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Laboratory Evaluation of Some Botanicals against the Honey Bee Ectoparasitic Mite *Varroa Destructor* (Acari: Varroidae) and its Impact on Honey Bees (*Apis cerana indica*)

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ABSTRACT

Background: Honey bees are one of the most important and efficient insect pollinators of food crops on earth providing honey, bee wax, bee pollen, royal jelly, propolis, *etc*. *Varroa destructor* is the most obnoxious pest of honey bees infliciting damage to the colony vis-à-vis transmitting viral diseases. The repeated use of synthetic acaricidal treatment results in developing resistance against varroa mite followed by residue hazards in bee products. This leads to an urgent need to develop alternate eco-friendly methods to manage the pest.

Methods: The experiment was designed to evaluate the relative efficiency of six botanical extracts *viz*. Sweet basil (*Ocimum basilicum* L.), turmeric (*Curcuma longa L.*), Coleus (*Coleus aromaticus*), Sweet flag (*Acorus calamus* L.), Tulsi (*Ocimum sanctum* L.) and goat weed (*Ageratun conezoides* L.) against Varroa mite (*Varroa destructor*) under laboratory conditions. After preliminary dose-setting experiments, mites and honeybees were exposed to 2% ethanol extracts of the plants, with 24 hours' exposure time.

Result: 2% concentration of Coleus, Sweet flag and Tulsi registered cent percent mortality of mites within 24 hours after treatment followed by turmeric (93.33%), sweet basil (86.67%) and goat weed (80%). All the extracts exhibited <30% mortality of honey bees (*Apis cerana indica*) whereas turmeric showed the lowest efficiency (14.29%).

Key words: *Apis cerana indica,* Botanical extracts, Per cent mortality, *Varroa destructor.*

INTRODUCTION

Honey bees are of great economic importance to agriculture, not only by providing honey but also by pollinating crops. Apart from honey, they also provide bee wax, bee pollen, royal jelly, propolis, *etc*. However, domesticated honeybees started declining all over the world, due to colony collapse disorder (CCD), a syndrome caused by different types of stress as well as pathogens and pests (Cox-Foster *et al*., 2007). Among these, the ectoparasite mite *Varroa destructor* (Mesostigmata:Varroidae) has been considered as the most obnoxious enemy by most honey bee researchers. (Dietemann *et al*., 2012).

Varroa mites cause damage to the honey bee colony directly through feeding on the haemolymph of adults and brood, preferring the later, especially drone brood (Plate 1) and indirectly by transmitting viral diseases such as Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), deformed wing virus (DWV), *etc*. (Boecking and Genersch, 2008). Infested colonies may die or migrate, resulting in economic loss and decreased honey production (Needham, 1988).

Several acaricides of natural origin are used for the control of Varroa mites in domesticated colonies (Calderone and Spivak, 1995; Sammataro *et al*., 1998). Organophosphates such as coumaphos; pyrothroids such as tauflavinate; and formamidine such as amitraz (Miani and Llob,1998, Li *et al*., 2017) were some of the commonly used synthetic acaricides against honey bee ectoparasitic mites. The repeated use of synthetic varroacides results in the development of resistance in the varroa mite to these products (Thomson *et al*., 2002). Apart from this, the use of synthetic acaricides causes residue hazards as traces of these pesticides are reported in bee products (Wallner, 1999; Howis and Nowakowski, 2009). Therefore, it is very pertinent

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that beekeepers have rule out new control tactics that is not detrimental to bees as well as contaminate hive products.

Plant derived products have shown toxicity, repellence, antifeedant and growth regulatory properties against insect pests and mites (Ntalli *et al*., 2022; Aivazi and Vijayan 2009; Ciccia *et al*., 2000; George *et al*., 2014); thus can be thought of as a desirable alternative. Therefore, the present work has been designed to screen and evaluate six easily available botanical extracts that have varroicidal action with little or no hazardous effects on honeybees.

MATERIALS AND METHODS

Experimental location

The present study was conducted in the Apiary unit located at Tamil Nadu Agricultural University, Coimbatore (11°N, 77°E), Tamil Nadu, India during 2022-2023.

Preparation of botanical extracts

The plant samples (Table 1) were obtained from the field of germplasm collections maintained by the department of medicinal plants and aromatic crops, TNAU, Coimbatore. The collected plant samples were shade-dried and pulverised using an electrical mixer. Microwave-assisted extraction is used for extracting the samples. 70% ethanol was used as the solvent. The extract thus obtained was filtered with Whatman No.1 filter paper and allowed to dry following evaporation. The crude extractives are then stored in glass vials and are further used for making stock solutions, from which further working solutions of different concentrations are formed.

Mites and bees

The mites (*V. destructor*) were collected from colonies of *Apis mellifera* and *Apis cerana indica* maintained at Tamil Nadu Agricultural University, Coimbatore. To collect Varroa mites from adult bees, bees were collected in a screen capped plastic can and shaked vigorously with powdered sugar to dislodge mites. Mites from broods were collected carefully with the help of forceps and a “00” camel hair brush by opening drone brood cells and removing the larvae or pupae (Plate 2).

Screening tests

The bio-effectiveness of essential oils against *Varroa* has been determined using a complete exposure method (Lindberg *et al*., 2000). The experiment was conducted in a completely randomised design under laboratory conditions (28±1°C, 70±5%) with three replications. Each replication consisted of an insect rearing petri dishes (10 cm diameter × 4 cm height) with filter paper treated with botanical extract placed at the bottom. Each filter paper was treated with 500 microliters of plant extract diluted with ethanol and kept for 5-10 minutes to evaporate the ethanol. Another similar container treated with 70% ethanol was used as a check. After complete evaporation of solvent, five adult *Varroa* mites were released in each container. A pupa of *Apis mellifera* is kept as a host and the lid is closed. Humidity is maintained by keeping wet filter paper below the lid of the

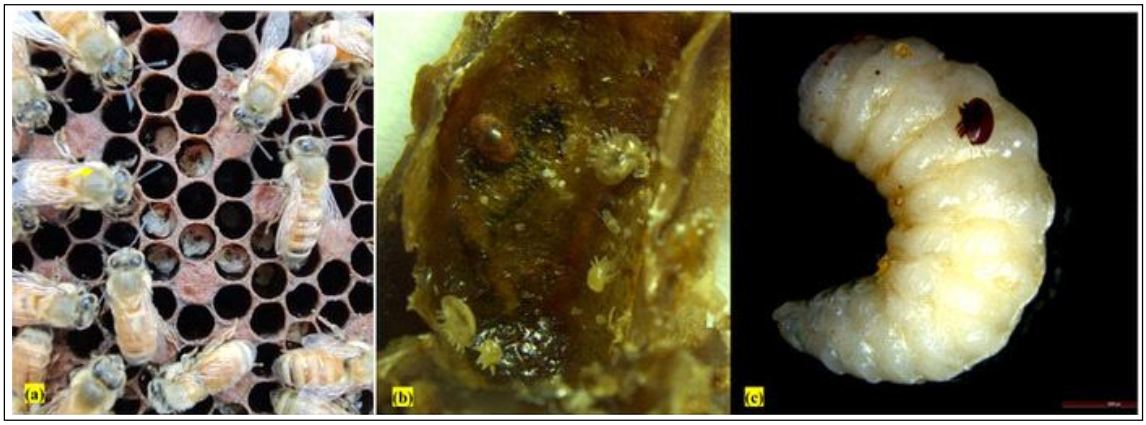

Plate 1: (a) Varroa mite damage symptoms in *A. mellifera*. (b) A family of Varroa mites in a drone brood cell (c) Adult gravid female of *Varroa destructor* on larvae of *A. mellifera.*

Table 1: Plants and plant parts evaluated for varroicidal activity.

Scientific name	Common name	Famliy	Parts used for extraction
Ocimum *basilicum* L.	Sweet basil	Lamiaceae	Leaves
Curcuma longa L.	Turmeric	Zingiberaceae	Rhizome
Coleus aromaticus	Coleus	Lamiaceae	Leaves
Acorus calamus L.	Sweet flag	Acoraceae	Rhizome
Ocimum sanctum L.	Tulsi	Lamiaceae	Leaves
Ageratun conezoides L.	Goat weed	Asteraceae	Leaves

container. Afterwards the mortality of mites was recorded at 1, 2, 4, 6, 12 and 24 hours after treatment (HAT). Mites were considered dead when they became immobile and showed no signs of activity even after being stroked with a single hair brush. Similarly, the mortality of honeybees has also been recorded giving the same treatment followed by releasing five adult worker bees in insect rearing container (diameter = 10 cm, height= 4 cm). The bees present in insect rearing cages was provided with sugar solution and the number of dead bees after 24 hours of treatment was recorded to evaluate relative efficiency of botanicals on honey bees (Plate 3).

Data analysis

The per cent corrected mortality of honeybees was done using Abott's formula and *arc sin* transformation is adopted for the data transformation of percentage (Abott,1925). Analysis of variance (ANOVA) was done in IBM SPSS v.20 statistical program and used Tukey's honestly significance difference (HSD) ($p<0.05$) for the comparison of means.

RESULTS AND DISCUSSION

The enumerated cumulative mortality value recorded at each observation time against the treatments are presented in Table 2. Extracts of sweet basil, turmeric, coleus, sweet flag, tulsi and goat weed showed significant variation in mite mortality from control. At one HAT, only sweet flag and tulsi plant extracts registered mite mortality, with a mean mortality of 33.33% and 6.67%, respectively. At 2 HAT, the order of efficiency was sweet flag (60%), followed by coleus (33.33%), turmeric (20%), goat weed (13.33%) and tulsi (6.67%). Sweet basil extract didn't show any mortality and did not varied significantly from control. Likewise, at 4 HAT,



Plate 2: Varroa mites collected for bioassay.

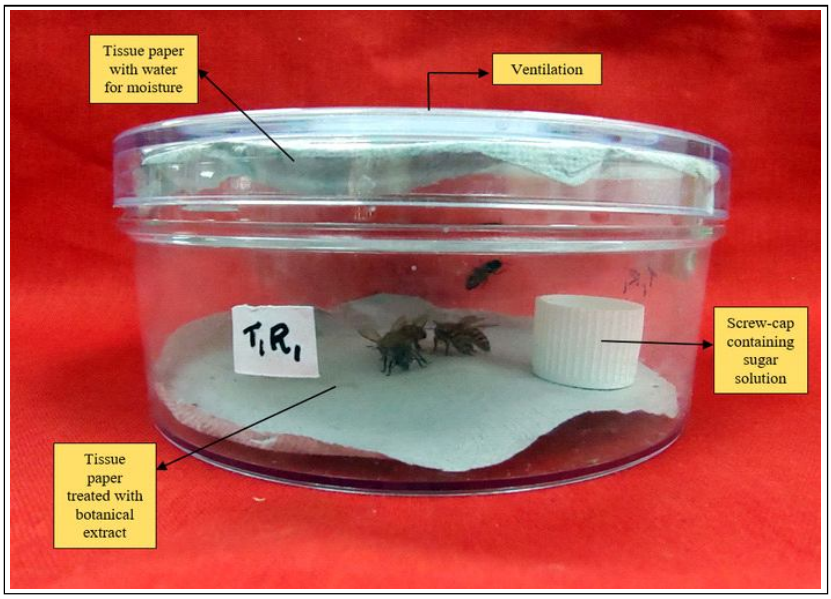

Plate 3: Experimental set up for bioassay of bees.

sweet flag registered more than 90% mortality (93.33%), followed by coleus with more than 50% mortality (66.67%). At 6 HAT, the order of descending toxicity was: sweet flag (100%), followed by coleus (86.67%), turmeric and tulsi (53.33%), goat weed (40%) and so on. All the treated plant extracts registered more than 50% mortality, whereas coleus and sweet flag showed resulted in to mortality to the tune of 100% at 12 HAT. At 24 HAT, coleus, sweet Flag and tulsi extracts recorded 100% mortality, followed by turmeric (93.33%), sweet Basil (86.67%) and goat weed (80%). There was a noticeable change in mortality with prolonged exposure in all the treatments and no mortality was observed in the untreated control up to 24 HAT.

Mean per cent mortality of *Apis cerana indica* workers after exposure to 24 h of botanical extracts have been depicted in Table 3. All the extracts registered <30% mortality of honey bees. The lowest mortality was to the tune of 14.29% in case of turmeric followed by sweet basil and coleus (19.05%). Goat weed (28.57%), sweet flag (28.57%) and tulsi (23.81%) showed relatively high toxicity to bees.

The bioassay experiment was conducted through a simple and rapid methodology to rule out some of the botanical extract for their varroicidal activity. High varroicidal activity was recorded in Sweet flag, more than 50% mortality at 2 HAT and also 100% mortality at 3 HAT followed by coleus which registered a 100% mortality at 12 HAT. Perusal of available literatures reveal that, scanty information are available in this direction on the varroicidal action of sweet flag (*Acorus calamus*) and coleus (*Coleus aromaticus*), though there are numerous studies conducted with the aforesaid ingredients for the management of storage pests (El-Nahal *et al*., 1989; Singh *et al*., 2021; Shukla *et al*., 2016) and plant mites (Pushpa and Nandihalli, 2008; Thevan *et al*., 2005; Janlaor and Auamcharoen, 2021). Swamy *et al*. (2006) reported that sweet flag (*Acorus calamus*) and tulsi (*Ocimum sanctum*), along with some other botanicals, can be used for the effective management of greater wax moth, which is another serious pest of honey bees. Dusting of sugar powder, sulphur and *Acorus calamus* as a method to control varroa mites is also stated by Tej *et al*. (2017). Many researchers reported δ-Asarone as one of the major constituents of *Acorus calamus*, which has insecticidal and miticidal action (Venskutonis and Dagilyte, 2003; Liu *et al*., 2013). According to Dutta (1959), *Coleus aromaticus*, native of India, is rich in carvacrol, thymol, eugenol and chavicol. Thymol is a volatile monoterpenoid and a natural constituent of thyme *(Thymus vulgaris)*. It is one of the most widely used essential oils for varroa mite management (Glavan *et al*., 2020; Gregorc and Planinc, 2012). Vimla and Khan (2013) reported mite mortality ranging from 56.85 to 75.03 per cent when treated with tulsi oil and turmeric oil in the hive. In one of the experiments, hives treated with garlic oil, turmeric oil, tulsi oil, ajwin oil, cinnamon oil and clove oil showed mite mortality ranging from 65 to 77% with no bee mortality in any of the treatments (Goswami *et al*., 2014).

Table 2: Mean per cent mortality of *Varroa* mites after exposure for 24 h in case of various botanical extracts under laboratory ambient condition.

Treatment	Cumulative mean per cent mortality** at HAT					
	1	2	4	6	12	24
Sweet Basil (*Ocimum basilicum* L.	0(1.28)^{b}	0(1.28)^{d}	13.33(18.14)^{bc}	13.33(18.14)^{cd}	60(51.15)^{a}	86.67(71.86)^{ab}
Turmeric (*Curcuma longa* L.)	0(1.28)^{b}	20(26.57)^{bc}	40(39.23)^{bc}	53.33(46.92)^{bc}	80(72.22)^{a}	93.33(80.29)^{ab}
Coleus (*Coleus aromaticus*)	0(1.28)^{b}	33.33(35.01)^{ab}	66.67(55.37)^{ab}	86.67(71.86)^{ab}	100(88.72)^{a}	100(88.72)^{a}
Sweet flag (*Acorus calamus* L.)	33.33(35.01)^{a}	60(50.77)^{a}	93.33(80.29)^{a}	100(88.72)^{a}	100(88.72)^{a}	100(88.72)^{a}
Tulsi (*Ocimum sanctum* L.)	6.67(9.71)^{b}	6.67(9.71)^{cd}	20(22.36)^{bc}	53.33(46.92)^{bc}	73.33(63.8)^{a}	100(88.72)^{a}
Goat weed (*Ageratun conezoides* L.)	0(1.28)^{b}	13.33(18.14)^{bcd}	26.67(26.58)^{bc}	40(39.23)^{c}	53.33(46.92)^{a}	80(63.44)^{b}
Control	0(1.28)^{b}	0(1.28)^{d}	0(1.28)^{c}	0(1.28)^{d}	0(1.28)^{b}	0(1.28)^{c}
SEd	5.04	6.76	11.85	8.85	12.42	6.37
F value	12.535	14.767	9.814	22.607	11.891	48.627
p(≤0.5) (Significance)	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

Values are mean of three replications; Figures in parentheses are *arc sin* transformed values; Mean(s) followed by same letters within the column are not significantly different according to Tukey's test at p≤0.05; HAT- Hours after treatment; SEd-Standard error of the difference; **Highly significant at p (≤0.5).

Table 3: Mean per cent mortality of worker honeybees after exposure to 2% concentration of plant extracts under controlled conditions.

Target	Mean percent mortality at 24 HAT							SEd	F value	p(≤0.5)
	Sweet Basil	Turmeric	Coleus	Sweet flag	Tulsi	Goat weed	Control			
Honey bee	19.05	14.29	19.05	28.57	23.81	28.57	0.00	5.24	4.82	0.007*
(*Apis cerana indica*)	(17.95)^{ab}	(13.26)^{ab}	(17.95)^{ab}	(25.98)^{a}	(22.63)^{a}	(25.98)^{a}	(1.28)^{b}			

Values are mean of three replications; Figures in parentheses are *arc sin* transformed values; Mean(s) followed by same letters are not significantly different according to Tukey's test at p≤0.05; HAT- Hours after treatment; SEd-Standard error of the difference; *Significant at p (≤0.5).

The variability of the effects of these botanicals and essential oils on mites and bees in various studies is perhaps due to the change in plant constituents at different locations as well as the age of the plant. Also, differences in concentration and application methods may play a role. (Ariana *et al*., 2002). In the present experiment, mortality of the mites and bees was the only observation taken, but there is also a chance that some of the constituents of plant extract may also have sub lethal effects on Varroa as well as on honeybees. Colin (1990) and Kraus *et al*. (1994) reported the inhibitory action of thymol and menthol on the growth and reproduction of insect and mite pests. Further investigations should be done to study these effects on mites and bees.

CONCLUSION

The present study gave a relative efficiency analysis of some of the plant extracts for the management of varroa and suggests the possibility of using them in the integrated management of varroa mites in honey bee colonies; however, more work need to be done to optimise the correct dose of treatment and application method at field level. Further research should be done to study its effect on bee survival and reproduction for a prolonged exposure.

Conflict of interest

All authors declared that there is no conflict of interest.

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