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Taye Beyene
Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center, Zeway, Ethiopia

Mekonen Woldatsadik
Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center, Zeway, Ethiopia

Corresponding Author:
Taye Beyene
Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center, Zeway, Ethiopia

Laboratory evaluation of the effectiveness of some botanical extracts against the larvae of greater wax moth, *Galleria mellonella* (L.)

Taye Beyene and Mekonen Woldatsadik

Abstract
Wax moths are serious pests of beeswax worldwide. The wax moth belongs to the subfamily *Galleriinae* of the family Pyralidae, order Lepidoptera. The current study was conducted to evaluate and identify the effectiveness of some plant based biocides to develop alternative method of controlling greater wax moth. For this purpose, *Azadirachta indica, Ocimum basilicum, Calpurnia aurea, Vernonia amygdalina* and *Vebascum sinatticum benth* were collected, dried, grinded and plant extracts were prepared by soxhlet extraction method. The oil extract were applied on the larvae of greater wax moth, *Galleria mellonella* at concentrations of 5%, 10%, and 15%. Completely randomized design was used with 3 replicates, each containing 10 larvae. Mortality was recorded at 3h, 12h, 24h and 48h post-treatment. The plant extracts showed significant ($p<0.05$) level of toxicity to the larvae of greater wax moth, *Galleria mellonella* at different exposure times and concentrations. Results showed that *Azadirachta indica* and *Ocimum basilicum* caused (100%) mortality in the larvae of greater wax moth, *Galleria mellonella* after application of 15% extract within 48h, while the least larvae mortality (42.82%) was obtained from *Vebascum sinatticum bent* within 48h exposure time. From different extracts, 15% concentration was the most toxic causing (100%) larvae mortality within 48h exposure time. The results indicated that the percentage of larval mortality was significantly increased vertically by increasing the concentration levels from 5% to 15% and horizontally by increasing exposure times from 3h to 48h. The results from the laboratory test showed that *Azadirachta indica* and *Ocimum basilicum* extracts is promising biological control agent against the larvae of greater wax moth, *Galleria mellonella***.** However, these results need further research in order to identify its effectiveness on the field and honeybee.

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

Introduction

Honeybees are well known and economically beneficial insects not only for the production of honey, wax and other valuable products but also for crop pollination and environmental stability. The essential and valuable activities of bees depend upon beekeepers maintaining a health population of honeybee, because like other living organisms, bees are subjected to many disease and pest (Ritter and Akratanakul, 2006). Many developing countries are trying to improve the quality of their honey products but they frequently encounter widespread obstacle in apiculture. Among many interrelated factors, infestation of honeybee colonies by pathogens and pests is the prominent ones that inflict enormous loss to the potential beekeeping production. The important pests of honeybees are small hive beetle, bee louse, Mediterranean flour moth, the lesser and greater wax moths.

The wax moth belongs to the subfamily *Galleriinae* of the family Pyralidae, order Lepidoptera. The order Lepidoptera contains two species of wax moths; the greater, *Galleria mellollella* L. and the lesser, *Acllroia grisella* .These two species of wax moth occur naturally or have been introduced by man in almost all regions of the world where apiculture is practiced. The greater wax moth is the most dangerous pest of honeybee combs (Hachiro and David, 2000) [10]. This moth is a serious problem in tropical and sub-tropical climate, where warm temperature favours their rapid development. Females of the moth laid their eggs in cluster usually in the cracks or between wooden parts of the hives. The larvae mainly eat and destroy beeswax combs where the bees can store pollen, honey and lay eggs for their normal activities. The larvae of these moths form a silken feeding tunnel, which enlarge along the mid-rib of the comb or make borings through the thin wax caps of honey cells causing honey to leak out. Adults of the wax moth can also transfer pathogens of serious diseases like foulbrood as they move from hive to hive (Tompkins and Griffith, 1977; Singh, 1982;

Crane, 1990; Chaniere and Imdorf, 1999; MAAREC, 2000) [15, 8, 7, 14]. 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.

In Ethiopia, both the greater and lesser wax moths are widely distributed throughout the country and are believed to be the most troublesome of the pests of honeybees though their economic injury level has not yet been estimated (Ayalew Kasaye, 1983) [4]. Distribution of these two species of wax moths varies i.e. the lesser is predominant in mid-highland areas like Holeta (Suba) and Gedo, the greater at lowland areas like Nazareth, Bahir-dar, Hawasa, and Bako. In some places both species are found (Desalegn, 2001) [9]. Using synthetic pesticide against bee pests is not essential in order to absence the risks of contaminating honey and beeswax with residue.

The development and promotion of alternative means of technologies against bee pests is so extremely important. 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 contaminating honey and other hive products, and promote the well-being of honeybee. Botanical pesticides are safer to user and environment because they are biodegradable and break down into harmless compounds within few hours in the presence of sunlight (Buss A and Park-Brown S., 2002) [6].

Crude extracts and essential oils have been explored for repellent, fumigant, larvalcidal and adulticidal activities against the various insect orders. Available literature shows that essential oils from thymol, eucalyptus and wintergreen have been commonly used for treating honeybee afflictions, including infestations of parasite mites (Khater, H. F., 2012) [12]. 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) [2, 5]. However, very little information is available on the comparative efficacy of different plant products against the larval mortality of the greater wax moth, *Galleria mellonella* L. Therefore, this study adopted to evaluate and identify the effectiveness of some plant extracts against the larvae of greater wax moth, *Galleria mellonella* under laboratory conditions.

Materials and Methods

Study location

The experiment was conducted in the laboratory of Holeta Bee Research Center during the year of 2016- 2018.The objective of this study was to evaluate and identify the effectiveness of some plant extracts against the larvae of greater wax moth, *Galleria mellonella.*

Collection of plant materials

Fresh leaves for extraction were collected from mature plants 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.

Table1: Plant names and parts used for essential oils extraction and treatment of *Galleria mellonella*

Scientific name	Common name	Family name	Parts of plant used
Azadirachta indica	Muka Niimii	Meliaceae	Leaves
Ocimum basilicum L	Damaa kasee	Lamiaceae	Leaves
Vernonia amygdalina	Eebicha	*Asteraceae*	Leaves
Calpurnia aurea	Ceekaa	Fabaceae	Leaves
Verbascum sinaiticum benth	Gurra harree	*Scrophulariaceae*	Leaves
Cymbopogon citratus	Xajji saara	*Poaceae*	Leaves
Maesa lanceolata Forssk	Abayyii	Myrsinaceae	Leaves

Processing of plant materials

Fresh leaves of *Azadirachta indica, Ocimum basilicum* L, *Vernonia amygdalina, Calpurnia aurea, Verbascum sinaiticum benth, Cymbopogon citratus and Maesa lanceolata Forssk* were shade-dried and pulverized into fine powder using electric grinder. The resulting powder was sieved using 2mm mesh size and stored in pre-labeled clean plastic bags prior to oil extraction.

Soxhlet extraction of oils

Extraction of essential oils from selected plant materials was carried by soxhlet extraction method using n-hexane as organic solvent at Holeta Agricultural Research Center food science laboratory. 50g of each plant powder was introduced separately into the Soxhlet chamber for oil extraction. In the round-bottom flask, 80ml of n-hexane introduced as solvent. The extraction was done at 60-80 ^{0}C until the solvent in the Soxhlet chamber became transparent. The flask was connected to hexane containing at 2/3 of total volume the extractor until 4 hours. The filtrate was then transferred to rotary evaporator to separate the solvent from the extract. Extracted plant oils were stored in separate labeled bottles until required for bioassay. Then, the extracts were dissolved in distilled water to obtain different extracts of 5%, 10% and 15% for bioassay test on the larvae of greater wax moth, *Galleria mellonella*. Distilled water and n- hexane solvents were used as negative and positive control respectively.

Screening of plant extracts

From the total seven tested plant materials, only five plant materials having positive effect on the larvae of greater wax moth, *Galleria mellonella* were selected and tested.

Collection of the larvae of greater wax moth

Samples of wax combs infested with the larvae of greater wax moth 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.

Bioassay

For the implementation of the study, three different concentrations (5%, 10% and 15%) of each tested plant were prepared in distilled water. The contact toxicity was tested by spraying plant extracts on the larvae of greater wax moth. Treated larvae were kept inside closed petri dish to prevent escaping. Larvae mortality was recorded by counting dead by probing them with a blunt object. The control group was treated with distilled water and n-hexane. A larval was considered dead if it did not move after being probed with a pin turn to black and became softness. Mortality was recorded at 3h, 12h, 24h and 48h post treatment.

Statistical analysis

Data from the three experiments were pooled and to correct for control mortality, mortality, all data was corrected using Abbots formula (1925):

Corrected % mortality =100 x (T %-C %)/(100 %-C %).

Where T % = the percentage of the dead test larvae and C % = the percentage of dead control larvae. The data obtained were subjected to one-way analysis of variance using SPSS version 10.0 (SPSS, 1999). Two-way analysis of variance was used to find out if there is interaction between the plant type, concentrations and exposure times. Tukey's Test was used to separate the means.

Results and Discussion

Effect of tested materials against the larvae of greater wax moth, *Galleria mellonella*

Percent mortality regarding the efficacy of different plant extracts against the larvae of greater wax moth, *Galleria mellonella* was recorded and subjected to statistical analysis. An ANOVA revealed that the interaction of plant type and concentration levels significantly influenced larval mortality of greater wax moth, *Galleria mellonella* ($p< 0.05$). The highest larvae mortality (94.22%) was obtained from *Azadirachta indica* after application of 15% extract with 48h from the interaction effects of plant type and concentration levels followed by *basilicum* (83.76%), *Vernonia amygdalina* (77.37%) and *Calpurnia aurea* (68.33%), while the least larvae mortality (43.66%) was obtained from *Vebascum sinatticum benth* extracts (Table 3). So, the percent larval mortality was greater by the application of higher plant extracts. The larval mortality rate was increased with raising extracts from 5% to 15%. *Azadirachta indica* and *Ocimum basilicum* caused (100%) mortality in the larvae of greater wax moth, *Galleria mellonella* after application of 15% extract within 48h, while the least larvae mortality (42.82%) was obtained from *Vebascum sinatticum bent* within 48h exposure time from the interaction effects of plant type and exposure times (Table 4). Plant extract at higher concentrations caused significantly higher mortality to the larvae of greater wax moth than the lower concentrations (Table 5).

Table 2: Mean square error of plant type, concentration, exposure time and their interaction on larval mortality of greater wax moth, *Galleria mellonella*

Source of variation	Mean value of larval mortality
Type of plant	84.726***
Concentration	42.784**
Time	22.721***
Type of plant * Concentration	17.972*
Type of plant * Time	9.875*
Concentration * Time	8.639*
Type of plant * Concentration * Time	22.095*

Standard error in parentheses (**) =P<0.01 ;(*) =P<0.05; (***) =p<0.001

Table 3: The interaction effects of plant type and concentration level on the larval mortality of greater wax moth, *Galleria mellonella*.

Type of plant	Mean value± SE (%)		
	5%	10%	15%
Azadirachata indica	64.25±1.4^{c}	74.33±3.18^{d}	94.22±0.99^{a}
Ocimum basilicum	52.31±1.8^{b}	63.18±3.85^{c}	83.76±0.35^{b}
Vernonia amygdalina	41.06±2.2^{a}	53.26±4.97^{b}	77.37±3.55^{c}
Calpurnia aurea	29.01±1.2^{c}	48.34±0.31^{b}	68.33±3.3^{c}
Vebascum sinatticum benth	26.67±5.5^{c}	32.90±0.00^{a}	43.66±2.90^{d}
Distilled water (-Ve control)	0.0±0.00^{a}	0.0±0.00^{b}	0.0±0.10^{c}
Hexane (+Ve control)	46.0±0.01^{b}	83.32±0.11^{b}	98.0±3.57^{e}
LSD (5%)	4.70	6.76	9.34
CV (%)	3.56	5.12	7.12

Means followed by different superscript for the given main effect are significantly different (P<0.05).

Table 4: The interaction effects of plant type and exposure time on the larval mortality of greater wax moth, *Galleria mellonella*.

Type of plant	Mean value ± SE (%)			
	3hr	12 hr	24 hr	48 hr
Azadirachata indica	62.23±0.9^{a}	83.52±3.45^{b}	88.12±0.63^{c}	100.00±5.34^{c}
Ocimum basilicum	54.92±0.6^{b}	72.68±0.50^{c}	76.30±1.42^{d}	100.00±2.31^{c}
Vernonia amygdalina	43.16±4.6^{c}	57.61± 1.57^{d}	68.62± 2.49^{d}	83.73± 5.55^{b}
Calpurnia aurea	25.23±1.2^{d}	46.33±0.31^{e}	54.32±3.3^{e}	61.33±2.49^{a}
Vebascum sinatticum benth	18.32±5.5^{e}	24.90±0.01^{f}	35.66±2.90^{f}	42.82±1.47^{d}
LSD (5%)	5.70	9.76	10.52	12.04
CV (%)	2.3	1.8	6.6	7.2

Means followed by different superscript within a column significantly different (P< 0.05)

An ANOVA revealed that the interaction of plant type and exposure times significantly influenced larval mortality of greater wax moth, *Galleria mellonella* (P< 0.05). The highest larvae mortality (100%) was achieved with Azadirachta *indica* and *Ocimum basilicum* at 48h exposure time followed by *Vernonia amygdalina* (83.73%) and *Calpurnia aurea* (61.33%), while the least larvae mortality (42.82%) was recorded from *Vebascum sinatticum bent.* Larval mortality of the greater wax moth increased gradually raising exposure times from 3h to 48h.

Table 5: The interaction effects of concentration and exposure time on the larval mortality of greater wax moth, *Galleria mellonella*.

Conc (%)	Mean value ± SE (%)			
	3hr	12 hr	24 hr	48 hr
5	26.22±0.9[a]	60.00±3.45[b]	68.81±0.63c	79.36±5.34[b]
10	32.41±0.6[b]	68.22±0.51a	84.22±1.42[b]	91.48±2.31[c]
15	52.76±4.6[c]	82.03± 1.57[c]	88.54± 2.49[b]	100.00± 5.42[c]
LSD (5%)	6.5	7.6	8.0	10.87
CV (%)	3.72	4.54	6.25	7.18

Means followed by different superscript within a column significantly different ($P < 0.05$)

An ANOVA revealed that the interaction of concentration levels and exposure times significantly influenced the larval mortality of greater wax moth, *Galleria mellonella* ($p < 0.05$). At 3h post-treatment period, the lowest mortality (26.22%) was achieved at concentration of 5%, while the highest mortality (52.76%) was observed at concentration of 15% of plant extract. At 12h post treatment time, concentration 5% (60%), 10% (68. 22%) and 15% (82.33%) were significantly different ($p < 0.05$). Highest mortality (82.03%) was observed at concentration of 15%. At 48h post-treatment period, mortality ranged from 52.76% to 100%. The percentage of larval mortality was significantly increased vertically by increasing the concentration levels from 5% to 15% and horizontally by increasing exposure times from 3h to 48h. These results indicate that plant extracts at higher concentrations caused significantly higher mortality to the larvae of greater wax moth than the lower concentrations and untreated controls. The current result is in line with the findings of Mohamed (2012) who reported that the effect of plant extract on the percentage of larval mortality of Greater wax moth, *Galleria mellonella* increased by increasing the period horizontally and the extract concentration vertically.

Table 6: Main effects of plant type, exposure times and extract concentrations on the larval mortality of greater wax moth, *Galleria mellonella*

Plant type	Mean value (%)
Azadirachata indica	96.82[a]
Ocimum basilicum	89.14[b]
Vernonia amygdalina	84.52[b]
Calpurnia aurea	79.21[d]
Vebascum sinatticum benth	54.62[e]
ES	1.72
LSD (5%)	2.6
Exposure times (hours)	
3	56.42[b]
12	82.65[c]
24	86.11[d]
48	91.68[a]
ES	0.89
LSD (5%)	3.14
Extracts (%)	
5	48.72[c]
10	88.74[b]
15	100.00[d]
SE	1.23
LSD (5%)	2.27
CV (%)	10.2

An ANOVA revealed that the main effects of plant type, exposure time and concentration significantly influenced larval mortality of greater wax moth, *Galleria mellonella* ($P < 0.05$). The highest larvae mortality (96.82%) was obtained from the main effect of *Azadirachata indica* and the lowest larvae mortality (54.62%) was recorded from *Vebascum sinatticum benth.* Furthermore, the highest larvae mortality (100%) was recorded from the main effect of extract concentration rate of 15%, while the least larvae mortality (48.272%) was obtained from the main effect of 5% extract concentration. 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.

The oil extracts from these plant materials were very potent in controlling the larvae of greater wax moth, *Galleria mellonella. Azadirachta indica* Oil extract was highly active in killing the larvae of greater wax moth, *Galleria mellonella.* This is in agreement with (Mohamed, 2012) who reported that crude oil extract of the *Azadirachta indica* at different concentrations was effective against the larvae of greater wax moth, *Galleria mellonella.* This may be attributed to the fact that neem tree (*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. Essential oils from some medicinal and aromatic plants are known to possess bioactive compounds that are either toxic to a number of insects at various stages of life or elicit anti feedant properties (Huang *et al.*, 2000). According to Asawalam *et al.* (2007), insecticidal activity of any plant extract depends on the active constituents of the plant extract. 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 Recommendation

Plant extracts exhibited variable responses to the larvae of greater wax moth, *Galleria mellonella.* The results from the laboratory test showed that *Azadirachta indica and Ocimum basilicum* extracts caused the highest larval mortality after application of 15% plant extracts within 48h. The effectiveness of these plant oil extracts in controlling larvae of wax moth is probably a reflection of their insecticidal activities. The increasing of the concentration of plant extracts with exposure time increase the larvae mortality of *Galleria mellonella*. That means, the mortality effect depends on type of plants, level of concentrations and exposure of time. Therefore, *Azadirachta indica* and *Ocimum basilicum* extracts can be developed as sources of bio-pesticide for the management of larvae of greater wax moth, *Galleria mellonella*. However, these results need further research in order to identify its effectiveness on the field and on honeybees. Also identification of bioactive compounds from the effective plant oils for the control of wax moth is necessary.

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