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Research Article

Evaluating the Effect of Plant Extracts against Greater Wax Moth, *Galleria Mellonella* (L)

Taye Beyene[1*] and Mekonen Woldatsadik[1]

[1]Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center, Zeway, Ethiopia

***Corresponding Author**
Taye Beyene
Email: tayebeye@yahoo.co.uk

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Abstract: Beekeeping is an important activity to increase the family income in rural areas. However, honey bees pests and their control measurements is a great challenge. Hence, this experiment was conducted to evaluate and identify the effectiveness of essential oils extracted from neem *(Azadirachta indica),* basil *(Ocimum basilicum), Calpurnia aurea, Cymbopogon citratus, Vernonia amygdalina, Vebascum sinatticum benth and Maesa lanceolata Forssk* against the larvae of greater wax moth, *Galleria mellonella.* Plant species were collected, dried, grinded and plant extracts were prepared from the selected plants using soxhlet extraction method. Botanical extracts were dissolved in distilled water to obtain 5%, 10%, and 15% concentrations and contact toxicity assay was conducted in the laboratory on the larvae of greater wax moth, *Galleria mellonella.* The experiment was laid out in Complete Randomized Design (CRD) and replicated three times. The results showed that, plant extracts tested at various concentrations exhibited variable responses to the larvae of greater wax moth, *Galleria mellonella* as compared to the controls. The highest larval mortality (94.22%) was recorded from neem (*Azadirachta indica) leaf extract* followed by basil (*Ocimum basilicum)* (83.76%), *Vernonia amygdalina* (77.47%), *Calpurnia aurea* (66%) and *Vebascum sinatticum benth* (43.66%) after application of 15% dose of plant extract within 48 hrs as compared with controls.This study concluded that, neem (*Azadirachta indica)* and *Ocimum basilicum* extracts had showed best result against the larvae of greater wax moth, *Galleria mellonella* compared to other botanical extracts. Therefore, neem (*Azadirachta indica*) and *Ocimum basilicum* oil extracts can be developed as sources of natural pesticides for the management of larvae of greater wax moth, *Galleria mellonella*. However, further research should be carried out in order to identify its effectiveness on the field and on honeybees.

Keywords: *Galleria mellonella*, wax moth, plant extracts, mortality, larvae.

INTRODUCTION

Honeybees are highly valued resource-insects around the world, prized not only for production of honey, wax and other products. The essential and valuable activities of bees depends upon beekeepers maintain a health population of honeybee because like other living organisms, bees are subjected to many diseases and pests (Ritter and Arkratanakul, 2006). The pests and diseases have severely reduced the number of healthy colonies available for beekeeping as well as the hive products harvested from the colonies (Desalegn, 2015). The diseases and pests not only cause economic loss, but also cause ecological problems related to the role of honeybees. In tropical countries pests and predators of honeybees are more prevalent and affect honeybees than diseases and cause loss of colonies and their products(Morse and Nowogrodzki 1990). Among the interrelated factors, wax moth infestation is the prominent ones that inflict enormous loss to the potential beekeeping production. Wax moths are potentially very troublesome to beekeepers all over the world especially in warm climate region of the world (Calvert: 1982: Harchiro and Knox, 2000). Two species of wax moth known to be harmful to bee colonies and stored beeswax are greater wax moth (Lepidoptera: Pyralidae, *Galleria mellonella*) and lesser wax moth (Lepidoptera: Pyralidae, *Achroia grisella)*. Both of these species have the same type of tunneling habits, but greater wax moth, *Galleria mellonella*, causes the greatest damage in apiaries. The larvae of both moths feed on wax, pollen and cocoons of the bee larvae (Ellis *et al.,* 2013). Unless controlled at early stage, this leads to the destruction of honeycombs and subsequent deterioration of the weakened colonies and can cause significant damage to stored beekeeping equipment.

Wax moth is widely distributed in Ethiopia and was reported the most troublesome pest of honeybees. According to (Amsalu, and Desalegn, 2001), wax moths cause loss of 2.5, 2.9 and 2.5 tons of pure beeswax in West Shoa, South West Shoa and East Shoa Zones, respectively. To overcome the problems of wax moth infestation, several intervention methods have been attempted for a century worldwide. Among these, using synthetic pesticides is the most used globally for the control of wax moth pest. However, using synthetic pesticides have serious drawbacks such as toxicological hazards to beekeepers and bees, risks of contaminating hive products are a global challenge (Pirali and Silva, 2010). Besides, beekeepers in developing countries may not be able to afford synthetic pesticides. Development and promotion of alternative means of technologies against bee pest is

compulsory in recent years. This would have advantages of enhancing environmental quality and economic viability of individual beekeeping operations, protect human health and safety by preventing the risk of contamination honey and other hive products. Most plant derived products are presumed to be less toxic to non target organisms, easily biodegradable and therefore do not persist in the environment as opposed to synthetic products which often end up being pollutants. Plant products are also cheap especially if they are locally available (Isman, 2006). Moreover, botanical pesticides are encouraged over synthetic pesticides because, they effective in small quantity and safe environment life (Pedigo, 1998). For these reasons, the more recent approach to control to control honeybee pests and diseases was by the use of relatively safe natural products, especially volatile oils and/or mechanical and biological managements (Currie, R.W. and P. Gatien, 2006).

Plant based pesticides have been adopted in different countries and cultures with their own specific indigenous knowledge and parallel standards and methods for evaluation. Available literature shows that volatile oils from *Eugenia aromatic* (Clove) and basil *(Ocimum basilicum)* were applied to control wax moth larvae and highly effective (Zaitoun, 2007). However, the safety, effectiveness and quality of botanical products depend on the quality of their source materials and how elements are handled through production processes.

Ethiopia has a vast flora and fauna that have potential for developing natural products into commercial technologies. Traditional use of plants and plant derivatives for pest control and medicinal value is long time established in the country. A diversity of plant species are traditionally used as repellents and insecticidal effect in Ethiopia (Abebe *et al.,* 2003; Berhanu *et al*., 2006). However, studies about their potential effects against honeybee pests, including wax moth is lacking. So, the aim of present study was to evaluate and identify the effectiveness of some plant extracts against larvae of greater wax moth, *Galleria mellonella* under laboratory conditions.

MATERIALS AND METHODS

Study Location

An experiment was conducted at the laboratory of Holeta Bee Research Center during2016- 2018. This study aimed to evaluate and identify the effectiveness of some plant extracts against larvae of greater wax moth, *Galleria mellonella.*

Plant Material Collection and Identification

Essential part, leaves of the selected plants were collected from different localities of central rift valley of Oromia, Ethiopia based on indigenous knowledge, previous research work and literature information. The location, taxonomy and common name of the plant were recorded, labeled and kept separately in plastic bags. Then the collected plant part was kept in plant presser and stored in freezers like cool box or uncryogenic envelopes or liquid nitrogen (<-4^{o}C).

Table1. List of Plants Species Collected and Tested against the Larvae of Greater Wax Moth, *Galleria mellonella*

No.	Scientific name	Vernacular name (Afan Oromoo)	Plant habit	Family name	Plant part used
1	*Azadirachta indica*	Muka Niimii	Tree	Meliaceae	Leaves
2	*Ocimum basilicum L*	Damaa kasee	Herb	Lamiaceae	Leaves
3	*Vernonia amygdalina*	Eebicha	Shrub	Asteraceae	Leaves
4	*Calpurnia aurea*	Ceekaa	Shrub	Fabaceae	Leaves
5	*Verbascum sinaiticum benth*	Gurra harree	Herb	Scrophulariaceae	Leaves
6	*Cymbopogon citratus*	Xajji saara	Herb	Poaceae	Leaves
7	*Maesa lanceolata Forssk*	Abayyii	Shrub	Myrsinaceae	Leaves

Sample Preparation

The collected plant leaves were kept at room temperature. Then, plant parts were dried under shade and mechanically grinded with a grinding machine into powder and passed through a sieve with fine mesh to obtain fine powder.

Preparation of Extracts

Plant extracts were prepared from the selected plant species by soxhlet extraction method at Holeta Agricultural Research Center food science laboratory. A sample of 50 gram of each plant powder was weighed and put into the thimble of soxhlet extractor and shaked to dissolve it in the extractor unit. The flask was connected to hexane containing at 2/3 of total volume the extractor until 4 hours. After completed the extraction process, the hexane was evaporated by rotor evaporation under vacuum and the extracts was picked in vial. Then, the extracts were dissolved in distilled water to obtain different concentration levels (5%, 10% and 15%) for bioassay test on the larvae of greater wax moth, *Galleria mellonella* L. Distilled water and hexane solvents were used as negative and positive control respectively.

Screening of Plant Extracts

From seven tested plant species, only five plant species have positive effect on the larvae of greater wax moth, *Galleria mellonella* were selected.

Collection of Wax Moth Larvae

Honey combs infested by wax moth and contain wax moth larvae were collected from Adami Tulu Agricultural Research Center apiary site. Then, the larvae were transported carefully to the Holeta Bee Research Centre, Bee Health Laboratory with infested honey combs.

Testing for Bioassay

Three different concentration levels (5%, 10% and 15%) of each tested plant were prepared in distilled water. The contact toxicity was tested by immersing 10 larvae in petri dishes containing 5%, 10% and 15% plant extracts. Larvae mortality was recorded by counting dead by probing them with a blunt object. A larva was considered dead if it did not move after being probed with a pin turn to black and became softness. The corrected mortality rate was reported as the percent mortality rate of pests calculated as per Abotts (1925) as mentioned by equation below:

$$\text{Abbott's formula} = \frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

Experimental Design and Statistical Analysis

The experiment was laid out in Complete Randomized Design (CRD) with three replications. Data were analyzed using the one way ANOVA procedure of SAS (2004) and comparison of the means was done using Tukey's HSD Test, with 5% error level. Mortality rate was calculated as mortality is equal to after treatment the number of dead wax moth larvae by before treatment the number of wax moth larvae times 100 percent.

RESULTS AND DISCUSSIONS

Extracts of five plant species including neem (*Azadirachta indica), Ocimum basilicum*, *Vernonia amygdalina*, *Calpurnia aurea* and *Vebascum sinatticum benth* at concentration of 5%, 10% and 15% were used in this experiment to determine their effectiveness against the larvae of greater wax moth, *Galleria mellonella* larvae in laboratory. The mortality percent of greater wax moth, *Galleria mellonella* treated with plant extracts is

depicted on Table 2. The results showed that, the tested plant extracts exhibited variable efficiencies against the larvae of greater wax moth, *Galleria mellonella*. The highest larval mortality (94.22%) was recorded from neem (*Azadirachta indica)* leaf extract followed by basil (*Ocimum basilicum)* (83.76%), *Vernonia amygdalina* (77.47%), *Calpurnia aurea* (64.33%) and *Vebascum sinatticum benth* (43.66%) after application of 15% dose of plant extract within 48 hrs as compared with the controls (Table 2).

Table 2: Percent mortality of larvae of greater wax moth, *Galleria mellonella* (L) treated with plant extracts

Treatment	N	Mean ± SE mortality rate at different concentration levels		
		5%	10%	15%
Azadirachata indica	10	62.40±0.42^{b}	74.33±3.18^{b}	94.22±0.99^{a}
Ocimum basilicum	10	50.31±1.8^{a}	63.18±3.85^{c}	83.76±0.35^{a}
Vernonia amygdalina	10	41.0±2.2^{d}	53.26±4.97^{c}	77.47±3.55^{a}
Calpurnia aurea	10	29.01±1.2^{c}	48.34±0.31^{cb}	64.33±3.3^{b}
Vebascum sinatticum benth	10	26.67±5.5^{cd}	32.90±0.00^{f}	43.66±2.90^{d}
Distilled water (-Ve control)	10	0.0±0.00^{b}	0.0±0.00^{a}	0.0±0.10^{c}
Hexane (+Ve control)	10	4.6±0.01^{b}	13.8±0.11^{a}	26.24±3.57^{e}
LSD (5%)		8.70	10.76	12.77
CV (%)		3.56	5.12	7.12

Means followed by the same letters within a column are not significantly different from each other using Tukey's HSD Test, with 5% error level.

Efficacy of botanical extracts of plants used in this study against larvae of greater wax moth, *Galleria mellonella were Azadirachta indica, Ocimum basilicum, Vernonia amygdalina*, *Calpurnia aurea*, and *Vebascum sinatticum benth* in decreasing order. Neem (*Azadirachta indica)* aqueous leaf extract performed better than other botanical extracts against larvae of greater wax moth, *Galleria mellonella.* The results are in close proximity with the study of (Mohamed, 2012) who declared that, *Azadirachta indica* leaf extract was more effective against Greater wax moth, *Galleria mellonella*. This may be attributed to the fact that neem (*Azadirachta indica)* leaf extract has been reported to possess insecticidal, growth regulatory and antifeedent properties against insects (Larry, 2004). These natural plant products are more economical when compared to other chemicals. In general, the use of natural products as an insecticide may help us to minimize the problem environmental pollutions as result of synthetic insecticide applications.

CONCLUSIONS AND RECOMMENDATIONS

Plant extracts tested exhibited variable responses to the larvae of greater wax moth, *Galleria mellonella* as compared with the controls. The study results showed that, *Azadirachta indica* and *Ocimum basilicum* extract caused the highest percent of larvae mortality after application of 15% concentration within 48 hours. Therefore, neem (*Azadirachta indica*) and *Ocimum basilicum* oil extracts can be developed as sources of bio-pesticide for the management of larvae of greater wax moth, *Galleria mellonella*. However, further research should be carried out in order to identify their effectiveness on the field and on honeybees. Identification of bioactive compounds from the effective plant oils for the control of wax moth is also necessary.

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Disclosure Statement

The authors declare that they have no conflict of interests.

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