

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, 2 % 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 them 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 doses 1, 3 and 6 µl. 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 μ l) converted to give equivalent fumigant concentrations of respectively 25, 75, 150 and 250 μ l/l air) for 24 and 48 hours. Each concentration and control was replicated four times. After 24 or 48h, 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 (LC_{50}).

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 μ l/l air), *C. sinensis* and *A. herba-alba* achieved more than 40% of larvae (L5, L4 and L3) mortality after 24 h of exposure.

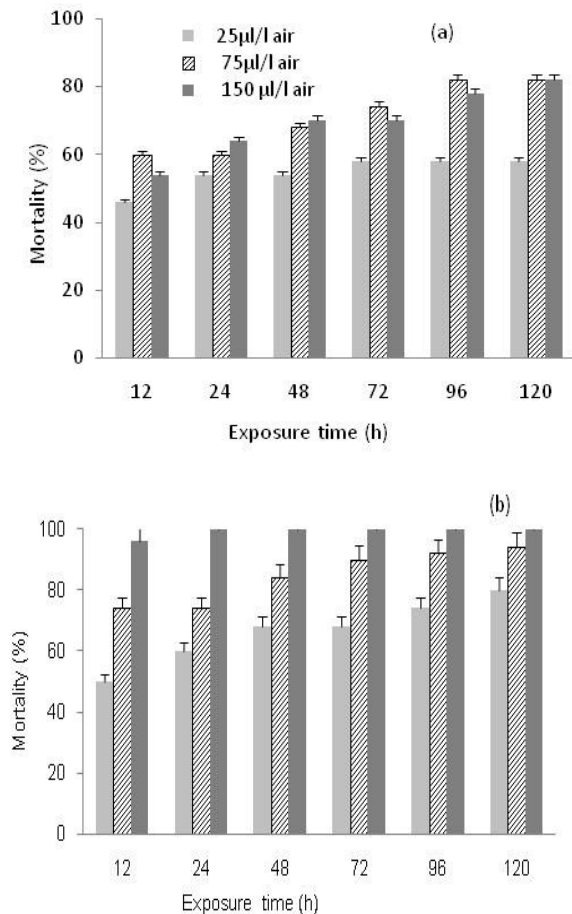


Fig. 1. Percentage of mortality of *E. ceratoniae* larvae (L5) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba*.

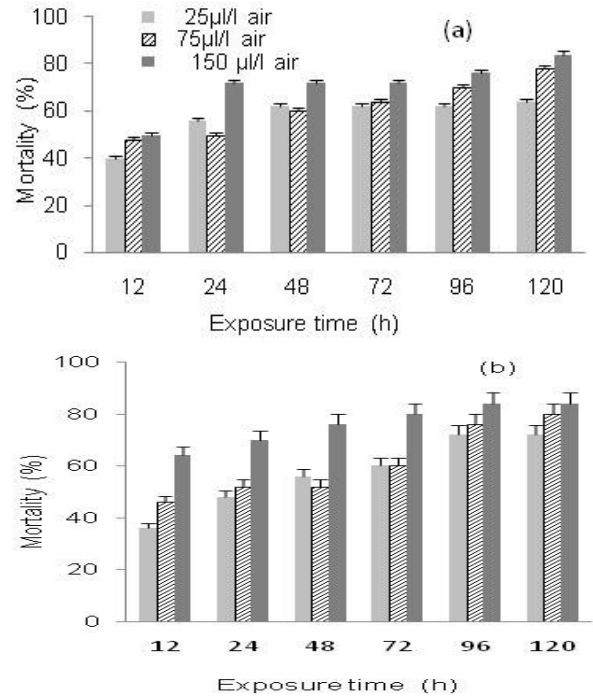


Fig. 2. Percentage of mortality of *E. Ceratoniae* larvae (L4) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba* (b).

At the highest concentration (150 μ l/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*.

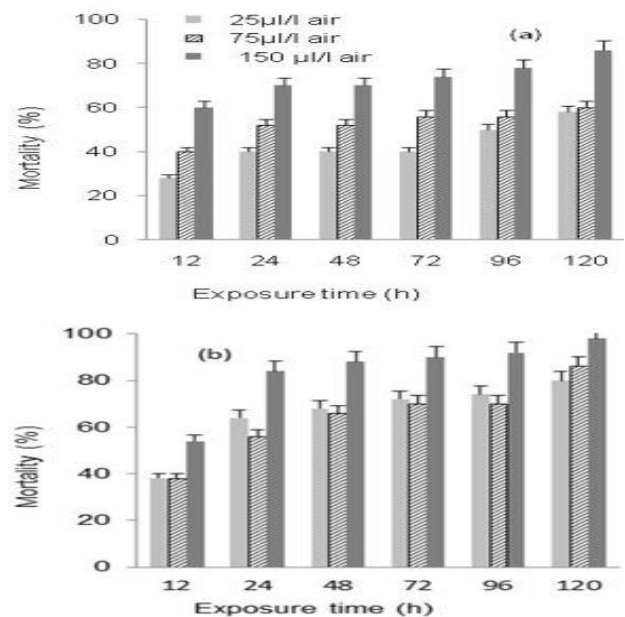


Fig. 3. Percentage of mortality of *E. ceratoniae* larvae (L3) exposed to various doses and periods of time of essential oils from *C. sinensis* (a) and *A. herba-alba* (b).

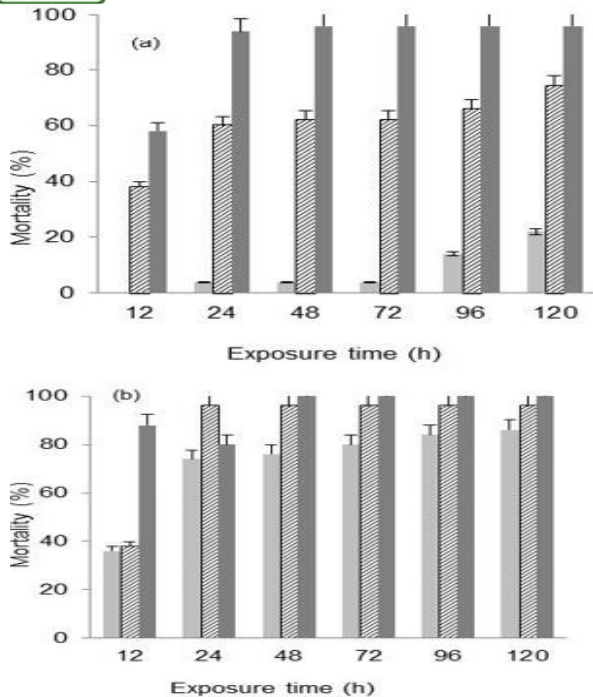


Fig. 4. Percentage of mortality of *E. ceratoniae* adults exposed to various doses and periods of time of essential oil from *C. sinensis* (a) and *A. herba-alba* (b).

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 $\mu\text{l/l}$ 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.

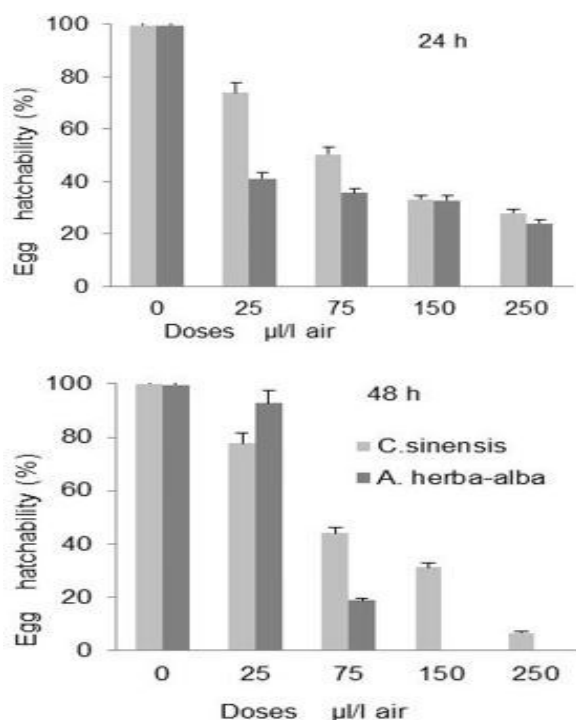


Fig. 5. Percentage of *E. ceratoniae* egg hatchability exposed to various doses and exposure time of essential oil from *C. sinensis* and *A. herba-alba*.

Table 1. LC₅₀ values ($\mu\text{l/l}$ air) of *C. sinensis* and *A. herba-alba* essential oils against *Ectomyelois ceratoniae* larvae (L3, L4, L5) and adults.

| | <i>Citrus sinensis</i> | <i>A. herba-alba</i> |
|-------------|------------------------|----------------------|
| larvae (L3) | 3.23 (2.29 -4.981) | 0.82 (0.07 - 1.50) |
| larvae (L4) | 1.181 (0.0- 2.329) | 1.46(0.00-2.94) |
| larvae (L5) | 0.501 | 0.89 |
| Adults | 2.6 (2.21- 3.01) | 0.31 (0.004 -0.76) |

In the current study, the essential oils obtained from *C. 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 *C. 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 sulfuryl 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], investigating the fumigant toxicity of *L. officinalis* essential oil against *E. ceratoniae* obtained 100% mortality after 18 h of exposure at 91 $\mu\text{l/l}$ 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 $\mu\text{l/l}$ air [19]. Additionally, Mediouni Ben

Jemâa et al, [14] indicated that essential oils from five Eucalyptus essential oils namely *E. astringens*, *E. lehmannii*, *E. camaldulensis* and *E. leucoxylon* *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

- [1] GIF. Inter-professional groupings for fruits, Rapport compagne de dattes, Ministry of Agriculture and Water Resources, Tunisia, 2016.
- [2] M.H. Dhoubi, 1989. Biology and ecology of *Ectomyelois ceratoniae* Zeller (L. pyralidae) in two different biotopes in southern Tunisia and search for alternative methods of control. These de Doctorat d'Etat Science Université de Paris VI 189 pp.
- [3] A. Jarraya and G. Vinson, Contribution to the study of the pistachio entomofauna. Ecological and ecological observations on *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), Annales INRAT, Vol. 53, pp 1- 42, 1980.
- [4] T. Mokhtar, A. Salem, S. Zehdi, E. Cherif, S. Rhouma, A. Othmani, S.D.K. Dakhlaoui and M. Morrakchi, Genetic resources of date palm in Tunisia, palm date Biotechnology. Research Institute for the Development, pp. 23-32, 2006.
- [5] E.J. Bond, Manual of Fumigation for Insect Control. Food and Agricultural Organization of the United Nations, Rome, 1984.
- [6] P.G. Fields and N.D.G. White, Alternatives to methyl bromide treatments for stored-product insect and quarantine insect, Annual Review of Entomology, Vol. 47, pp.331-359, 2002.
- [7] B.H. Lee, P.C. Annis, F.C. Tumaalii and W.S. Choi, Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects, Journal of Stored Products Research, Vol. 40, pp. 553-564 2004.
- [8] M.A.G. Pimentel, L.R.D.A. Faroni, R.N.C. Guedes, AH. Sousa and M.R. Totola Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research, Vol. 45, pp. 71-74, 2009.
- [9] P.J. Collins, G.J. Daghli, H. Pavic and R.A. Kopitke, Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzoperthadominica*, to phosphine at a range of concentrations and exposure periods, Journal of Stored Products Research, Vol. 41, pp. 373-385, 2005.
- [10] M.B. Isman, Plant essential oils for pest and disease management, Crop Protection, Vol. 19, pp 603-608, 2000.
- [11] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, Biological effects of essential oils: a review, Food and chemical Toxicology, Vol. 2, pp. 446-475, 2008.
- [12] D.R. Batish, P.H. Sing , K.R. Kohli and S. Kaur, Eucalyptus essential oil as a natural pesticide, Forest Ecology Management , Vol. 256, pp. 2166-2174, 2008.
- [13] S. Haouel, J. Mediouni-Ben Jemâa and M.L. Khouja, Postharvest control of the date moth *Ectomyelois ceratoniae* using eucalyptus essential oil fumigation, Tunisian Journal of Plant Protection, Vol. 5, pp. 201-212, 2010.
- [14] J. Mediouni Ben Jemâa, S. Haouel, M. Bouaziz M and M. Larbi Khouja, Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia, Journal of Stored Products Research, Vol. 48, pp. 61-67, 2012.
- [15] W.S. Abbott, A method of computing the effectiveness of an insecticide, Journal of Economic Entomology, Vol. 18, pp. 265-267, 1925.
- [16] D. L. Finney, Probit Analysis. Cambridge University Press, UK, 125 pp. 1971.
- [17] I. Tunc, B.M. Berger, F. Erlerand F. Dagli, Ovicidal activity of essential oils from five plants against two stored-products insects, Journal of Stored Products Research, Vol. 36, pp.161-168, 2000.
- [18] H. Chiasson, A. Belanger, N. Bostanian, C. Vincent and A. Poliquin, Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction, Journal of Economic Entomology, Vol. 94, pp.167-171, 2011.
- [19] O. Bachrouch, J. Mediouni-Ben Jemâa, A. Wissem, T. Talou, B. Marzouk and M. Abderraba, Composition and insecticidal activity of essential oil from *Pistacialentiscus* L. Against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller(Lepidoptera: Pyralidae), Journal of Stored Products Research, Vol. 46,pp. 242-247, 2010.
- [20] Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisiaabsinthium*, *A. dracunculus*, *A. santonicum*, and *A. spicigera* essential oils. Journal of Agriculture and food chemistry. 53: 9452-9458.
- [21] A. Delimi, F. Taibi, A. Fissah , S. Gherib, M. Bouhkari and A. Cheffrou, Bio-activity of essential oils of *Artemisia herba alba*: effect on reproduction and adult mortality of a stored product pest *Ephestia kuehniella* (Lepidoptera), Africa science, Afrique science, Vol. 09 Issue: 1, pp.82-90 , 2013.
- [22] M.M. Abbasipour, M. Basij, M.H. Hosseinpour, F. Rastegar and M. Bagher Nasiri, Fumigant toxicity of some essential oils on adults of some stored-product pests, Chilean Journal of Agricultural Research, Vol. 71Issue: 1, pp.83-89, 2011.
- [23] C. Kamaraj, A. Abdul Rahuman and A. Bagavan, Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), Syleptaderogata (F.) and *Anopheles stephensi* (Liston), Parasitology Research, Vol. 103, pp. 1361-1368, 2008.
- [24] E.E. Kenaga, Time, temperature and dosage relationships of several insecticidal fumigants. Journal of Economic Entomology, Vol.54, pp. 537-542, 1961.
- [25] D.L. Lindgren and L.E. Vincent, The susceptibility of laboratory-reared and field collected cultures of *Tribolium confusum* and *T. castaneum* to ethylene dibromide, hydrocyanic acid, and methyl bromide, Journal of Economic Entomology, Vol. 58, pp. 551-555, 1965.
- [26] J. Wang, F. Zhu, X.M. Zhou , C.Y.Niu and C.L. Lei, Repellent and fumigant activity of essential oils from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), Journal of Stored Products Research, Vol. 42, pp. 339-347, 2006.
- [27] S. Mansouri, Biotechnological methods for controlling the date moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae): fumigation with essential oils. Master's thesis. Institut Supérieur Agronomique de, Chott Meriem. 43, 2009.
- [28] S. Ben Chaaban, I. Chaieband K. Mahjoubi. Composition and insecticidal activity of essential oil from *Eucalyptus occidentalis* and *Eucalyptus camaldulensis* against *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyraliae). 12th Arab Congress of Plant Protection, Egypt, 4-10 November 2017.