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Evaluating the Effect of Plants Extracts Against Varroa Mites (Varroa Destructors) of Honeybees (Apis Mellifera)

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Abstract
The honeybee is the most useful insect to man but their productive potential is reliant on their health conditions. The mites not only cause economic loss but also cause ecological problems related to the role of honey bees, as they are most vital pollinators of the World. The aim of this study was to evaluate and identify the efficiency of some plant-based biocides to develop alternative method of controlling *Varroa destructors* that is the safest to hive products. For this purpose, *Euclaptus globulus, Calpurnia aurea, Ocimum basilicum, Allium sativum, Cymbopogon citratus, Mentha piperita* and *Ocimum gratissimum* were collected, dried under shade and grinded and their oils were extracted by using Soxhlet apparatus and n-hexane as an extractor. The botanical extracted oils were prepared in different concentrations and applied on *Varroa destructors* and *Apis mellifera*. The effectiveness of the extracts against the mites and the safety to the honeybees was observed after 24 and 48 hours, respectively. Plants extracts showed variable responses to *Varroa destructors*. Results showed that *A. sativum*, *E. globulus* and *C. aurea* caused 100±0.0 percent mortality on *V. destructor*s after 24 hours. *C. citratus* extracts of 100 percent dose caused 100+0.0 percent mortality of the mites after application on the mites. *C. aurea* leaf extract may be safer to *A. mellifera*. Bio pesticides can be upcoming pest managing healthier choice. The n-hexane extracts of the *E. globulus, A. sativum* and *C. aurea* plants had insecticidal effects on the insect pests of bees and could be considered as bioactive candidate for management of the pests.
Keywords: Effectiveness, Honeybee, Plant oils, *Varroa destructors*
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1. Introduction

The honeybee is considered of great economic importance 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 the health status of honeybees, because like other living organisms, bees are subjected to many diseases and pests (Ritter and Akratanakul, 2006). Many developing countries are trying to improve the quantity and 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. 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).Using synthetic pesticide against bee pests and diseases cause the risks of contaminating honey and beeswax with residue .Furthermore, beekeepers in developing countries may not be able to afford synthetic pesticides.

Thus, the development and promotion of alternative means of technologies against bee pests is so extremely important. This would have advantages of enhancing environmental quality, economic viability, protect human health and safety by preventing the risk of contaminating honey and hive products, and promote the well-being of honey bees. Botanical pesticides are safer to users 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). Furthermore, botanical pesticides are encouraged over conventional pesticides because, they affect only target pest, effective in every small quantity, decompose quickly and provide the residue free food and a safe environment to life (Pedigo, 1998). When incorporated with integrated pest management programs, botanical pesticides can greatly decrease the use of conventional pesticides (Parmar and Devakumar, 1993; Khater, 2012). For these reasons, the more recent approach to control honey bee pests and diseases has been inclined to use relatively safe natural products of plants and plant derivatives.

Plant based pesticides have been adopted in different countries and cultures with their own specific indigenous knowledge and parallel standards and methods for evaluation. The plants of families Myrtaceae, Lamiaceae, Asteraceae, Apiaceae and Rutaceae are highly targeted for anti-insect activities against specific insect orders like Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera (Khater, 2012).

Crude extracts and essential oils have been explored for repellent, fumigant, larvicidal and adulticidal activities against the various insect orders. Evidence showed that some botanical pesticides are effective against

bee pests and disease (Stanghelliniand Raybold, 2004; Zaitoun, 2007). Essential oils from thymol, eucalyptus and wintergreen have been commonly used for treating honey bee afflictions, including infestations of parasitic mites (varroa and Acarine l) (Khater, H. F. 2012). 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). Plant species including *Olea europae, cuspidata*, *Otostegia integrifolia*, *Azadirachta indica*, *Silenema croserene* and *Echinop* spp. are known to be the most common traditionally used insects repellent plants (Karunamoorthi et al., 2009).

However, scientific evidence about the safety and effectiveness of traditionally used plant products against major pests and pathogens is very limited. Studies to explore the existing traditionally used plant species to the more advanced practice against honeybee pests is absent in the country. Therefore, the aim of this study was to evaluate the effectiveness of the most common traditionally used plant species oil extracts against major pests (*V. destructors, A.* and *A. mellifera)*.

2. Materials and methods

2.1. Study area

The experiments were conducted at laboratory of Holeta Bee Research Centre Oromia Agricultural Research Institute, Oromia regional state in Ethiopia during July 2016–July 2018. Honeybee *A. mellifera* colonies were transferred in Langstroth beehives and naturally infested with *Varroa* destructors.

2.2. Plant parts collection and identification

Seven plant species essential parts were collected based on information obtained from documented indigenous knowledge and literature. The plants selected for the purpose were *E. globulus* (leaves), *C. auria* (leaves), *O. basilicum* (leaves), *A. sativum* (bulbs), *C. citratus* (leaves), *M. piperita* and *O. gratissimum* (leaves). The bulbs of the onion and leaves of the rest selected plants were collected from areas of Oromia region.

2.3. Sample preparation

The collected plants parts were kept at room temperature. Then, the leaves and bulbs were dried under shade and grinded with ultra-centrifugal mill in Holeta Agricultural research Centre animal nutrition laboratory and the grinded sample was stored in a plastic bottle.

2.4. Plant oil extraction

Three gram (3g) of dry sample was weighed to within milligrams in an extraction thimble; it was placed and shaked to dissolve it in the extraction unit. The parts were dissolved in organic solvent (hexane).The flask was connected to hexane containing at 2/3 of total volume to the extractor until 4 hours. When finished, the hexane was evaporated by in rota evaporator. Hexane was removed by rotor evaporation under vacuum and the oil was picked in a vial. The extract was put in vial and stored at 0^{o}C until time for use.

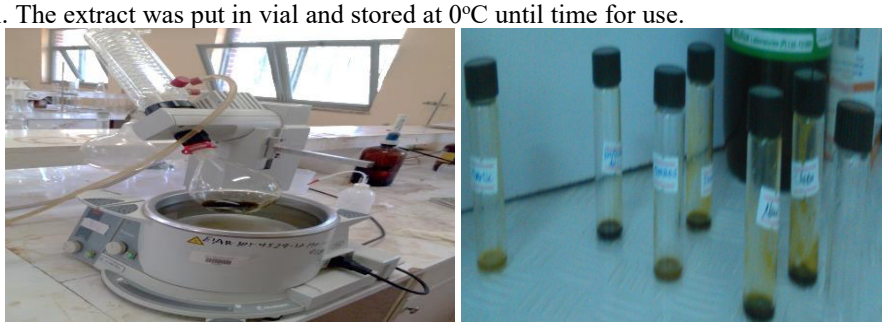


Figure 1. Plant oil extraction (left) and extracted plant oils (right)

The flasks were cooled in a dryer and weighed them then , the extracts were dissolved in distilled water to obtain different concentrations (25%, 50%, and 100%) on Varroa mites for bioassay tests while 100% were used to detect the safety of the extracts on honey bees. The base for concentration levels preparation is literature information. Distilled water and hexane were used as positive and negative control, respectively. The controls were without plant extracts however distilled water was used for concentration levels preparation purpose.

2.5. Screening of plant extracts oils

Even though 10 plant extracts oils were tested, only the seven for *Varroa destructors* with a positive effect were

mentioned in this paper which was effective on the mites and beetles in laboratory.

2.6. Collection of Varroa mite

2.6.1. Adult Varroa mites collection method

To collect the required sample of adult varroa mite for the treatment test (bio-assey test), the standard icing sugar rolling methods was applied according to (Dietemann *et al*., 2013). Samples of 250-300 adult bees was collected from randomly selected bee colonies and brushed off directly into a wide mouth jar with the number 8-mesh lid. About 1 teaspoon powdered icing sugar (at least 7 g) was poured through the mesh and vigorously rolled the jar for 1 minute to distribute the sugar over all the bees. Then, the jar was allowed to sit for a few minutes, and then invert it, and was shacked over a piece of paper to recover the mites.The fallen mites and sugar were placed in a sieve and rinsed with 1x phosphate-buffered saline (or other similar saline solution) to rid them of icing sugar particles, allowing the sugar to escape, but dump the mites on a clean sheet of paper. Finally, the mites were counted and collected in a mite-tight container.

2.6.2. Testing for Bioassay on Varroa destructors

Laboratory bioassay was tested against Varroa mites of honeybees. The experiment was tried on five Varroa mites (*Varroa destructors)*. Fumigation technique was used in all approach. Collection of data was carried out at different time interval after application of different concentrations of plant extracts during bioassay testing of the mites. The population of pests were noted and recorded after each plant extract applications.

Biopesticidal activity of 7 plant extracts was performed using adult fumigation test according to (Drummond et al., 1976). Serial dilutions of plants extracts oils (25%, 50% and 100%) were prepared in distilled water. Antipest effects of each dilution were tested by immersing a group of 5 pests in a Petri dish containing the extracts of the plant species. The experiment was performed in Petri dishes for the times in three replications for each concentration levels. The mortality was recorded by counting dead Varroa mites (alive and dead) and the corrected mortality rate reported as the percent mortality rate of the pests calculated as (Abotts, 1925) as mentioned by equation below:

$$\text{Corrected Mortality}=\frac{\%\text{Treated mortality}-\%\text{control mortality}}{100-\%\text{control mortality}}\text{x}100$$

2.7. Testing of effectiveness against honeybee safety

Laboratory bioassay was tested not only on Varroa mitesbut also on safety of honeybees. The toxicity effect of the plant extracts on honeybees was seen by direct feeding, contact or fumigation. Three hours prior to each feeding trial, 10 adult bees for each treatment were sampled from healthy colonies and brought to the laboratory in cages. Comparative efficacy of the botanical extracts on safety of the plants extracts were also tested on honeybees (*A. mellifera*).

2.8. Statistical analysis

The experiments were conducted in petri dishes to test the efficacy of the plant extracts against Varroa mites and safety of the extracts on honeybees. The obtained results were submitted to one way ANOVA and the mean values were compared by Tukey's test (P=0.05) calculated by the program SPSS 20). Mortality rate was calculated as; mortality is equals to after treatment the number of died mites divided by before treatment the number of mites times 100 percent.

3. Results and Discussions

A total of seven extracts from different medicinal plants were tested for efficacy against V. *destructors*. The efficacy of plants extracts of *E. globulus* (leaves), *C. aurea* (leaves), *O. basilicum* (leaves), A. *sativum* (bulbs), *C. citratus* (leaves), *M. piperita* and *O. gratissimum* (leaves) at a concentration of 25, 50 and 100 % against *V. destructors* (Table 2) doses of the extracts were tested.

The result also showed that *A. sativum*, *E. globulus* and *C. aurea* extracts have pronounced effective followed by *C. citratus* and *O.gratissimum*. Hundred percent mortalities were recorded by *A. sativum*, *E. globulus* and *C. aurea* against *V. destructors* within 20 hours of exposure at a concentration of 25, 70 and 100%. The mite's mortality rates increased as concentration and exposure time increased during the efficient plants extracts were applied.

3.1. Varroa Mites (*Varroa destructors*)

Testing of laboratory bioassay was carried out on varroa mites using different concentration of different plant extracts. For this purpose, five *V. destructors* were collected from honey bees (*Apis melifera*). These extracts were dissolved in water to obtain the required concentrations. Different concentration levels of botanical extracts applied on the mites were 25%, 50% and 100%. The effect of the extracts was observed after 24 hours with three replications and the result is shown in Table 1. Results of this study revealed that *A. sativum*, *E. globulus* and *C.*

aurea extracts caused high percent mortality (100±0.00) of *V. destructors* after 24 hours when 50% concentrations of extracts application. *C. citratus* leave extract caused high percent mortality (100.00±0.00) of the mite population after 100 percent plant extract concentration application in 24 hour. *M. piperita* (93.33±6.67), *O. gratissimum* (86.67±6.67) and *O. basilicum* (80.00±0.00) leaf caused relatively less percent mortality after application on pests after the time interval of twenty four hours.

Table 1. Varroa mite's mortality rate at different concentration level of plant extracts

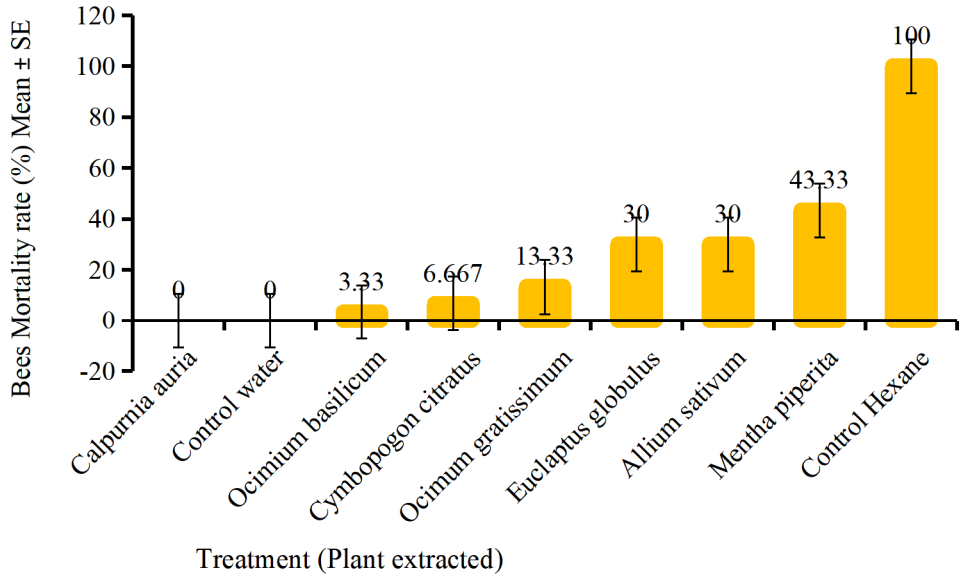
Treatment	N	Mean ± SE mortality rate at concentration level		
		25%	50%	100%
A. sativum	5	46.67±26.67^{b}	100.00±0.00^{a}	100.00±0.00^{a}
E. globulus	5	83.67±6.3^{a}	100.00±0.00^{a}	100.00±0.00^{a}
C.citratus	5	53.33±24.04^{b}	56.67±13.33^{c}	100.00±0.00^{a}
O. gratissimum	5	46.67±17.64^{b}	85.00±2.90^{b}	86.67±6.67^{ab}
O. basilicum	5	33.33±6.67^{b}	60.00±.00^{c}	80.00±0.00^{b}
C. aurea	5	100.00±0.00^{a}	100.00±0.00^{a}	100.00 ±0.00^{a}
M. pepirita	5	80.00±0.00^{b}	80.00±0.00^{b}	93.33±6.67^{ab}
Hexane (standard control)	5	100.0±0.0^{a}	100.0±0.00^{a}	100.00±0.00^{a}
Water (negative control)	5	0.00±0.0^{c}	0.00±0.00^{d}	0.00±0.00^{c}

Means with the same alphabets down the column are not significantly different from each other using Tukey HSD at 0.05.Different options for the control of Varroa mites are necessary because of the rapid and widespread development of pesticides resistant mite populations and because of the potential for pollution of hive products by these agro-chemicals. Essential oils, and especially components of essential oils, may serve as alternatives or as aides to traditional management measures. In extensive screening tests, many oils show significant acaricidal activity. Some oils are repellent to *V. distructors*, others are attractive and some cause mite mortality (Imdorf et.al, 1999).

According to this study results, the efficacy of the botanical extracts of plants used in the experiment against *V. distructors* was *C. aurea, E. globulus, A. sativum* and *C. citratus, M. piperita, O. gratissimum and O. basilicum* in decreasing order.

3.2. Testing safety of plants extracts on honeybees

Adult honeybees were kept at room temperature, without food to make them hungry. Each plant extracts were dissolved with distilled water to be tested. Each liquid sandwich was comprised 100% for each concentration of each extracts, 2 cages, each with 10 honeybees, were fed the liquid sandwiches. The bees were fed on control each time. During this time the numbers of dead and alive bees were monitored within time.



According to this study results, *C. aurea* extract was the safest of all plant extracts to honey bees while *O. basilicum* and *C. citratus* shown less mortality after application in 24 hours intervals. Present results also showed that the plant extracts such as *M. piperita, A. sativum and E. globulus* were unsafe to honey bees at 100 percent

doses after 24 hours' time interval, respectively. The plants extracts oil safety in decreasing order is *C. aurea*, *O. basilicum, C. citratus, O. gratissimum, E. globulus, A. sativum and M. piperita.* This result indicated that the safest and most effective plants species may be used to develop bio pesticides to control pests of honeybees.

5. CONCLUSIONS and RECOMMENDATION

Plant extracts exhibited variable responses to *Varroa destructors* population. *A. sativum (bulb), E. globulus* and *C. aurea* extract caused highest percent mortality on V*arroa destructor* population after application of the 50 percent concentration of plant extracts while C. citratus *leaves* extract killed the highest percent of the mites' population after application of the highest dose of the extracts. *M. piperita, O. gratissimum* and *O. basilicum* leaf extracts caused higher mortality after application on *Varroa destructors. A. sativum (bulb), E. globulus* and *C. aurea* extracts were screened as most effective plant species on *Varroa destructors* but among these plants extracts that of C. *auria* was shown the safest of all extracted oils on honeybees. Therefore, the plant species may be developed as botanical pesticides to control target honeybee pests. The efficacy should be also checked on pests-infested larvae of honeybees. Identification of active components from the effective plants oils for developments of the control of the mites of honeybees and of safety to the pollinators should also be studied in the future.

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