Assessing the Impact of Storage Temperature on the Stability and Biocidal Activity of Essential oils Formulated. Case *Tribolium Castaneum* (herbst). (insecta, tenebrionidae)

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Abstract: In this paper, authors describe how to carry out a research assessing the impact of storage temperature on the stability and biocidal activity of formulated essential oils. Cereals, like other stored food, currently experience serious health problems of storage. The offending agents are primary pests such as *Sitophilusoryzae* and secondary pests including *Tribolium*. This study focused on the evaluation of the biocidal effect on the essential oils of thyme and citrus, against red flour beetle adults (*Triboliumcastaneum*) under different temperature regimes. The results showed that different molecules produced a delayed effect (12 h-14 h-16 h-18 h) on the populations of *Triboliumcastaneum* at the storage temperature of 20 °C. The same results showed an early striking effect of thymol and carvacrol on *Triboliumcastaneum* compared to limonene. The toxicity of thymol and carvacrol was much more active than limonene at 12 h, which was very remarkable as limonene expressed its toxicity from 14 h with a deadly effect. When temperatures are under 20 °C and 25 °C, thymol showed a greater degree of efficiency followed by carvacrol and limonene which had lower efficiency. However, at 28 °C, carvacrol expressed a very significant biocidal effect compared to the other two formulations, thymol and limonene.

Key words: *Thymus fontanesii*, bio-efficacy, essential oils, bioactive molecules, *Triboliumcastaneum*, plant protection.

1. Introduction

In Algerian economy, grains occupy a strategic position because they are the staple food of the people. Cereals and their derivatives provide more than 60% of caloric intake and 75% to 80% of protein intake in the diet [1]. Everyday climatic hazards and the lack of control of tillage have led to a decline in yields, and thus the Algerian state has resorted to massive imports of exotic varieties of cereals which are unfortunately susceptible to pests and diseases when stored in silos. However, there are many methods of clean curative struggle such as the struggle with cold and heat, and the fight by ionizing radiation. Currently, radiation and

non-polluting methods are very expensive and little used in Algeria. The use of chemicals has led to the appearance of resistance phenomenon to insecticides by insects and an accumulation of pesticide residues in stored grains [2]. Faced with these problems, there is a great need to develop new strategies to implement. Many researchers in recent years have focused on a new alternative control method based on the use of plant extracts or their active ingredients that act as natural fumigant. The power insecticide is very important such as the Miliaceae the *Rutaceae*, *Asteraceae*, the *Anacaceae*, Labiateae and *canellaceae* [3-6].

The main aim of this work is to evaluate the value of Algerian aromatic and medicinal plants at stored cereal pest control in laboratory. Specifically, our study aims to evaluate the efficiency and stability of three formulations

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of organic essential oils of thyme (thymol, carvacrol) and citrus (Limonene) under different storage temperatures on the population of a pest of stored products, the *Triboliumcastaneum* (Herbst). The longer-term aims are to minimize the use of chemical pesticides and encourage a more diversified agriculture which is more respectful of ecosystems and natural resources.

2. Material and Methods

2.1 Preparation of the Entomological Equipment

The biological material for the assessment of the effectiveness of applied organic products is restricted to individuals of *Triboliumcastaneum* (Herbst) originated in the commercial flour stocks. Following the method described by Laviolette and Nardon (1963), a rearing of insect was carried out in glass jars containing 250 g of commercial flour. Each jar was infested with 60 adults of *Triboliumcastaneum* and closed by a fine-mesh net held by an elastic band to allow the insects were incubated at ($30\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$) and relative moisture ($60 \pm 5\%$), for a period of two months. The age of the insect was adults of 2 to 3

weeks on their usual development substrates.

2.2 Formulations of Organic Pesticides

The treatments used in our study are organic pesticides formulated with two chemo types, carvacrol and thymol to 14% of extracted essential oils of thyme (*Thymus fantanasii*) and a comprehensive oil, 33% limonene extracted fruit Citrus (*Citrus Limonium*). The formulation is comprised of an Active ingredient (essential oil or chemo typed completed, a surfactant and a protector) [7].

2.3 Experimental Organic Assays

The tests were performed in the laboratory of the department at the National School of Agronomy.

From biological material we tried to estimate the impact of temperature, temporal stability and biocidal activity of the three formulations based on two essential oils of thyme and lemon on the populations of *Triboliumcastaneum*.

The better visualize the different components of the study we have illustrated (Fig. 1):

Fig. 1 Experimental organic assays.

Each essential oil extract formulated was uniformly spread on a type of Whatmanpaper's filter washer 9 cm diameter (63.62 cm²). The next method was to determine the toxicity of the essential oil contact. For this, *T. castaneum* of adult individuals are withdrawn from the breeding jar and placed separately in the petri dish. These are closed to ensure a good efficiency of treatment to estimate the influence of temperature on the formulated product, placed in prepared boxes, labeled and placed in an oven at three different temperatures, 20 °C, 25 °C and 28 °C.

The evaluation of the biocidal effect of the biological treatment made on individuals *T. castaneum* was conducted by counting the number of dead individuals every two hours during a period of 10 hours. This technique was repeated 9 times for each treatment used. The same method was applied to estimate the biocidal effect of the essential oil and the thyme essential oil to Limonène.

2.4 Estimated Mortality Rate

According to Mumbling et al. [8], the mortality rate is the rate of deaths of individuals under conditions of environmental data and varies according to the population considered and environmental factors. It is given by the decrease in population mortality compared time variation.

2.5 Estimation of the Corrected Mortality

The efficacy of a biocidal product is evaluated by the death of the target organism. However, the number of dead individuals enumerated in a population treated with a toxic substance is not the actual number of people killed by this substance. There are also a number of mortalities due to natural causes within a specific population, which adds to the mortality caused by toxic substances. Mortalities were corrected for control mortality using Abbott's formula [9] to determine if there were any detrimental sublethal effects.

2.6 Statistical Analysis

GLM analysis of variance was performed by using the F test for variables following the normal distribution. Comparisons of average corrected mortalities are followed by the Tukey test. Among the variables involved more often than the total variance, those whose contribution is significant at the 0.05 level were selected. The test was conducted by the SYSTAT software (SPSS 12, 2009).

3. Results and Discussion

3.1 Estimation Of *Triboliumcastaneum* Corrected Mortality at Different Storage Temperatures under the Effect of Essential Oils Formulated

The graph (Fig. 2) shows the time evolution of the adjusted mortality rate of *Triboliumcastaneum* adults. The overall vision of this graph shows that the molecules (thymol and carvacrol) exert a more significant biocidal effect in relation to limonene. Rising temperatures causes a delay in the expression of the effect of bio products.

At 20 °C, authors see the same trends in the efficiency of thymol and carvacrol on the corrected mortality *Triboliumcastaneum* of individuals other than those reported in the limonene with progressive lethal effect comparable to 18 h. However, during this period, dissimilarity is observed between the three treatments until the end of the investigation (Fig. 2a).

The results of the corrected mortality show that essential oils (thyme and citrus) are clearly formulated activated from 2 hours of treatment in the three selected temperatures (a, b, c). Carvacrol is manifested more effective at 28 °C. At 25 °C the three organic pesticides express the same toxic effect after 6 hours of exposure. These results show an increase in mortality of *Triboliumcastaneum* of individuals up to 50% throughout the test period.

The graph (Fig. 1b) shows that the insecticidal effect of thymol is stronger compared to those of limonene and carvacrol. It shows a less efficiency

Fig. 2 Estimated adjusted mortality *Triboliumcastaneum* to different storage temperatures under the effect of essential oils formulated. (a): 20 °C, (b): 25 °C, (c): 28 °C.

beyond 14 h and 18 h to stabilize. The graph (Fig. 1c) shows the time evolution of corrected mortality rate of *Triboliumcastaneum* adults. The tested biopesticide have a biocidal effect quite similar to that of 8 h to 16 h on individuals of *Triboliumcastaneum*, but after 16 h, the efficiency decreases. Carvacrol and thymol differ from limonene in having a high toxicity vis-a-vis these insects. However carvacrol showed a greater

effect than thymol and limonene at 8 h.

3.2 Comparative Study of the *Tribolium Castaneum* Corrected Mortality at Different Storage Temperatures under the Effect of Essential Oils Formulated

The general linear model (GLM) was used to evaluate the temporal variation of the corrected mortality based essential oils (thymol, carvacrol and

limonene) to determine their precise effects on *Triboliumcastaneum* adult populations under different temperature regimes. This model allows us to study the individual effect of each factor without the variable of interactions between them (Fig. 3).

According to the results obtained by the GLM model on the toxic effect in adults of *Triboliumcastaneum*, authors record that time and process factors have a highly significant difference with respective associated probabilities (F ratio = 2.64, $p = 0.000$, $p < 1\%$) (F-ratio = 4.25, $p = 0.000$, $p < 1\%$). For the treatment factor we report the presence of a highly significant difference with respective associated probability (F-ratio = 4.42, $p = 0.000$, $P < 1\%$) (F-ratio = 13.28, $p = 0.000$, $p < 1\%$).

Fig. 3a includes the effect of different treatments applied on biopesticide populations of *Triboliumcastaneum*. The authors find that the lethal effect appears larger from 12 h of exposure. At temperatures between 20 °C and 25 °C, thymol showed a greater degree of efficiency followed by carvacrol and limonene which has a low efficiency. However at 28 °C, carvacrol expresses a very significant biocidal effect compared to the other two formulations, and thymol Limonene (Fig. 4c).

Overall testing evaluations of the effectiveness of bio-based essential oils clearly shows a significant expression of the mortality of 12 h with acute toxicity in carvacrol and thymol and finally a higher mortality at 20 °C (Fig. 3).

Fig. 3 Comparative study of the *Triboliumcastaneum* corrected mortality at different storage temperatures under the effect of essential oils formulated. Carv: Carvacrol, Lim: Limonene, Thy: Thymol.

Fig. 4 Model GLM applied to the corrected mortality *Triboliumcastaneum* according to the treatment time and temperature.

Fig. 5 ANOVA test applied to salaries tests on populations of *Triboliumcastaneum* applied to the corrected mortality and the effect of treatments depending on temperatures (20 °C, 25 °C and 28 °C).

In order to appreciate the fluctuation (successive variations) mortalities individuals under different thermal regimes. The ANOVA model was used, and the results (Fig. 5) show that each formulation has an individualized reaction of the toxic effect relating to different temperatures. For limonene, the temperature of 25 °C give the greatest toxic effect on individuals of *Triboliumcastaneum*, followed by 20 °C and 28 °C respectively. Carvacrol was represented by the ambient temperature of 20 °C which gives the greatest biocidal effect on individuals of *Triboliumcastaneum*, followed by 28 °C and 25 °C which shows the lowest toxicity. Finally, thymol shows the same biocidal effect at temperatures of 20 °C and 25 °C, which is greater than the effect at 28 °C.

4. Conclusion

The insect pests of stored products are now resistant to most insecticides. For food safety reasons, some pesticides may be used to eradicate these pests. The development of new drugs to control these insects is long and it is very expensive to overcome these disadvantages and to prevent damage from the environment and human health through the use of increasing doses of insecticides. Therefore research interests are focusing on integrated management approaches including non-biocidal substances or predators of the target species. The implementation of these methods requires early detection of pests [10, 11]. The essential oils of plants in recent years are one of the most explored ways of regulating pests; their

application in the protection of stocks has been the subject of numerous studies. Their toxicity is expressed in different ways: ovicidal and larvacidal activities, by inhalation [4, 6, 8, 12-20].

The work that the authors have adopted was mainly based primarily on a multidisciplinary action for recovery of endemic plants pursuant to link the information provided by the ethno-botanists, the work of chemists and biologists. In this context the present study aims to develop new formulation based on essential oils. The results of the evaluation of the effectiveness of essential oils formulations have enabled us to identify the following assumptions:

4.1 Effect of Essential Oils on Tribolium Castaneum Formulations

The results show that different molecules had a delayed effect (12 h, 14 h, 16 h and 18 h) on populations of *Triboliumcastaneum* at the storage temperature of 20 °C. The same results show a striking early effect of thymol and carvacrol on individuals of *Triboliumcastaneum* compared to limonene. The toxicity of thymol and carvacrol stands limonene at 12 hours and exert a remarkable killing effect as limonene exerts its toxicity that from 14 hours.

At 25 °C, the same effect is reported in terms of late effectiveness of bioproduct, except that carvacrol is different thymol by his early disruptive marked that identifies at 12 h exposure. At 28 °C, in adult mortality somewhat late with a similarity at 12 h effect for bioproduct made from limonene and carvacrol, thymol but presents its toxic affect that after 14 h of exposure.

The inhibitory activity of bioactive molecules in most individuals tested in this study seems to be explained by the presence of molecules limonene, carvacrol and thymol. These compounds would act according to their ability absorbed through the cuticular barrier of the insect. Thus carvacrol and thymol are phenolic compounds known to have the highest efficiency of insecticide and a broader biocidal

spectrum on the pests of stored food. They are able to reduce their cuticular permeability. With regard to the formulation based on limonene, despite its low toxicity, this compound also showed insecticidal efficacy and this assumes that limonene contains bioactive molecules which are expressed late [19].

Ismanm, et al. [11] argues that the essential oils act directly on the cuticle of insects and soft-bodied mites. It makes this hypothesis because several essential oils seem more effective on soft-bodied arthropods. This is the case with carvacrol which exerts a satisfactory suppression of the beetle and was less effective with hard-shelled insects such as beetles and wasp adults and some predatory mites. Regnault-roger [21] reports that the insecticidal activity of thymus vulgaris oil was effectively evaluated by these two components of thyme 2%, thymol and carvacrol against the different larval stages of the mealworm (*Alphitobiusdiperinus* Panzer, *Coleoptera*, *Tenebrionidae*). The high bioactivity of thyme was attributed mainly to its high carvacrol and thymol which are identified as major compounds responsible for the great power of this insecticide.

The evaluation of the smoke effect of the essential oil of pistachio mastic, *Pistacialentiscus* on the third instar larvae and adults of *Triboliumcastaneum* has shown vis-à-vis toxicity of larvae and adults in terms of Mortality according to its stage of development, the concentration of oil and description. Many times work has demonstrated the effectiveness of carvacrol, camphor and terpenes; these molecules have a very wide spectrum of antiparasitic activity. This toxicity of variability is due to the presence of thymol, carvacrole as the major compounds in the essential oil composition [22]. The toxic effects by contact on several essential oils and *Triboliumcastaneum*, *Rhyzoperthadominica* and other insects of stored foodstuffs have been demonstrated by many authors. According to the work of [23] on the toxic effect of essential oils of thyme, contact on *Rhyzoperthadominica* induced a mortality of 96% to 100%.

4.2 Effect of Storage Temperature on the Biocidal Effect of the Essential Oil Formulations

The results for the effect of different treatments applied on organic pesticides to *Triboliumcastaneum* populations. The authors have found that the lethal effect appears larger from 12 h exposure. At temperatures between 20 °C and 25 °C, thymol showed a greater degree of efficiency followed by carvacrol and limonene which has a low efficiency. However, at 28 °C, carvacrol expresses a biocidal effect very appreciable compared to the other two thymol and limonene formulations. Overall testing evaluations of the effectiveness of bio-based essential oils clearly show a significant expression on the mortality of 12 hours with acute toxicity of carvacrol and thymol and authors have watching a higher mortality at 20 °C.

Indeed, each formulation has an individual reaction of the toxic effect next to different temperatures. For limonene, a temperature of 25 °C shows the greatest toxic effect against *Triboliumcastaneum* individuals, followed by a temperature of 20 °C, and then 28 °C. Carvacrol, represented by the ambient temperature of 20 °C as many biocidal effect on individuals of *Triboliumcastaneum*, followed by the temperature 28 °C and then 25 °C, which shows the lowest toxicity.

Finally, thymol at a temperature of 20 °C and 25 °C exert the same biocidal effect greater relative to the temperature 28 °C. This difference in biocidal activity reported for the same formulation in different thermal regimes allows us to estimate the degradation of certain compounds of chemo types of essential oil. This is at the root of this efficacy which increases with the changing temperatures.

Few works are devoted to the study of the stability of formulations of essential oils, but instead authors publish the search results [17] which were interested in the formulation of powdered insecticides by adsorption of essential oils *Xylopiiaethiopica* and *Ocimumgratissimum* cameroonian on modified clays. The study is devoted to the evaluation of the qualitative

composition of the essential oil after storage at 25 °C, 40 °C and 100 °C during the 1st and 8th week. It appears from this study that the essential oil, stored for eight weeks at 25 °C does not undergo any change in its composition. However, at 40 °C there is already a degradation of certain terpene compounds after the 4th week such as α - β -ocimene, carvacrol and δ - cardinène, indicating instability of these terpene compounds.

However, at the same temperature, the β -pinene of thymol are still present in the essential oil until the 8th week which the terpene compounds are known for their insecticidal properties [13]. The results obtained in Ref. [18] showed that the insecticidal property of essential oils confirms a synergistic effect in the terpene compounds of the constituents.

For the qualitative study of the chromatograms, it was clear at this point the deterioration in essential oil temperature leads to the formation of new products that authors have not identified in this work. Nonetheless, these results show that terpene compounds are not resistant to a temperature rise. This thermal instability varies from one group to another compound, it is much more important on monoterpenes than sesquiterpenes at 100 °C.

There are mainly sesquiterpenes in the essential oil (made less volatile than monoterpenes). This instability of terpene compounds was also observed by Marmonnier [8]. In this case, the degradation is due to hydrolysis reactions, degradation of proteins and cyclization. Denaturation and degradation of terpene compounds observed are only due to the combined effects of acidity and heat that would result in degradation of proteins reactions and reclosing. These results thus confirm the thermode gradability, and thermode naturability of terpene compounds and their thermal instability.

4.3 General Conclusion

The results of evaluating the efficacy of three formulations of organic essential oils of thyme (thymol and carvacrol) and citrus (Limonene) under different

storage temperatures against the main bioaggressor of stored products *Triboliumcastaneum* (Herbst), show that each formulation has an individual reaction of the toxic effect at each of the three temperatures (20, 25 and 28 °C). The thymol and carvacrol have shown a greater biocidal effectiveness in relation to limonene. For limonene, a temperature 25 °C showed its supremacy of toxicity, followed by that of 20 °C and 28 °C. Regarding carvacrol, it showed greatest toxicity at 20 °C, followed by 28 °C and then the temperature 25 °C which shows the lowest toxicity. However, thymol at a temperature of 20 °C and 25 °C exerted the same biocidal effect which was greater relative to a temperature of 28 °C. These results show essential oil of the *Thymus* could be applicable to the populations of *Triboliumcastaneum* management which could be a basis for further studies.

Algeria conceals a rich plant heritage, but it is unfortunately not exploited. It is therefore necessary to carry out ethnobotanical surveys of Algerian flora for better use of insecticidal plants. It is in this context that the subject of our contribution to the knowledge of the insecticidal effect of our chemo types can be considered useful in actual storage situations.

Through this work authors have tried to help grow the way on agri-pest potential of Algerian herbs that can be used locally as biopesticides for the protection of stored products that specifically affect targeted insect pests. This is a field of research and development that merits the interest of scientists, public authorities and economic actors especially ecological and environmental concerns of the world of living beings.

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