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(RESEARCH ARTICLE)



Ovicidal and larvicidal effects of two essential oils (*Citrus sinensis* and *Ocimum basilicum*) on the development cycle of wax moth *Galleria mellonella* in tropical hives

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Abstract

The greater wax moth, *Galleria mellonella*, is one of the ubiquitous pests of honey bee colonies worldwide. The economic importance of moths has led to a number of studies on the life cycle, biology, behavior, ecology, molecular biology, physiology, and control of the wax moth. Our study aims to verify the effectiveness of two essential oils against the greater moth *Galleria mellonella* L. The essential oils of sweet orange (*Citrus sinensis*) and basil (*Ocimum basilicum*) tested on the greater wax moth, have presented various degrees of activity against *G. mellonella* eggs and larvae. The highest aborted egg rate and the highest mortality were recorded with the mixture of sweet orange oil (*Citrus sinensis*) and basil (*Ocimum basilicum*) (respectively 91.27% and 65.33%) while the lowest aborted egg rates and mortality were recorded with basil oil (*Ocimum basilicum*) (respectively 75.77% and 15.67%).

These treatments appear to be safe for pollinators, humans, and the environment, and the essential oils applied are readily available and inexpensive.

Keywords: Bees; Wax Moth; Eggs; Larvae; Essential Oils; Efficiency

1. Introduction

Bees and beekeeping contribute to the livelihoods of people in almost every country in the world. Human societies have always known about honey and other products derived from bees. Bee species vary between regions and beekeepers depending on the conditions and resources of their living environment. Thus, the beekeeping practiced in Europe, where the climate is temperate, is very different from that practiced in tropical Africa, even if the honey bees belong to the same species "*Apis mellifera*" and appear identical, their biology and behavior actually differ significantly [1, 2].

In Benin, beekeeping is an economic activity "having positive repercussions on agricultural production and the income of rural households", but is little valued by the public authorities [3, 4]. Yet it has "positive ecological repercussions" by providing economic, social, and cultural benefits [5]. The conditions there are favorable and the production of honey allows the actors to create important value chains. A recent study revealed that in Benin, more than 70% of honey production comes from honey hunting, a devastating practice of bee colonies [6].

Despite the important role that honey bees play in the economy and the environment, there is a worldwide decline in the populations of managed honey bees and wild pollinators [7, 8].

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The populations of honey bees are confronted with a number of interrelated stressors, including diseases and pests, exposure to pesticides, habitat reduction, and climate change [9].

Many studies have shown a large number of causes of bee colony mortality [10, 11] including pathogenic viruses, bacteria agents of bacterial diseases), and pathogenic fungi. In addition, some insects such as *Galleria mellonella* and *Achroea grisella* vampirize brood and adult bees. As for the butterfly *Galleria mellonella*, it is a ubiquitous pest of honeybee *Apis mellifera* Linnaeus and *Apis cerana* Fabricius. Greater moth larvae burrow into the edge of unsealed cells with pollen, bee brood and honey through the midrib of the bee comb. Then, they cause *galleriasis* and later the flight of the colonies. Damage from *G. mellonella* larvae is severe in tropical and subtropical regions and is thought to be one of the factors contributing to the decline of wild and feral bee populations. It is currently widespread, particularly in Africa, and its potential for transmitting viruses to bees has raised legitimate concerns. Chemical insecticides are currently the most practical method of controlling insect pests, due to their effectiveness and easy application. However, the intensive and inconsiderate use of these insecticides has unfortunately led to scientifically proven harmful effects on human and environmental health.

It is, therefore, necessary to continue studies to find control strategies that are harmless to bees. In Africa, plants and derivatives are widely used in veterinary ethnomedicine. [12].

Sweet orange essential oil (*Citrus sinensis*) and basil essential oil (*Ocimum basilicum*) are characterized by their local availability. In addition, note that they are among the plants often used for their larvicide, insect repellent, and nematocidal properties but also used in the treatment of skin conditions and various other diseases [13, 14, 15]. Moreover, these essential oils do not present any toxicity hazards to bees in particular. [16, 17].

Our study, therefore, proposed to evaluate the larvicidal and ovicidal properties of these two essential oils on the greater wax moth.

2. Material and methods

2.1. Experimental materials

Galleria mellonella eggs have been treated with essential oils in culture areas (in jars), in the laboratory;

The larvae at various pre-imago stages were directly exposed to the treatments within hives fulfilling their development conditions (hives provided with a temperature of 29-35 °C and empty combs and sheltering young colonies at the beginning of establishment, that is to say in full activity of building future brood nests). For this, 4 Kenyan hives were used. The dimensions of each were: length: 51cm, width: 30cm, and height: 29cm. 4 incubation jars, each containing three clusters of 40 fresh eggs of *Galleria mellonella* laid.

Inside the hives, the wooden floor was slightly surmounted by a custom-made mesh frame, the openings of which remained inaccessible to the bees.

2.2. Monitoring and measuring equipment

Some laboratory instruments were used:

- A microscope and a binocular magnifying glass to observe the rate of emergence of larvae or induced abortion in eggs exposed to the three types of treatment, and during the incubation period (8 days)
- A dropper to quantify the doses of essential oils to be applied in the hives.

2.3. Harvesting plants

Two identified aromatic plants and essential oils: *Citrus sinensis* and *Ocimum basilicum* were collected in the botanical garden of the University of Abomey- Calavi.

2.4. Extraction of essential oils

The aerial part of the plants and fruit pericarp (orange) harvested and dried was extracted by steam distillation. The used device has a separate steam generator. This generator is a balloon containing boiling water. The balloon is surmounted by a tank filled with the biomass (leaves, seeds, flowers, etc.) to be extracted. A cooler has been adapted to

this tank. The water vapor-essential oil mixture is condensed in the cooler and the oil is then collected by decantation in an evaporator. The oil was collected and weighed for yield determination.



Figure 1 Essential oil extraction device, Anagonou, 2022

2.5. Study Methods

A total of 480 fresh eggs of *Galleria mellonella* were used on the one hand for the in vitro tests, i.e. a lot of 120 fresh eggs per jar, each jar subjected to one of the three treatments each (T 1, T 2, & T 1+ T 2) over a period of 24 hours for 8 days (which corresponds to the number of days before hatching under optimal conditions); the last jar has not undergone any treatment (T0) and constitutes the control lot; and on the other hand 480 24-hour/one-day old larvae introduced in lots of 120 into each of the four hives, precisely on the floor (in the crevices of the hive) treated with essential oil, an essential oil applied for each hive using a dropper and at increasing doses according to the evolution of the pre-imago stage of the larvae (day 1; day 5; day 8; day 15) over a period of 16 days which corresponds to the intermediate phase of the transition from the larval stage to the last pre-imago stage.

2.6. Data analysis

For this analysis, it was for us to evaluate the rate of abortion or emergence of the larvae at the level of the eggs following the various in vitro treatments, and also to evaluate the rate of larval mortality within the hives to each treatment and after comparison to retain the most effective, i.e., the one which induces the most larvicidal or ovicidal action.

For the calculation of the parameters mentioned above, the formulas below were used:

$$aer = naec * 100 / ine$$

$$lmr = ndlc * 100 / inl$$

Where;

aer: aborted egg rate

naec: number of aborted eggs counted

ine: Initial number of eggs

lmr: larval mortality rate

ndlc: number of dead larvae counted

inl: Initial number of larvae

These parameters were calculated taking into account the time of exposure to the treatment and the dose of essential oil applied.

3. Results

The rate of eggs in the colonies studied is reported in Table 1. The application of the different oils, as well as their combination, could not totally eliminate the eggs of *G. mellonella*. About the treatment with essential oil of sweet orange tree *Citrus sinensis*, the aborted egg decreases considerably already the first day: 67.40%. It continues to decrease in this way on the following days, and, on the 7th day, it reaches 90.47%. For treatment with *Ocimum basilicum* basil essential oil, the egg rate of *G. mellonella* aborted was 40.87 after the first day of treatment. On the 7th day, it was noted 75.77% of eggs of *G. mellonella* aborted. The highest rate of aborted eggs was recorded with treatment T3, composed of essential oil of sweet orange tree *Citrus sinensis* and basil essential oil *Ocimum basilicum*, 91.27 on the 7th day.

Table 1 Mortality rates of *G. mellonella* eggs at various post-treatment intervals

Treatments	Post treatment intervals			
	aer at 24h	aer at 48h	aer at 72h	aer at D7
T1 _	67.40	70.22	84.62	90.47
T2 _	40.87	54.60	66.34	75.77
T3 _	69.33	70.50	83.22	91.27
T 0	10.11	12.41	12.89	15.10

T 1: treatment with sweet orange essential oil *Citrus sinensis*; T 2: treatment with essential oil of basil *Ocimum basilicum*; T 3: treatment with essential oil of sweet orange tree (*Citrus sinensis*) + essential oil of basil *Ocimum basilicum*; T 0: no treatment

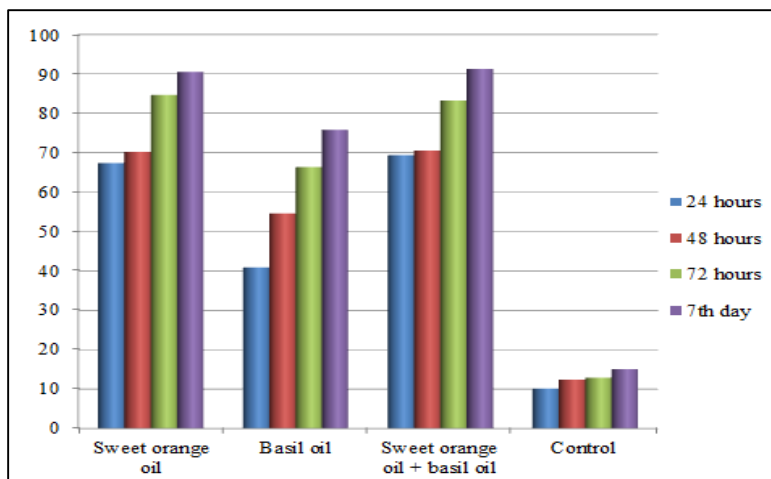


Figure 2 Egg mortality at various post-treatment intervals

Table 2 *G. mellonella* larval mortality rates at various post-treatment intervals

Treatments	Post treatment intervals			
	lmr at 24h	lmr at D5	lmr at D8	lmr at D15
T1	49.33	30.38	54.76	52.34
T2	2.69	4.56	6.22	15.67
T3	42.55	54.30	63.22	65.33
T 0	2.12	3.84	4.11	10.11

T 1: treatment with sweet orange essential oil *Citrus sinensis*; T 2: treatment with essential oil of basil *Ocimum basilicum*; T 3: treatment with essential oil of sweet orange tree (*Citrus sinensis*) + essential oil of basil *Ocimum basilicum*; T 0: no treatment

The rate of larval mortality of greater wax moth is recorded in Table 2. The different oils had a remarkable effect on the larvae, but none could eliminate them completely. Treatment with sweet orange essential oil *Citrus sinensis* and basil *Ocimum basilicum*, recorded the highest mortality rate on the 15th day with 65.33 %. The T2 treatment with basil oil *Ocimum basilicum* was the least effective with a mortality rate of 15.67% on the 15th day.

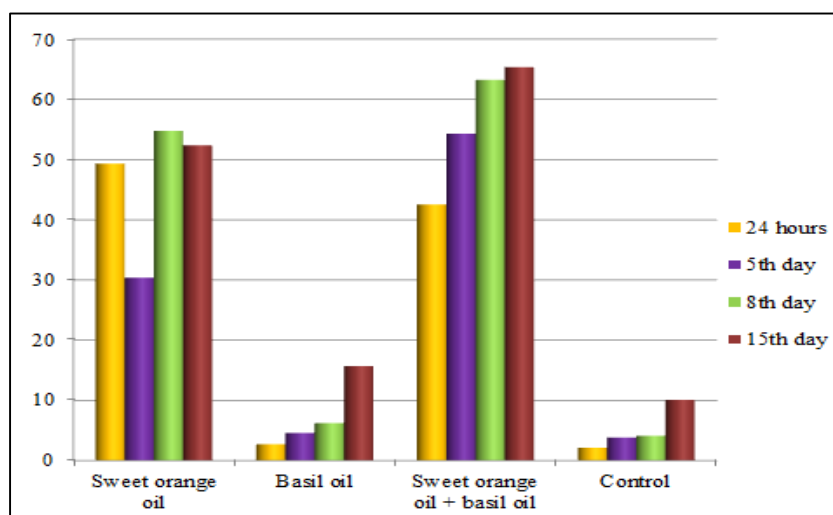


Figure 3 Larval mortality at various post-treatment intervals

4. Discussion

Wax moth (*Galleria mellonella*) is a considerable danger to hives. The galleries dug by the caterpillar can lead to the complete destruction of the attacked frame, then of the neighboring frames [18, 19].

Beekeepers use different products and treatments to fight against diseases that affect the bee colony, such as synthetic veterinary drugs. But the latter constitute a danger for bees as well as for humans and the environment [20]. Medicinal plants have long proven their effectiveness [21]. Essential oils have mechanisms of action that make them very promising for research, particularly their insecticidal effect [22]. Most of the prior studies were already focused on mosquitoes, as well as on the repellent effect of essential oils [23] and their ovicidal [24] or larvicidal [25] effect. Many studies on the use of essential oils and their mode of use in bee colonies have been carried out [26, 27,28].

The essential oils tested in this study were found to show varying degrees of activity against *G. mellonella* eggs and larvae. The highest rate of aborted eggs was observed with the combination of both essential oils used in our study (rate of aborted eggs 91.27 on D7), followed by sweet orange oil *Citrus sinensis* (rate of aborted eggs increases to 90.47% on D7). The group treated by basil essential oil *Ocimum basilicum* and the control group, showed the lowest mortality percentage throughout the observation period. The peaks in the graph clearly show which treatment leads to the high percentage of mortality (Table 1 and Fig. 2). In regards to the activity of essential oils on *G. mellonella* larvae, the results were not as strong as those of the eggs. But these results are still conclusive and could be more satisfactory with a higher concentration of these essential oils. These results support those of Sayah in 2014 who confirmed the larvicidal activity of essential oils of *Citrus aurantium*, *Citrus sinensis*, *Pistacia lentiscus* at lethal doses of DL50 and DL80, which were tested on *Culex pipiens* larvae [29].

It appears from the results obtained that there is no significant difference between the effectiveness of natural treatment and the mixture of oils. Indeed, the essential oil of *citrus sinensis* applied on the 7th day has an efficiency of 90.47% and the treatment efficiency of the mixture of essential oils of *citrus sinensis* and *Ocimum basilicum* is 91.27%.

This result confirms that of NÉBIÉ et al. who proved that the mixture of essential oils, in order to have a higher insecticidal activity is not more effective than the most active essential oil of this mixture [30].

In the control group, although it did not undergo any treatment, we had noticed a rate of aborted eggs and larval mortality, if even they are almost negligible. We believe that this situation may be explained by the result of Frey et al, who had proved in 2011 that each colony is, in fact, infested according to its own tolerance and resistance factors [31].

Thus, depending on many factors (hygienic behavior, for example), the infested bees colonies may be able themselves to fight the invasion of the eggs and larvae.

5. Conclusion

The effectiveness of two essential oils against the *Galleria greater moth mellonella L.*, was studied, and the results showed that the activities of the oils tested were different. The most effective was *Citrus sinensis* essential oil. Its combination with *Ocimum basilicum oil* may slightly increase the ovicidal /larvicidal activity, but without significant difference, in reality.

These essential oils are available at a lower cost and can give beekeepers good results in protecting wax combs. Further studies are needed to assess harmless doses, as well as the effects of its oils on bees and different hive products.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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