series. The EO treatment induces a reduction in the content of vitellins at the two doses tested for (G0). The mean values recorded are of the order of  $143.4 \pm 5.51$  and  $82.38 \pm 7.05$   $\mu\text{g}/\text{mg}$  respectively for the  $\text{DI}_{25}$  and  $\text{DI}_{50}$ . Vitellins amounts in *E. kuehniella* females were estimated to be  $213.84 \pm 8.38$  in control series for (G1), the EO treatment induces a reduction in the content at the two doses tested for (G1), the mean values recorded are of the order of  $166.44 \pm 4.01$  and  $150.74 \pm 8.26$   $\mu\text{g}/\text{mg}$  respectively for the  $\text{DI}_{25}$  and  $\text{DI}_{50}$  (Fig. 2). This EO induced a significant reduction in the Vitellins content in adult females treated with the 2 inhibition doses for both G0 and G1. The adult females of G1 show an increase in the values of ovarian Vitellins compared to the adult females treated with G0, a return to physiological norms seems to appear. Analysis of variance using ANOVA 2 reveals a generation effect ( $F(1, 30) = 171.9$ ) a treatment effect ( $F(2, 30) = 622.4$ ) and a generation-treatment interaction effect ( $F(2, 30) = 85.52$ ). Therefore, the EO of *T. munbyanus* affects the vitellogenesis process over two successive generations of *E. kuehniella* following treatment.



**Figure 2:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly

molted pupae of *Ephestia kuehniella* ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ) on the vitellin content ( $\mu\text{g}/\text{mg}$ ) at the adult stage of generation 0 (G0) and generation 1 (G1) ( $m \pm \text{SD}$ ,  $n = 12$ ). Different lowercase letters above the same series indicate a significant difference ( $p < 0.05$ ).

Regular monitoring, of couples treated with *Thymus munbyanus* EO ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ) topically on the newly molted pupae of *Ephestia kuehniella*, allows to determine the effects *in vivo*, on the reproductive potential of adult females of G0 and G1. In particular the duration of the preoviposition and oviposition periods, the number of eggs laid (fecundity) plus percentage of egg hatching (viability), the fresh weight of ovaries and the number of basal oocytes per pair of ovaries. The EO treatment in generation 0 ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ), induced a highly significant decrease ( $P < 0.0001$ ) over the preovipositionary period and it varies with a dose-response relationship ( $p < 0.0001$ ) (Table 1). A highly significant decrease ( $P < 0.0001$ ) in the duration of oviposition was also recorded at the two doses tested, with a dose-response relationship ( $P = 0.0133$ ). The EO treatment ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ) the adult females in generation 1 induced a significant decrease ( $P < 0.0001$ ) only with the  $\text{ID}_{50}$ , tested over the preovipositionary period. A significant decrease ( $P < 0.0001$ ) in the duration of oviposition is also recorded only at the  $\text{ID}_{50}$  tested and this with a dose-response effect ( $P = 0.0133$ ).

Comparison of the effects of *T. munbyanus subsp. Coloratus* EO, on the reproductive potential during the adult stage of *E. kuehniella* for the two generations G0 and G1, show a significant difference on the duration of the preovipositionary and ovipositionary periods of G0 and G1 between the treated series.

**Table 1:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephestia kuehniella* ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ) on the duration (days) of the preoviposition and oviposition periods (days) for G0 and G1 ( $m \pm \text{SD}$ ,  $n = 6$ ). Different lowercase letters above the same parameter indicate a significant difference ( $p < 0.05$ ).

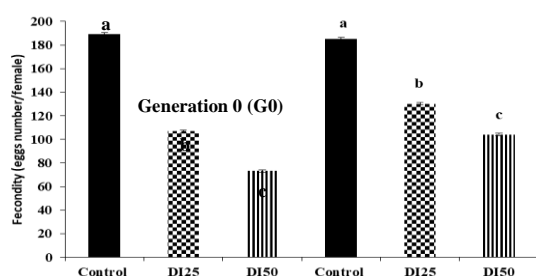
Treatment	G0	G1	G0	G1
	Preoviposition	Preoviposition	Oviposition	Oviposition
Control	$1.34 \pm 0.005\text{a}$	$1.34 \pm 0.05\text{a}$	$3.54 \pm 0.1\text{a}$	$3.54 \pm 0.019\text{a}$
DI <sub>25</sub>	$0.68 \pm 0.012\text{b}$	$1.1 \pm 0.02\text{a}$	$3.21 \pm 0.041\text{b}$	$3.4 \pm 0.04\text{a}$
DI <sub>50</sub>	$0.37 \pm 0.004\text{c}$	$0.77 \pm 0.04\text{b}$	$2.52 \pm 0.02\text{c}$	$2.77 \pm 0.20\text{a}$

This EO induces a decrease in the length of the preovipositionary period in adult females of *E. kuehniella*

for the two tested doses ( $\text{ID}_{25}$  and  $\text{ID}_{50}$ ) ( $P < 0.0001$ ). This reduction was also recorded in the next generation



in adults of *E. kuehniella* ( $P < 0.0001$ ). Statistical analysis shows that the reduction in the duration of the preovipository period induced by HE is significantly higher at G0 compared to G1. Analysis of variance indicates high significant effects for generation ( $F(1, 20) = 1753$ ), treatment ( $F(1, 20) = 1068$ ), moreover no significant difference is noted for the generation-treatment interaction ( $F(1, 20) = 1.043$ ). The EO induces a decrease in the duration of the ovipository period in adults in *E. kuehniella* for the two doses tested (ID<sub>25</sub> and ID<sub>50</sub>) ( $P < 0.0001$ ). This reduction is also found in the next generation in adults of *E. kuehniella* ( $P < 0.0001$ ). Based on statistical study, the reduction caused by EO is higher at G0 than it is at G1. Analysis of variance indicates highly significant effects for generation ( $F(1, 20) = 5.748$ ), treatment ( $F(1, 20) = 51.73$ ), furthermore no significant difference is noted for the interaction generation-treatment ( $F(1, 20) = 0.1069$ ). Therefore, EO affects the length of the ovipository period in two successive generations of *E. kuehniella*. Furthermore, the tukey HSD test revealed that for the tested doses (ID<sub>25</sub> and ID<sub>50</sub>) of essential oil *T. munbyanus* exhibited persistent effects on the fertility of adult females. However, statistical analysis shows that the reduction in eggs laid is greater at G0 compared to G1. This may be in favour of a restoration of physiological normality. Analysis of variance indicates highly significant effects for generation ( $F(1, 30) = 129$ ), treatment ( $F(2, 30) = 1596$ ) and the generation-treatment interaction ( $F(2, 30) = 52.54$ ). EO therefore affects the number of eggs laid per female in two successive generations of *E. kuehniella*.

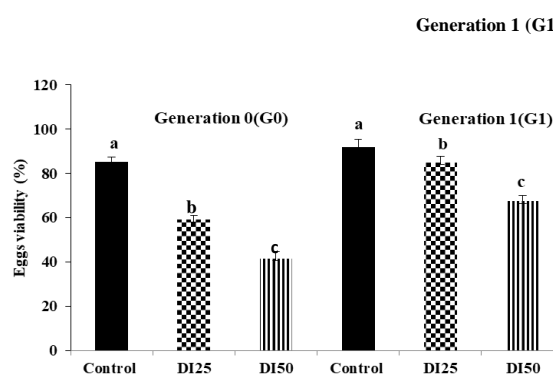


**Figure 3:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephesia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on fecundity (number of eggs) of females for G0 and G1 ( $m \pm SD$ ,  $n = 6$ ). Different lowercase letters above the same series indicate a significant difference ( $p < 0.05$ ).

Concerning the egg viability, the obtained results indicate a highly significant reduction ( $p = 0.0024$ ) in the number of eggs hatched by females following exposure to the EO of *Thymus munbyanus* (ID<sub>25</sub> and ID<sub>50</sub>) for G0. The mean value, of the eggs hatched in the control, is  $85.25 \pm 2.36$ , in the treated  $59 \pm 2.16$  and  $41.5 \pm 3.10$  for the ID<sub>25</sub> and ID<sub>50</sub> respectively (Fig. 3). The results obtained for G1, indicate a highly significant reduction ( $p = 0.0024$ ) in the number of eggs hatched by females following exposure to the EO of *T. munbyanus* (ID<sub>25</sub> and ID<sub>50</sub>). The mean value of the eggs hatched in the controls is  $92 \pm 2.60$ , in the treated  $85 \pm 3.55$  and  $67.5 \pm 2.38$  for the ID<sub>25</sub> and ID<sub>50</sub> respectively (Fig. 3).

The essential oil of *T. munbyanus subsp. Coloratus* induces in G0 a decrease in the viability of eggs laid in *E. kuehniella* for the two doses tested (ID<sub>25</sub> and ID<sub>50</sub>) ( $P < 0.0001$ ;  $P < 0.0001$ ). This reduction is also found in the next generation in adults of *E. kuehniella* ( $P < 0.0001$ ). This may be in favor of restoring physiological normality. Analysis of variance indicates highly significant effects for generation ( $F(1, 30) = 461.5$ ), treatment ( $F(2, 30) = 467.2$ ) and the generation-treatment interaction ( $F(2, 30) = 49.55$ ). EO therefore affects the viability of eggs laid by females in two successive generations of *E. kuehniella*.

The treatment with EO of *Thymus munbyanus* (ID<sub>25</sub> and ID<sub>50</sub>), induced a highly significant decrease ( $P < 0.0001$ ) in the number of basal oocytes for the treated by comparison to the controls for G0. In fact, the mean values recorded are  $131.16 \pm 3.13$  for the ID<sub>25</sub> and  $92 \pm 1.58$  for the ID<sub>50</sub> against values of the controls  $184.8 \pm 2.38$  (Fig. 4).

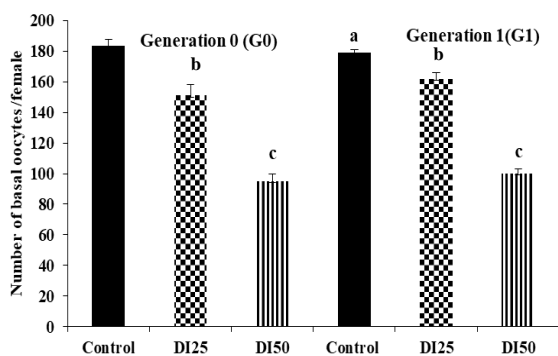


**Figure 4:** Effect *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of



*Ephestia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on egg viability for G0 and G1 ( $m \pm SD$ ,  $n = 6$ ). Different lowercase letters above the same series indicate a significant difference ( $p < 0.05$ ).

The treatment with EO of *Thymus munbyanus* (ID<sub>25</sub> and ID<sub>50</sub>), induced a highly significant decrease ( $P = 0.0015$ ) in the number of basal oocytes only for the ID<sub>50</sub> by contribution to the controls for G1. Indeed, the mean values recorded are  $151.5 \pm 2.64$  for the ID<sub>25</sub> and  $95.5 \pm 3.41$  for the ID<sub>50</sub> against values of the controls  $183.8 \pm 4.54$ . (Fig. 5). The *T. munbyanus* EO induced a decrease in the number of basal oocytes in the ovaries of adult female *E. kuehniella* treated with the two tested doses (ID<sub>25</sub> and ID<sub>50</sub>) ( $P < 0.0001$ ). This reduction is also found in the next generation in adults of *E. kuehniella* ( $P < 0.0001$ ). Analysis of variance indicates highly significant effects for generation ( $F(1, 30) = 54.75$ ), treatment ( $F(2, 30) = 2584$ ). Therefore, no significant difference is noted for the interaction generation-treatment ( $F(2, 30) = 39.84$ ). Also, the EO affects the number of basal oocytes in the ovaries of adult females of the two successive generations of *E. kuehniella*.

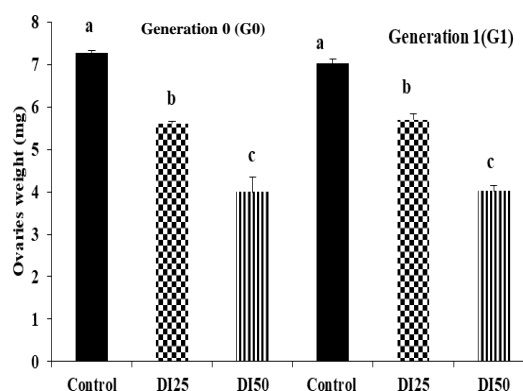


**Figure 5:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephestia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on basal oocyte count for G0 and G1. ( $m \pm SD$ ,  $n = 6$ ). Different lowercase letters above the same series indicate a significant difference ( $p < 0.05$ ).

The effects of *Thymus munbyanus* EO administered *in vivo*, by topical application, on newly molted pupae in the day of pupal exuviation, 0 day (<8h), were evaluated on the morphometry of the ovaries (fresh weight and number of basal oocytes) in adult female *E. kuehniella* 1 day after emergence of G0 and G1.

The results obtained show that the EO causes a very significant reduction ( $p = 0.0002$ ) in the fresh weight of the ovaries measured at the emergence of adults (<8h), since it varies from  $7.28 \pm 0.06$  mg in the controls to  $5.61 \pm 0.05$  and  $4 \pm 0.02$  mg in ID<sub>25</sub> and ID<sub>50</sub> treated respectively for G0 (Fig. 6). The results obtained show that the EO causes a significant reduction ( $p < 0.0001$ ) in the fresh weight of the ovaries only for the ID<sub>50</sub> measured at adult emergence (<8h). It goes from  $7.04 \pm 0.34$  mg in the controls to  $5.7 \pm 0.15$  and  $4.05 \pm 0.12$  mg for ID<sub>25</sub> and ID<sub>50</sub> respectively for G1 (Fig. 6).

The *T. munbyanus* EO induces a decrease in fresh ovarian weight in G0 in adult female of *E. kuehniella* with the two doses tested (ID<sub>25</sub> and ID<sub>50</sub>) ( $P < 0.0001$ ). This reduction is also found in the next generation in adults of *E. kuehniella* ( $P < 0.0001$ ). Statistical analysis shows that the reduction in ovarian weight in adult *E. kuehniella* is greater at G0 compared to G1. Analysis of variance indicates highly significant effects for generation ( $F(1, 30) = 1.347$ ), treatment ( $F(2, 30) = 3973$ ) and the generation-treatment interaction ( $F(2, 30) = 13.1$ ). Therefore, the EO affects the fresh weight of the ovaries in adult females of the two successive generations of *E. kuehniella*.



**Figure 6:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephestia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on the fresh weight (mg) of the ovaries measured at emergence of adult females of the G0 and G1 ( $m \pm SD$ ,  $n = 6$ ). Different lowercase letters above the same series indicate a significant difference ( $p < 0.05$ ).

The pupae of *E. kuehniella* were processed on the day of pupal exuviation and then the surviving adults were removed on the day of emergence; males and females were then mated in control individuals and treated with



the used doses. After 48 hours of mating, the various pairs formed are removed and generation 1 or G1 is monitored daily. The delayed effect of *T. munbyanus* EO could be assessed by counting the number of eggs, larvae (L5), pupae and adults exuviated for each series of experiments. In the control series, the number of eggs laid is  $187.66 \pm 7.09$ . This number decreases and reaches respective values of  $104 \pm 7$ ;  $72.33 \pm 4.50$  for the couples M ID<sub>25</sub> x F ID<sub>25</sub>; M ID<sub>50</sub> x F ID<sub>50</sub>. In the control series the number of larvae (L5), pupae and adults, are in the order of  $79 \pm 4$ ;  $72 \pm 5$  and  $69 \pm 3.33$  respectively. This number decreases and reaches respective values of  $51 \pm 3$ ;  $39 \pm 3$  and  $36 \pm 4$  for the couples M ID<sub>25</sub> x F ID<sub>25</sub> and the respective values of  $30 \pm 3$ ;  $25 \pm 3$  and  $21 \pm 3$  for the M ID<sub>50</sub> x F ID<sub>50</sub> pairs. Statistical analysis of the data (Table 02), compared to controls, revealed a decrease ( $p < 0.05$ ) in the number of eggs, larvae, pupae and adults for the 2 series, from the surviving adults from the treated pupae. The comparison between the treated series for each stage of development reveals a significant dose effect between the different couples formed ( $p < 0.0001$ ).

**Table 02:** Effect of *Thymus munbyanus* EO administered *in vivo*, by topical application to newly molted pupae of *Ephesia kuehniella* (ID<sub>25</sub> and ID<sub>50</sub>) on the number of offspring (progeny G1) from adults who survived after treatment of *Ephesia kuehniella* pupae ( $m \pm SD$ ;  $n = 3$ ). Comparison of the means at different couples for the same stage of development. Means followed by the same letter are not significantly different ( $p > 0.05$ ).

Stage	Control	ID <sub>25</sub>	ID <sub>50</sub>
Eggs	187±7.09a	104±7 b	72.33±4.50c
Larvae	76.66±4.93a	66.66±5.50b	60.33±7.57b
pupae	54.33±3.05a	39±20 b	33±3.60 b
Adults	33.33±3.51a	24±3.60 a	19±2 b

## 5. DISCUSSION

The development and reproduction of insects are controlled by external factors (temperature, nutrition, photoperiod) but also internal factors such as hormones and neurohormones [27]. The two main hormones, coordinating in these physiological processes, are ecdysteroids and juvenile hormone (JH). In insects, the fatty body, analogous to adipose tissue and vertebrate

liver, governs most of the metabolic and synthetic processes [28], it is involved in reproduction by participating in vitellogenesis [29]. This process, controlled by 20 hydroxyecdysone (20E) and HJ which play a crucial role in the metamorphosis and reproduction of insects [30], is a pre-requisite for the production of eggs (oocytes) of insects and the embryonic development after oviposition. Through this process which requires considerable energy and which must constantly adapt to fluctuating nutritional parameters [31], the precursor of the vitelline protein, vitellogenin synthesized in the adipose body is transported by hemolymph and absorbed by maturing oocytes [30]. Indeed, vitellin (yolk cells) play an essential nutritional role during embryogenesis [7]. The obtained results, with *E. kuehniella* after treatment with essential oil of *T. munbyanus* at inhibitory doses (ID<sub>25</sub> and ID<sub>50</sub>), showed a reduction in the levels of vitellogenins in the pupal stage and of vitellins in the adult stage for G0 and G1 compared to the control ones.

these results are in agreement with those observed in *Podisus nigrispinus* [32] and in mulberry pyralid (*Glyphodes pyloalis*) following the ingestion of *Artemisia annua* EO which caused a reduction in vitellin granules in the oocytes of females emerging from treated larvae [33]. In addition, an inhibition of vitellogenesis has also been reported in *E. kuehniella* [21]. Thus, the reduction could be attributed to increased metabolic activity due to the consumption of body fat in the detoxification processes leading to the inhibition or reduction of vitellogenesis, the process which is under the control of JH on body fat [33]. Presumably, it would appear that EO can act secondarily as an endocrine disruptor affecting vitellogenesis and therefore reproduction via neurohormones and hormones which play an essential role in these processes [34].

The reproductive potential of insects remains the most important factor in their undeniable proliferation. The study of reproduction is therefore essential in any reasoned approach to significantly reduce the damage caused by insects [15]. The present results showed a decrease in the preovipository and ovipository periods, as well as fertility, viability, the fresh weight of ovaries and the number of basal oocytes in the 2 generations



following the treatment with *T. munbyanus* EO at the inhibitory doses (ID<sub>25</sub> and ID<sub>50</sub>). We also noted a reduction in the number of offspring (eggs, larvae, pupae and adults) (G1) from parents who survived treatment on pupae (G0) at the two inhibition doses (ID<sub>25</sub> and ID<sub>50</sub>). The reduction in fertility observed in *E. kuehniella* confirms an effect of ecdysteroid agonists on the process of vitellogenesis via the production of molting hormone. In agreement with our results similar works on *E. kuehniella*, in fact various essential oils (*Satureja thymbra*, *Origanum onites*, *Myrtus communis*, *Origanum majorana*, *Laurus nobilis*, *Citrus limon*, *Chenopodium botrys* and *Tanacetum armenum*) caused a decrease in the percentage of development of adults (F1 offspring) from treated eggs of *E. kuehniella* [35]. In this regard, [36] pointed out that essential oils extracted from the cortex of *Pinus pinea* and *Pinus brutia* exhibited ovicidal activity on *E. kuehniella*. EO of *Artemisia herba alba* applied to pupae of *E. kuehniella*, disrupted adult reproduction, prolonging the pre-oviposition period and reducing the egg deposition period as females fertilized, reducing the number of eggs deposited. Moreover, in a previous study, the EO of thyme (*Thymus vulgaris* L) exerted a significant influence on the spawning deterrent index thus affecting the survival of adults [37]. the F1 offspring of *Acanthoscelides obtectus*. Thymol; an isomer of carvacrol, is able to inhibit the reproduction of adults of *Helicoverpa armigera* under different regimes of application (oral and topical). Indeed, it causes the reduction in the number of eggs laid (fertility), the viability of the eggs and the preovipositionary and ovipositionary periods [38]. The *Myrtus communis* EO was tested against the fourth instar larvae of *Cx. pipiens*, exhibited a reduction in fecundity and the number of eggs [39]. Other works showed a reduction of weight ovaries and number of oocytes per pair of ovaries on

*Drosophila melanogaster* due a treatment by Spiromesifen [40].

In conclusion, the results suggested that *T. munbyanus* EO can be proposed in integrated pest management programs because it has great effects on the reproduction of Mediterranean flour moth, *Ephestia kuehniella* zeller.

#### ACKNOWLEDGMENTS

The National Fund for Scientific Research (Excellence Laboratory of Applied Animal Biology), Ministry of High Education and Scientific Research, Algeria supported this work. (PRFU Project N° D01N01UN230120230005 to Pr. S. TOUIKER).

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