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Exploring the acaricidal potential of 11 ethanolic plant extracts against *Varroa destructor* in *Apis mellifera* colonies

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Abstract
This study explores the efficacy of botanical extracts as sustainable alternatives for managing *Varroa destructor*, a major parasitic threat to honeybee colonies. Bioactive compounds were identified through phytochemical screening, revealing the presence of key constituents like eugenol, cinnamaldehyde, menthol, and curcumin. Laboratory bioassays assessed acaricidal activity, with several extracts showing significant mite mortality while ensuring minimal impact on honeybee health. The research employed maceration techniques for extract preparation, serial dilution for concentration standardization, and bioassays for evaluating efficacy and safety. These findings highlight the potential of plant-based treatments as eco-friendly and sustainable solutions for apiculture, reducing reliance on synthetic chemicals and contributing to the health and productivity of honeybee populations.

Keywords: *Varroa destructor*, honeybee health, phytochemicals, botanical extracts, acaricidal activity, maceration, sustainable apiculture, eco-friendly pest management

Introduction

Honey bees (*Apis mellifera*) are vital to ecosystems, agriculture, and environmental conservation as key pollinators, supporting plant reproduction, biodiversity, agricultural productivity, and global food security (Hung *et al.*, 2018) [9]. Apiculture also contributes significantly to the economy by generating employment and billions in revenue (Karaman *et al.*, 2009) [12]. However, honeybee populations face threats from *Varroa destructor*, a parasitic mite that weakens bees by feeding on their hemolymph, spreading viral pathogens, and causing developmental abnormalities (Le Conte *et al.*, 2010) [15]. Originally native to Asia, this mite has spread globally via international trade, leading to colony health issues, reduced honey production, pollination inefficiency, and colony collapse disorder (CCD) (Flores *et al.*, 2021) [8].

Conventional control strategies like chemical acaricides and cultural practices have limitations, including resistance development, contamination of hive products, and negative effects on bee health. Integrated Pest Management (IPM), combining chemical, biological, and cultural controls, offers more sustainable solutions (Jack *et al.*, 2021) [11]. Recently, plant-based extracts have gained attention as eco-friendly alternatives due to their low resistance risk and suitability for organic beekeeping. Ethnobotanical research has identified bioactive plant compounds as promising acaricides for sustainable mite management, crucial for protecting honeybee populations, ecosystems, and agricultural sustainability (Bava *et al.*, 2023) [3].

This study aims to evaluate the acaricidal effects of 11 ethanolic plant extracts on *Varroa destructor* to develop eco-friendly methods for managing this destructive mite in honeybee colonies.

Table 1: The 11 plants selected for study

Sl no.	Plant	Brief description
1	*Ocimum basilicum*	*Ocimum basilicum*, or Thai basil, is an herb with slender stems and oval-shaped leaves, known for its aromatic scent and culinary use. It contains essential oils rich in linalool and eugenol, which exhibit repellent properties against mites (Łyczko *et al.*, 2020) [16].
2	*Salvia spathacea*	*Salvia spathacea*, also known as Pitcher sage, is a perennial herb featuring lance-shaped leaves and reddish-purple tubular flowers. It contains compounds like flavonoids and terpenoids known for insect repelling effects (Sharma *et al.*, 2019) [23].
3	*Lantana camara*	*Lantana camara* is a perennial shrub with clusters of colorful flowers and ovate leaves. It contains bioactive compounds like flavonoids and terpenoids, that has insect repelling properties (Naz *et al.*, 2013) [19].
4	*Cinnamomum verum*	*Cinnamomum verum*, or true cinnamon, is a small evergreen tree native to Sri Lanka. Its aromatic bark contains cinnamaldehyde and eugenol, which are toxic to mites and disrupt their life cycle (Singh *et al.*, 2021) [24].
5	*Syzygium aromaticum*	*Syzygium aromaticum*, commonly known as clove, is an evergreen tree native to Indonesia. Its aromatic flower buds are high in eugenol, a potent compound, which is effective in controlling mite populations (Mittal *et al.*, 2014) [17].
6	*Mentha spicata*	*Mentha spicata*, or Spearmint, is a perennial herb known for its bright green leaves and aromatic fragrance. Essential oils, particularly carvone and limonene, act as natural repellents against several insects (Sulieman *et al.*, 2011) [25].
7	*Citrus limon*	*Citrus limon*, or Lemon, is a small evergreen tree native to South Asia. Lemon tree produces limonene and citral compounds that repel many pests (Pandey *et al.*, 2011) [21].

8	*Azadirachta indica*	*Azadirachta indica*, or Neem, is a versatile tree native to the Indian subcontinent. Rich in azadirachtin and limonoids, that disrupt mite reproduction and act as natural repellents (Susmitha *et al.*, 2013) [26].
9	*Ocimum sanctum*	*Ocimum sanctum*, or Holy basil, is a herb native to the Indian subcontinent. Contains eugenol and other phenolic compounds with strong miticidal properties (Baliga *et al.*, 2013) [2].
10	*Curcuma longa*	*Curcuma longa*, or Turmeric, is a flowering plant native to South Asia. Its bright orange-yellow rhizomes are used as a spice and in medicine due to high content of curcumin (Chanda *et al.*, 2019) [6].
11	*Pinus roxburghii*	*Pinus roxburghii*, or Chir pine, is a tall evergreen tree native to the Himalayas and surrounding regions. The resin, known as pine resin, containing pinene and terpenoids, have toxic and repellent effects on many insects (Kumari *et al.*, 2019).

Materials and methods

Study Area and Site

Apis mellifera workers and larvae, along with adult females of *Varroa destructor*, were collected in March 2024 from Nimit Singh's Madhumakkhiwala bee farm in Bishanpur, Rajauli, Uttar Pradesh (27°05'21.4"N, 81°10'48.0"E; 125m elevation). During this period, the average ambient temperature was 24.52°C (range: 21.6–32.8°C), and relative humidity averaged 65.38% (range: 61–68.5%). The experiments were conducted in the Zoology Department Laboratory at Babasaheb Bhimrao Ambedkar University, Lucknow.

Collection and processing of plant material

Essential parts from 11 plant species were collected based on indigenous knowledge and literature. The parts used included flowers and seeds from *Ocimum basilicum* and *Ocimum sanctum*, flowers from *Salvia spathacea* and *Lantana camara*, bark from *Cinnamomum verum*, buds from *Syzygium aromaticum* and *Citrus limon*, leaves from *Mentha spicata* and *Azadirachta indica*, rhizome from *Curcuma longa*, and resin from *Pinus roxburghii*. The plant parts were rinsed, shade-dried, oven-dried at 30°C for 12 hours, and ground into fine powder using a blender and mortar. The powder was sieved, weighed for uniformity, and stored in labeled airtight containers in a cool place.

Preparation of plant extract

The plant extract was prepared through maceration, mixing powdered material with ethanol (1:4 w/v) in sealed containers stored in a dark, cool place for several days to 2 weeks, with intermittent stirring (Lapornik *et al.*, 2005) [14]. After filtration, the ethanol was evaporated to obtain a concentrated extract, stored in amber vials at 4°C. Concentrations of 25%, 50%, and 100% were prepared using serial dilution: the 100% stock was diluted with ethanol to create 50%, and the 50% solution was further diluted for 25%. This standardized method ensured consistent concentrations for bioassays.

Table 4: Preliminary phytochemical screening for identification of bioactive compounds (secondary metabolites) in plant extracts

Name of bioactive compound	Test conducted
Eugenol (Phenolic compound)	Detected in Tulsi and Thai basil leaves via NaOH and $FeCl_3$ tests (DeFrancesco *et al.*, 2021) [7].
Salvinorin A (diterpenoid furanolactone) & Lantadene (diterpenoid furanolactone)	Identified using sulfuric acid, inducing color changes indicative of furan rings and sesquiterpenes, respectively (Praiwala *et al.*, 2018) [22].
Cinnamaldehyde (phenylpropanoid)	Confirmed with Fehling's solution, observing reactions in heated cinnamon bark extract (Verma *et al.*, 2021) [27].
Menthol (monoterpenoid)	Detected by cooling the ethanol extract of crushed leaves (Brignall *et al.*, 1941) [5].
Limonene (cyclic monoterpene) & Azadirachtin (limonoid or tertranortriterpenoid)	Identified via organoleptic tests by crushing citrus flowers and neem leaves to observe aromas.
Curcumin (polyphenol)	Verified by adding turmeric extract to NaOH, noting color change (Onuegbu *et al.*, 2023) [20].
Camphor (terpenoid ketone)	Detected using sulfuric acid and potassium permanganate, observing reactions (Mosaa *et al.*, 1984).

Collection of mites and bees

Varroa mites were collected from infested honeybee colonies by inspecting brood frames, particularly drone brood cells, which are preferred by mites due to their size and longer developmental period (Boot *et al.*, 1992) [4]. Brood cells were uncapped, and mites were collected with a fine-tipped brush. Additionally, mites on bee bodies were dislodged by shaking infected bees in a jar and separating them with forceps. This method ensured diverse sample for analysis.

The collection of bees involved selecting healthy colonies with robust populations. Bees were gently brushed from various hive locations, including brood frames, honey supers, and entrances to minimize harm. Sampling was conducted at different times of the day to capture variations in foraging behaviour, ensuring a diverse and representative sample of the colony's activities and demographics for analysis.

Laboratory lethality test on varroa mite and honey bees

Laboratory bioassays were conducted to evaluate the acaricidal efficacy of botanical extracts against *Varroa destructor* mites and the potential lethality to honey bees. For the mites, various concentrations of each extract were prepared by diluting in ethanol, and 1 ml of each extract was applied to filter paper in a petri dish. After the ethanol evaporated, five mites were placed on each plate along with bee larvae as food, and mite mortality was monitored over 6 hours. Control groups were treated with ethanol alone (Ariana *et al.*, 2002) [1]. For the honey bees, varying concentrations of the extracts were tested in petri dishes over 48 hours, with five worker bees per dish. A nylon mesh cover prevented escape, and bees were provided with a fresh 1:1 sugar solution every 6 hours. Bee behavior, mortality, and sublethal effects were observed and recorded during the bioassay. (Figure 1 & Figure 2)

$$Mortality(\%) = \frac{\text{no. of dead mites}}{\text{total no. of mites}} \times 100$$

If 5 to 10 % mortality occurs in the control, the percentage mortality in all the trial batches of mites were modified using Abott's calculation in (FAO Module-1, 2001).

$$Mortality(\%) = \frac{\%\ mortality\ in\ test\ concentration - \%mortality\ in\ control}{100 - \%\ mortality\ in\ control} \times 100$$

Fig 1: Lethality test on *Varroa destructor*

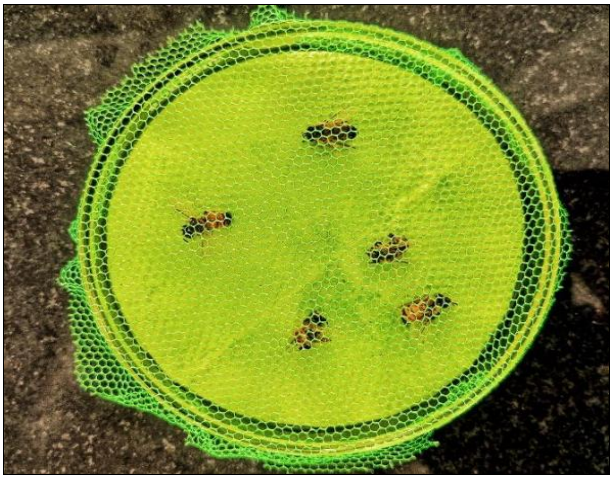


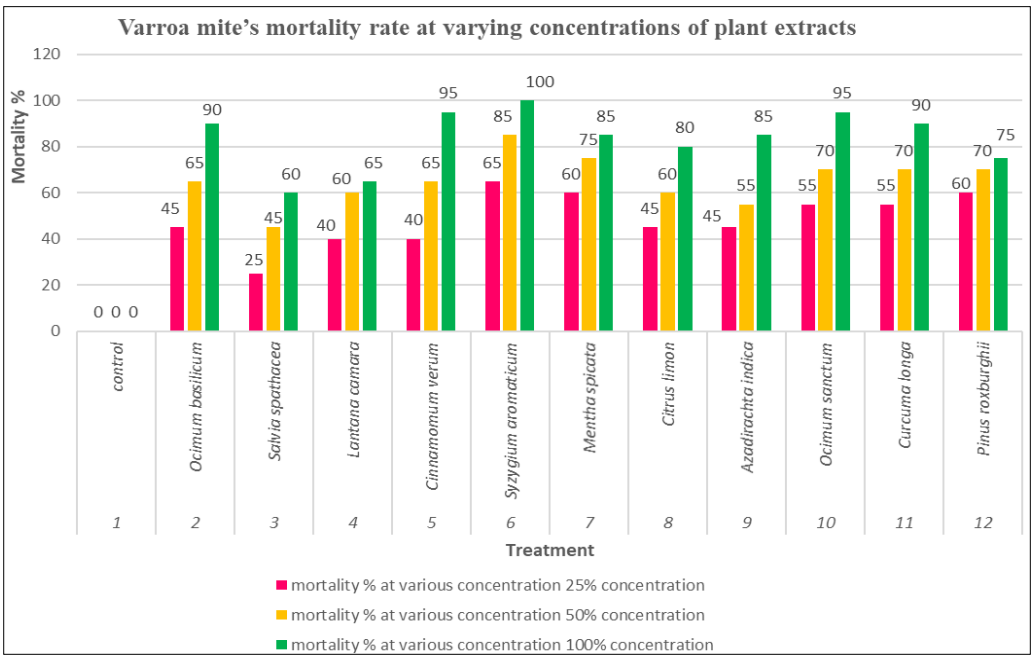
Fig 2: Lethality test on *Apis mellifera*

Results and Discussion

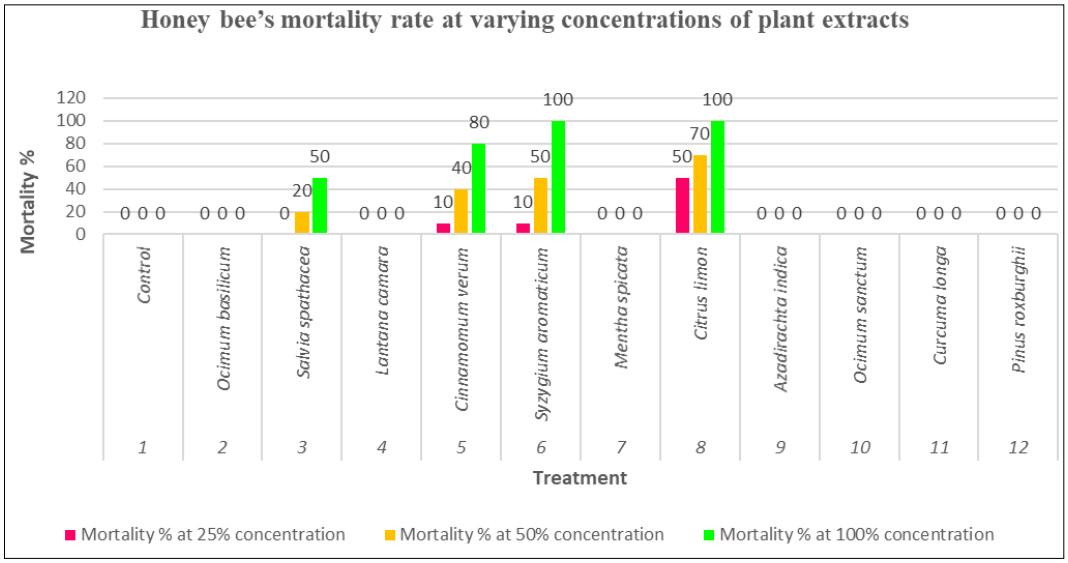
The preliminary phytochemical screening revealed several active compounds in the plant extracts. Eugenol, identified by reddish brown and bluish color changes with sodium hydroxide and ferric chloride respectively, suggests phenolic compounds. Salvinorin A was indicated by a green or blue color change with sulfuric acid, and lantadenes by an orange or purple color change. Cinnamaldehyde formed a brick-red precipitate in Fehling's solution. Menthol showed crystalline deposits upon cooling, while limonene gave a citrusy aroma and neem had a bitter taste and pungent odor. Curcumin reacted with an alkaline solution to form a reddish-brown color, and camphor produced a violet or blue coloration, indicating terpenoids. These findings highlight the potential bioactive constituents in the extracts.

Syzygium aromaticum exhibited the highest mortality which is in line with findings of Ismail *et al.*, 2006 [10], reaching 100% at 100% concentration, followed by *Ocimum sanctum* and *Cinnamomum verum* at 95% which are similar to findings of Vijayan *et al.*, 2023 [28]. *Pinus roxburghii* had the lowest mortality at all concentrations, with a maximum of 75%. Overall, treatments varied in their effectiveness against mite populations across different concentrations.

Salvia spathacea and *Syzygium aromaticum* showed increasing mortality with higher concentrations, reaching 50% and 100% at 100% concentration, respectively. *Citrus limon* exhibited the highest mortality across all concentrations, reaching 100% at 100% concentration. Conversely, some treatments showed no bee mortality at any concentration.



Graph 1: The above bar graph displays the mortality percentages of mites at different concentrations of treatments



Graph 2: The above bar graph summarizes bee mortality percentages at different concentrations for each treatment

Overall, the findings of this study contribute to the growing body of research on natural alternatives for Varroa mite control in beekeeping. The acaricidal properties of ethanolic plant extracts offer a promising avenue for developing sustainable and environmentally friendly strategies to mitigate the impact of *Varroa destructor* on honeybee populations. However, further research is warranted to elucidate the mechanisms of action of these botanical extracts and optimize their efficacy for practical application in beekeeping practices.

Conclusion

In this work, the acaricidal activities of ethanolic plant extracts on *Varroa destructor*, which is a significant hazard to honeybee colonies, were studied. Phytochemical screening showed active compounds such as phenolics, terpenoids, and alkaloids that have established acaricidal activities.

Bee lethality assays indicated that *Syzygium aromaticum*, *Cinnamomum verum, and Citrus limon* extracts registered impressive mite killing at higher concentrations. Repellency tests also indicated that *Ocimum basilicum* and *Syzygium aromaticum* exhibited intense mite-repelling activities.

Natural acaricides derived from plants have potential benefits including environmental safety, sustainability, and lowered chemical resistance. They are biodegradable, inexpensive, and less harmful to ecosystems and non-target organisms.

In general, the research provides evidence for the efficacy of plant-derived extracts as environmentally friendly *Varroa* mite control agents. More work is required to elucidate their mechanisms and make them more usable in beekeeping practice.

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