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Efficacy of plant extracts against Varroa mites (*Varroa destructor*) of honey bees (*Apis mellifera* L.)

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Abstract

The acaricidal and insecticidal effects of 12 selected botanicals against *Varroa destructor* and *Apis mellifera* were investigated. Crude ethanol leaf extract of botanicals was tested for acute toxicity using the full exposure method in a 16×4×3 Factorial Complete Randomized Design including negative control (water) and three commonly used chemical miticides (amitraz, tau-fluvalinate, and thymol). The results revealed four promising miticides whose efficacy of plant extract, as measured by LC_{50}, was in the following order of potency: *Ocimum basilicum (*biday*)(*15.80)> *Vitex negundo (*lagundi*) (23.79)* > *Cymbopogon citratus (*lemon grass*)* (27.73) >*Gliricidia sepium* (28.83). Due to their high selectivity index, they are also considered less toxic to honey bees.

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Introduction

Varroa destructor Anderson and Trueman (Parasitiformes: Varroidae) is an ectoparasitic mite of *Apis mellifera* L. (Hymenoptera: Apidae), that is currently regarded as the greatest threat to apiculture. It is considered as the major cause of colony collapse in various parts of the world. Mites feed on honey bee hemolymph and fat body cells/cellular components, causing physical harm and spreading lethal honey bee viruses such as Deformed wing virus (DWV) and Varroa destructor virus-1 (Posada-Florez *et al.*, 2020) necessitating Varroa treatments on a regular basis.

Throughout the years, numerous treatments, such as formamidine amitraz, pyrethroid tau-fluvalinate, organophosphate coumaphos, and even soft compounds, such as formic and oxalic acids, and thymol, have been developed to combat Varroa infestations. These are also the commonly used acaricides by local producers particularly in Region 1 Philippines, however, none of these alternatives provide honey bee's protection to Varroa destructor with long-term viability. On the contrary, it has been reported that V. destructor develops resistance to these chemicals. Miticide residue in wax can harm bees and make them more susceptible to disease, as well as reduce the value of honey and other bee products. Fluvalinate and coumaphos are the most widely used mite treatments; however, mites have developed resistance to them, and their residues remain in wax even in locations where they have not been used for years (Underwood *et al.*, 2022). Popular synthetic acaricide, amitraz may not persist as a contaminant in honey or wax in its original form, nonetheless, some amitraz metabolites have been found to persist, and a synergistic effect between amitraz and viruses has been linked to an increase in bee mortality (Underwood and López-Uribe, 2022). Soft acaricides, such as formic acid, caused short- and long-term memory impairment in bees at sub-lethal doses (Gashout *et al.*, 2020). High and sub-lethal doses of oxalic acid caused severe and irreversible internal tissue damage or disruption of the cuticle's proteolytic activity, impairing bee immunity (Rademacher *et al.*, 2017). According to Carayon *et al.* (2014), thymol disrupted honeybee phototactic behavior and remained in wax one year after treatment. Sub-lethal effects of thymol accumulation in honeybee wax have been discovered, including a low larval survival rate, delayed vitellogenin expression, and altered memory traces (Chapuy *et al.*, 2019). Thymol binds to dopamine receptors, causing honeybees to be irritated and changing the flavor of honey (Mondet *et al.*, 2011; Blenau *et al.*, 2012).

Currently, there is no viable, long-term method to reduce mite pressure without inducing honeybee resistance or harming them. Consequently, the potential for developing alternative tools to disrupt the parasitic life cycle remains open. In terms of identifying new options for Varroa mite control, the use of botanical pesticides (plant extracts) is a helpful tool to complement the control of V. destructor with tools other than chemically synthesized acaricides, a growing alternative for the management of this pest. Numerous studies have been conducted on pesticide-containing plant species known and unknown.

Plants are the most significant source of chemical compounds because they serve as natural laboratories where a variety of chemicals are produced. All plant species produce essential substances as a result of primary plant metabolism. On the other hand, the by-products of secondary metabolism are neither essential nor present in all plants. Alkaloids, steroids, tannins, flavonoids, terpenoids and saponins, phenols and resins are examples of secondary metabolites found in plants that have been shown to have insecticidal properties (Isman and Machial, 2006). These substances have the ability to poison arthropods through ingestion, contact, or inhalation. They also have the ability to repel and stop insects from feeding, which reduces their ability to reproduce and alters their normal behavior (Weaver and Subramanyam, 2000). The modes of action can include repulsiveness, inhibition, denatured proteins, and other consequences.

Plant extracts have the potential to be effective acaricides because they contain secondary

compounds that may be responsible for their acaricidal effects, such as terpenes, stilbenes, coumarins, acids, alcohols, sulfide compounds, tannins, and aldehydes (Gonzales-Lopes *et al.*, 2019). Benelli *et al.* (2016) identified compounds responsible for the acaricidal property that were specifically found in some botanicals. These compounds included eugenol from *Ocimum* and *Artemesia spp.*, pyrethrin from chrysanthemums, azardachtin from *A. indica*, citronellal from *C. nardus*, carvacrol and thymol from *Origanum spp.*, eucalyptol and linalool from *Ocimum spp*, and geraniol from *Cymbopogan spp.*

The acaricidal effect of 12 promising plants against the *V. destructor* mite was evaluated in vitro at various extract concentrations and contact times, and the insecticidal effect on honey bees was also evaluated.

Objectives

1. Determine the plant's secondary metabolite components;
2. Evaluate the effects of botanical extract concentration and exposure period on mite and bee mortality;
3. Determine the toxicity of botanical extracts to honey bees, and;
4. Select the most effective botanical extracts against Varroa mites of honey bees.

Material and methods

Research design

The experiment was conducted following 16 × 4 × 3 Factorial in CRD to screen 12 botanical extracts, with four concentrations and three contact times. Included in the treatments are four controls consisting of distilled water as the negative control and amitraz, apistan and thymovar as positive controls used according to the manufacturer's recommendation.

Selection and identification of the plant materials

The plants having insecticidal or acaricidal characteristics were chosen based on scientific and ethnomedical information in the literatures, preliminary interviews on traditional ethnobotanical knowledge, and familiarity with regional sources. The following are the candidate plants, along with their plant families and common names:*Premna odorata*: Lamiaceae *(Alagaw)*; *Ocimum bacilicum*: Lamiaceae (*Biday*); *Origanum vulgare*: Lamiaceae (Oregano); *Vitex negundo*: Lamiaceae (*Lagundi*); *Artemisia vulgaris*: Asteraceae (*Herbaka*); *Gliricidia sepium*: Fabaceae *(Kakawate)*; *Cymbopogon citratus*: Poaceae *(Lemon grass)*; *Cymbopogon nardus*: Poaceae (Citronella); *Swietenia macrophylla:* Meliaceae (Mahogany); *Azadirachta indica*: Meliaceae (Neem);; *Blumea balsamifera*: Asteraceae (*Sambong*); *Nicotiana tabacum*: Solanaceae (Tobacco).

Plants were collected from their natural growing soils in Bacnotan, La Union, Philippines in the months of May and June of 2020. Voucher specimens were brought to the College of Agroforestry and Forestry, of the Don Mariano Marcos Memorial State University (DMMMSU) for authentication of the identity of the plants.

Plant extract preparation

Fresh mature plant leaves were selected and rinsed with tap water to remove dirt and soil particles. Then leaves were air dried in the shade for two weeks followed by oven drying at 40° Celsius for one to two hours. After which the leaves were ground in an electric grinder and stored in a zip lock bag in the fridge until needed.

The powdered leaves were extracted with 95% ethanol. In an Erlenmeyer flask, 60 grams of powdered plant were placed, and ethanol was added until the contents reached 100 ml. The mixture was thoroughly mixed, then the flask was covered with an aluminum sheet to prevent direct light exposure and placed in a refrigerator for 48 hours for maceration. The mixture was then subjected to vacuum filtration using filter paper (Whattman No. 01) in a porcelain Buchner funnel filter (100mm diameter) connected to a 220 volts vacuum pump (GAST, USA) via 10mm diameter rubber hose. The extract was poured into a

15-mm Petri plate to let the solvent evaporate under fume hood for 24 hours to yield a raw thick extract. The extracts were kept in the fridge in labeled jars.

Phytochemical analysis

Chemical tests of ethanol extracts of plants were carried out in the College of Health Sciences, Mariano Marcos Memorial State University using commonly employed precipitation and coloration reaction to detect the presence of bioactive compounds believed to have acaricidal activities: alkaloids, saponins, phenols, flavonoids, steroids, anthraquinones and protein. It was done qualitatively, using standard procedures by Himesh *et al.* (2011) and Kaur *et al.* (2011).

Collection of parasites

Varroa mites were collected from heavily infested colonies using Dietemann *et al.* method (2013). Combs containing Varroa-infested broods were brought to the laboratory of the National Apiculture Research, Training & Development Institute (NARTDI). The cells were uncapped, the broods were removed, and the pupae with mites were collected and kept in a Petri dish on bee pupae to avoid starvation of mites.

Toxicity test of plant extracts against V. destructor and Apis mellifera

Plants extracts were diluted in distilled water to four treatment concentrations: 10%, 15%, 20%, and 25% w/v. The negative control was distilled water, while the positive controls were amitraz, apistan, and thymovar, all of which were used according to the manufacturer's instructions.

The acute toxicity test using the full exposure method began one hour after the mites were collected (Zaman *et al.*, 2011). In a Petri dish, 250 µl of a given concentration of leaf extract was pipetted onto a 3cm² paper towel tissue on which 10 active adult female mites, picked out of the pupae with fine bristle brush, were placed. This was replicated three times. The mites were exposed to the extract for 90 seconds, 3 minutes, and 6 minutes.

The mites were removed from the petri dish after the allotted time and placed on a paper towel for 1 minute to absorb excess fluid. Mites were placed on a white pad in a Petri dish and were checked every 15 minutes for the first two hours and every 30 minutes for the remaining two hours. The viability of mites was checked by prodding with a needle, if the inactivation lasted more than four hours after treatment, the mites were considered dead.

Treatments that killed mites in the acute toxicity test were used in subsequent mite and adult bee tests. Filter papers were treated with 0.5 ml of the prepared concentrations and dried at room temperature for 10 minutes before being placed inside glass jars. Ten bees and ten Varroa mites were placed in each 250-ml glass jar. The various botanical extract concentrations were tested in triplicate. In the control glass jars, filter papers were wetted with 0.5ml of water. The deaths were documented 48 hours after treatment. Mites that did not move when prodded were considered dead. The same method was used to assess the toxicity of plant extract to honey bees, but with bees that were not infested with Varroa.

Mortality effect of plants was classified as described by (Chungsamarnyart *et al.*, 1991): High (86-100%); relatively high (71-85%); Moderate (M) (56-70%); Low (31-55%); and Non-significant (NS) (0-30%).

Statistical analysis

The Microsoft Excel database was utilized to store and handle mortality data in its raw format. Version 27 of SPSS Windows was used for data analysis. Using descriptive statistics, the mean mite mortality and related results of the study were expressed in percentage. Mortality rates were subjected to ANOVA using 16 × 4 × 3 Factorial in CRD followed by Tukey's HSD multiple comparisons utilizing STAR Version 2.0.12014. (Biometrica and Breeding Informatics, PBGB Division IRRI, L.B.).

The 48h LC_{50} was obtained by plotting the mortality percentage against the extract concentration using Probit analysis. Selectivity Index is the mite-to-bee toxicity ratio calculated using the formula: SR = LC_{50} *A. mellifera*/LC_{50} *V. destructor*.

Results and discussion

Phytochemical analysis

The results of phytochemical tests in sample plants for alkaloids, saponins, phenolics, flavonoids, phytosterol, carbohydrates, and proteins, are shown in Table 1. Alkaloids were present (+) in *O. bacilicum* (*biday), G. sepium (kakawate)*, *S. macrophylla* (mahogany, neem, and with strong presence in tobacco (++) while absent (-) in alagaw, citronella, erbaka, lagundi, lemon grass, oregano, and sambong.Saponin was present in mahogany and tobacco only while phenolics were detected in *alagaw, biday, lagundi, herbaka*, citronella, mahogany, neem, and *sambong*. Flavonoids have strong presence in citronella and was found also present in *biday, herbaka*, and neem. Anthraquinones were not detected in all plant extracts. Phytosterol was strongly present in sambong while also present in alagaw, kakawate, lagundi, lemon grass, neem and oregano but absent in biday, citronella, herbaka, mahogany, and tobacco. All sample plants except *biday* and *kakawate* contained carbohydrates while protein was present in *alagaw*, biday, hebaka, lagundi, mahogany *oregano* and *sambong* but not detected in citronella, kakawate, lemon grass, neem, and tobacco.

Table 1. Qualitative determination for secondary metabolites of ethanol crude extracts of sample plants

Common name	Sample plants	Secondary metabolites						
	Scientific name	Alkaloids	Saponins	Phenolics	Flavonoids	Anthraquinones	Phytosterol	Proteins
Fragrant premna	*P. odorata*	_	_	+	_	_	+	+
Common basil	*O. basilicum*	+	_	+	+	_	_	+
Citronella	C. *nardus*	_	_	+	++	_	_	_
Common mugwort	*A. vulgaris*	_	_	+	+	_	_	+
Mexican Lilac	G. sepium	+	_	_	_	_	+	_
Lagundi	*V. negundo*	_	_	+	_	_	+	+
Lemon grass	*C. citratus*	_	_	_	_	_	+	_
Mahogany	*S. mahogany*	+	+	+	_	_	_	+
Neem	*A. indica*	+	_	+	+	_	+	_
Oregano	*O. vulgare*	_	_	_	_	_	+	+
Sambong	B.balsamifera	_	_	+	_	_	++	+
Tobacco	*N. tobacum*	++	+	_	_	_	_	_

Acaricidal activities of crude ethanol plant extracts to V. destructor

The mortality rates of Varroa caused by the interaction effects of botanicals and concentrations during a 90-second exposure are shown in Table 2. At 10%, *O. bacilicum* (*Biday*) had the highest mortality rate of 77.77% which is considered relatively high (RH). While statistically comparable to *O. basilicum, Gliricidia sepium* (*kakawate*) caused a lower mortality of 66.67% which is classed as moderate (M). *Artemisia vulgaris (herbaka)* did not caused any mortality hence; acaricidal activity is nonsignificant (NS) as water.

Almost all other treatments were found to be comparable, indicating that 10% (w/v) ethanol leaf extracts in a 90-second contact time were not lethal to Varroa mites. At15%, *Cymbopogon nardus* (citronella), *Vitex negundo* (lagundi) (77.77%), and *N. tabacum* (72.20%) exhibited relatively high (RH) acaricidal activity. At 20% *Ocimum tenuiflorum (biday)*, also caused relatively high mortality (77.77%). At 25% neem extract caused 61% death, indicating a moderate (M) effect. However, increasing the extract concentration to 20-25% did not increase mortality, which remained comparable to water in most treatments.

Table 2. Acaricidal activity of botanical extracts at different concentrations against *Varroa destructor* in 90-second of exposure.

Botanicals	Concentration							
	10%		15%		20%		25%	
P. odorata (Alagaw)	44.43 abc	L	38.87 ab	L	27.77 bcd	NS	00.00 c	NS
O. tenuiflorum (Biday)	77.77 a	RH	11.1 3b	NS	77.77 ab	RH	44.43 abc	L
C. nardus (Citronella)	28.00 abc	NS	77.77 a	RH	50.00abcd	L	44.43 abc	L
A. vulgaris (Herbaka)	0.00 c	NS	50.00 ab	L	49.97 abcd	L	0.00 c	NS
G. sepium (Kakawate)	66.67 ab	M	50.00 ab	L	27.77 bcd	NS	11.03 bc	NS
V. negundo (Lagundi)	27.77 abc	NS	77.77 a	RH	38.87 abcd	L	55.57 ab	L
C. citratus (Lemon Grass)	27.77 abc	NS	27.77 ab	NS	38.90 abcd	L	22.20 bc	NS
S. macrophylla (Mahogany)	27.80 abc	NS	49.97 ab	L	50.00abcd	L	0.00 c	NS
A. indica (Neem Tree)	27.77 abc	NS	38.90 ab	L	16.70 cd	NS	61.10 ab	M
O. vulgare (Oregano)	27.77 abc	NS	27.77 ab	NS	0.00 d	NS	38.90 bc	L
B. balsamifera (Sambong)	16.70 bc	NS	38.87 ab	L	22.23 cd	NS	27.77 bc	NS
N. tabacum (Tobacco)	38.90 abc	L	72.20 a	RH	55.53 abc	L	0.00 c	NS
Water (-)	0.00 c	NS	0.00 b	NS	0.00 d	NS	0.00 c	NS
Apivar®	22.23 bc	NS	44.47 ab	L	55.57 abc	L	16.67 bc	NS
Apistan®	33.33 abc	L	38.87 ab	L	55.57 abc	L	94.43 a	H
Thymovar®	44.43 abc	L	0.00 b	NS	83.33 a	RH	55.57 ab	L

[1]Based on 10 mites per replicate; each concentration was replicated four times.

Means followed by the same letter(s) in a column are not significantly different at 5% level (HSD).

Mortality ranked as follows: High (H) (86-100%; relatively high (RH) (71-85%); moderate (M) (56-70%); low (L) (31-55%); and non-significant (NS) (0-30%).

Table 3. Acaricidal activity of botanical extracts at different concentrations against *Varroa destructor* three-minute exposure.

Botanicals	Concentration[1]							
	10%		15%		20%		25%	
P. odorata (Alagaw)	10%		15%		20%		25%	10%
O. tenuiflorum (Biday)	22.23b	NS	5.57c	NS	33.33abc	L	16.67ab	NS
C. nardus (Citronella)	33.30b	L	33.30bc	L	44.47abc	L	33.33ab	L
A. vulgaris (Herbaka)	27.77b	NS	0.00c	NS	38.90abc	L	55.57a	L
G. sepium (Kakawate)	22.23b	NS	16.67bc	NS	38.90abc	L	44.43ab	L
V. negundo (Lagundi)	44.43ab	L	27.77bc	NS	77.77a	RH	16.67ab	NS
C. citratus (Lemon Grass)	38.87ab	L	22.23bc	NS	50.00abc	L	16.67ab	NS
S. macrophylla (Mahogany)	33.33b	L	0.00c	NS	22.20bc	NS	27.77ab	NS
A. indica (Neem Tree)	38.90ab	L	0.00c	NS	33.33abc	L	0.00b	NS
O. vulgare (Oregano)	16.67b	NS	16.67bc	NS	44.47abc	L	55.57a	L
B. balsamifera (Sambong)	38.90ab	L	27.77bc	NS	50.00abc	L	55.57a	L
N. tabacum (Tobacco)	33.33b	L	22.23bc	NS	33.33abc	L	55.57a	L
Water (-)	88.90a	H	66.67ab	M	72.23ab	RH	38.90ab	L
Apivar®	0.00b	NS	0.00c	NS	0.00c	NS	0.00b	NS
Apistan®	11.13b	NS	44.47bc	L	61.10ab	M	44.43ab	L
Thymovar®	33.33b	L	44.43bc	L	38.90abc	L	22.23ab	NS

1Based on 10 mites per replicate; each concentration was replicated four times.

Means followed by the same letter(s) in a column are not significantly different at 5% level (HSD).

Mortality rated as follows: High (H) (86-100%; relatively high (RH) (71-85%); moderate (M) (56-70%); low (L) (31-55%); and non-significant (NS) (0-30%).

Table 3 shows mortality effects of herbal concentrations in three-minute Varroa mite exposure. *N. tabacum* had the highest mortality at 10%, killing 88.90%, significantly higher than the synthetic miticides. When concentration was increased to 15%, again, tobacco showed the strongest killing action, notwithstanding its moderate efficacy. *O. basilicum* demonstrated low efficacy similar to Apivar and Apistan. The remaining botanicals showed acaricidal effects comparable to water, non-significant.

G. sepium was the most active at 20% concentration and 3-min contact time, with a death rate of 77.77%, followed by tobacco (72.23%), indicating relatively high miticidal effects that were as effective as Thymovar (83.33). All other botanicals caused low mortalities except *C. citratus* (lemon grass) which was non-significant.

At 25%*C. nardus (citronella), A. indica (neem),O. vulgare*, and *B. balsamifera (sambong)* had equally the highest mortality (55.57%), but were considered low similar to all other botanicals and Thymovar (66.67%) except *C. citratus, (lemon grass) V. negundo (lagundi), G. sepium (kakawate), P. odorata (alagaw), and S. macrophylla (mahogany),* which were non-significant.

At six minutes of exposure, Table 4 shows the interaction impact of botanical extracts and concentration on mortality of mites. At the lowest concentration of 10%, mortalities were low for *P. odorata*, *A. indica*, and *O. vulgare* and Apivar while non-significant for all other botanicals, including Apistan and Thymovar. At 15% extract, mortalities were generally non-significant, with the exception of *N. tabacum and V. negundo (lagundi),* with low miticidal action. With an increase of crude extract concentration to 20%, lethal effects of *N. tabacum and G. sepium* were relatively high while *O. basilicum* was moderate. *C. nardus* with no mortality like water was non-significant as were *V. negundo*, *C. citratus*, *A. indica*, *B. balsamifera* similar to Apivar.

Table 4. Acaricidal activity of botanical extracts at different concentrations against *Varroa destructor* after six-minute exposure.

Botanicals	Concentration[1]							
	10%		15%		20%		25%	
P. odorata (Alagaw)	38.90a	L	0.00c	NS	33.33bcd	L	44.43bcd	L
O. tenuiflorum(Biday)	16.70a	NS	0.00c	NS	61.10abc	M	50.00abcd	L
C. nardus (Citronella)	11.13a	NS	16.70bc	NS	0.00d	NS	72.20ab	RH
A. vulgaris (Herbaka)	16.70a	NS	5.57c	NS	49.97abcd	L	77.77ab	RH
G. sepium (Kakawate)	0.00a	NS	27.80bc	NS	72.23abc	RH	55.57abc	L
V. negundo (Lagundi)	11.10a	NS	33.33bc	L	22.23cd	NS	66.67ab	M
C. citratus (Lemon Grass)	22.23a	NS	5.57c	NS	27.77bcd	NS	33.37bcd	L
S. macrophylla (Mahogany)	16.67a	NS	0.00c	NS	44.43bcd	L	11.10cd	NS
A. indica (Neem Tree)	38.90a	L	11.13bc	NS	27.80bcd	NS	38.90bcd	L
O. vulgare (Oregano)	33.33a	L	22.23bc	NS	38.90bcd	L	72.23ab	RH
B. balsamifera (Sambong)	22.23a	NS	16.70bc	NS	22.23cd	NS	72.23ab	RH
N. tabacum (Tobacco)	11.13a	NS	50.00abc	L	77.80ab	RH	38.90bcd	L
Water (-)	0.00a	NS	0.00c	NS	0.00d	NS	0.00d	NS
Apivar®	33.33a	L	33.30bc	L	27.77bcd	NS	72.23ab	RH
Apistan®	16.67a	NS	61.10ab	M	33.33bcd	L	33.33bcd	L
Thymovar®	27.77a	NS	88.87a	H	100.00a	H	100.00a	H

[1]Based on 10 mites per replicate; each concentration was replicated four times.

Means followed by the same letter(s) in a column are not significantly different at 5% level(HSD).

Mortality rated as follows: High (H) (86-100%; relatively high (RH) (71-85%); moderate (M) (56-70%); low (L) (31-55%); and non-significant (NS) (0-30%).

At the highest concentration of 25 percent and six minutes of exposure, four botanicals demonstrated their potential as acaricides. *A. vulgaris* (herbaka) (77.77%), *O. vulgare* (oregano) and *B. balsamifera* (sambong) (72.23%) and *C. nardus* (citronella) (72.20%), previously deemed insignificant at lower concentrations, were comparably high and statistically comparable to Thymovar with 100% efficacy.

LC_{50} and Selectivity Index of plant extracts

The estimated median lethal concentration (LC_{50}) and selectivity index (SI) values for botanical extract after 48 hours are shown in Table 5. The LC_{50} values presented here are treatment concentration values. The results revealed that the bioactivity of the botanicals examined on *A. mellifera* and *V. destructor* differed. In the case of honeybees, the insecticidal effectiveness of plant extracts suggests the following potency order:

A. vulgaris (herbaka):19.55 >*O. vulgare* (oregano):21.20 >*O. tenuiflorum* (biday):23.41 >*S. macrophylla* (mahogany:25.34 >*P. odorata* (alagaw):25.55 > *V. negundo* (lagundi):25.98 >*N. tabacum* (tobacco):26.50 > *C. nardus* (citronella):26.56 > *C. citratus* (lemon grass):30.54 > *A. indica* (neem):32.36 >*B. balsamifera* (sambong):33.96 > G. sepium (kakawate):44.04.

Similarly, as indicated by the LC_{50} values, the miticidal efficacy of plant extracts against Varroa mites is consistent with the following potency order:

O. basilicum (biday):15.80> V. negundo (lagundi):23.79 > A. vulgaris (herbaka):24.47 > O. vulgare (oregano):25.18 > C. citratus (lemon grass):27.73 > G. sepium (kakawate):28.73 > S. macrophylla (mahogany):30.78 > A. indica (neem):32.23 > C. nardus (citronella):34.71 > N. tabacum (tobacco): 41.96 > P. odorata (alagaw):45.95 > B. balsamifera (sambong):49.09.

G. sepium (*kakawate*), *O. basilicum* (*biday*), *C. citratus* (lemon grass), and *V. negundo (lagundi)* have a higher selectivity index (> 1), indicating that they are more effective as a miticide while being less toxic to honey bees.

Table 5. Median lethal concentration (LC50) values and selectivity index of botanical extracts to *Varroa destructor* and *Apis mellifera*.

Botanicals	LC_{50} Estimates (%)		Selectivity Index*
	Honeybees (a)	Varroa (b)	(a)/(b)
P. odorata (Alagaw)	25.55	45.95	0.55
O. basilicum(Biday)	23.41	15.80	1.48
C. nardus (Citronella)	26.56	34.71	0.77
A. vulgaris (Herbaka)	19.55	24.47	0.80
G. sepium (Kakawate)	44.04	28.73	1.53
V. negundo (Lagundi)	25.98	23.79	1.09
C. citratus (Lemon Grass)	30.54	27.73	1.10
S. macrophylla (Mahogany)	25.34	30.78	0.82
A. indica (Neem Tree)	32.36	32.23	1.00
O. vulgare (Oregano)	21.20	25.18	0.84
B.balsamifera(Sambong)	33.96	49.09	0.69
N. tabacum (Tobacco)	26.50	41.96	0.63

*Selectivity index was calculated by dividing the LC50 for honey bee by LC50 for Varroa mite.A ratio of >1 is considered high and the extract is less harmful to honey bees.

Selected botanicals contain a variety of secondary metabolites, which aid in understanding the acaricidal and insecticidal activities investigated to identify effective miticides against *Varroa destructor* infesting *Apis mellifera*. Based on their high selectivity index and acaricidal efficacy there are four botanicals that provide promising natural products for controlling Varroa mites.

First is *O. basilicum (Biday)*, which contains a potent blend of alkaloids, phenolics, flavonoids, and proteins that demonstrated a relatively high level of acaricidal activity at the lowest tested concentration of 10% and after a 90-second exposure. It aligns with the findings of Veeramani *et al.* (2016) concerning chloroform extract of *O. basilicum* at concentrations ranging from 6% to 10% which caused 70% to 100% mortality of the ectoparasitic tick *Rhipicephalus (*Boophilus*) microplus*. *Biday* was found to have the highest efficacy, with a 15.80% LC_{50} against Varroa. Despite having a 23.41% LC_{50} against honeybees, its high selectivity index indicated that it was more harmless to honeybees than to mites. The toxic effect of *O. basilicum* was also tested to cause effective control of *Tropilaelaps* mites of honey bees (Noor Islam, 2017) and could induce 100% mortality in brown dog ticks *(Rhipicephalus sanguineus) (Manzoor et al., 2013)* with repellent and anti-reproductive effects *(Popović et al., 2006*). Moreover, Kostic *et al.* (2008) found that *Ocimum basilicum's* linalool content caused antifeedant activity against second-stage larvae, and on gypsy moth caterpillars of the second-instar *(*Popovic *et al.,* 2013*)*. Kloucek *et al.* (2012) quantified the primary bioactive compounds of *O. basilicum* as having linalool (60.1%), eugenol (11.1%) and estragole (92%) contents.

G. sepium (kakawate) that was positive for alkaloid and phytosterol was found to have the highest selectivity index of 1.53, comparatively the safest for honey bees. *Kakawate* had an efficacy of 28.73% LC_{50} against the mite. It was most active at 20% concentration and 3-min contact time, and relatively high acaricidal activity. Alkaloids have an impact on the functioning of entire organisms when they bind to receptors and disrupt nervous system function (Wink, 2000). The effects can range from stimulatory, i.e. narcotic, to toxic, causing death at extremely low doses (Diaz, 2015). Phytosterol, on the other hand, are plant-derived fatty compounds (steroids) which was isolated in *T. portulacastrum* by Thacker (1999) to be the chemically active fraction of a crude plant extract that had already been shown to be toxic to arthropod pest species. Phytosterol compounds extracted from *Mesembryanthemum forsskaolii* Hochst were found to have larvicidal and adulticidal effects in *R. annulatus* tick infesting cattle (Moawad *et al.*, 2017). Acaricidal activity of *G. sepium* leaf extract was also reported on other Acari species, such as the plant mite *Tetranychus cinnabarinus*, which caused 100% mortality when used at a concentration of 20% (Sivira *et al.*, 2011) and *Rhipicephalus* (Boophilus) *microplu*s (Rodriguez and Pulido, 2015). It was also reported to be effective against *Demodex canis* (Viste *et al.*, 2013). The ethanolic extract of *G. sepium* tested by Ravindran *et al.* (2017), on the other hand, resulted in very low mortality to adult *Rhipicephalus* (Boophilus) annulatus ticks.

Vitex negundo (lagundi), which contains phenolics, phytosterols, and protein, is also thought to be a promising miticide in honeybees. Lagundi showed relatively high acaricidal activity at 15% concentration and 90 seconds contact duration. Efficacy of Lagundi was at LC_{50} 23.79% and regarded as safer to honeybees with 1.09 selectivity index. This finding is parallel with Singh and Jyoti's (2014) results that ethanolic extracts of *V. negundo* leaves have a high acaricidal effect against resistant tick populations. Moreover, phenolic compounds could serve as both attractants for beneficial organisms and toxicants against invading pests and pathogens (Pratyusha, 2022). Most phenolic compounds are known as feeding deterrents. Plants with a high concentration of phenols are often less attractive hosts for many insects and mites than plants with a low content of these secondary metabolites which suggests the importance of phenolic compounds in defense mechanisms of plants against these pests. Protein content of lagundi might be toxic plant proteins regarded as valuable in crop protection because to their biological activity. Proteins could be harmful to animals, insects, bacteria, and fungi (Dang and Van Damme, 2015). Using genetic engineering, genes producing poisonous plant proteins have been inserted into crop genomes to boost disease resistance. Lectins, ribosome-inactivating proteins, enzymes inhibitors, arcelins, chitinases, ureases, and modified storage proteins are involved in plant insect defense (Carlini and Grossi-de-Sá, 2002).

While *C. nardus* (citronella), with its phenols and strong presence of flavonoids, and *N. tabacum*, which is rich in alkaloid and saponins, performed similarly to lagundi, both citronella and tobacco had low selectivity indices, indicating that they are harmful to honey bees. Flavonoids, as alternative to synthetic pesticides can inhibit enzymatic activity and prevent the growth of larvae of different insect species (Kim *et al.*, 2000). Some flavonoids interfere in the process of molting and reproduction of several insects, that is, they inhibit the formation of juvenile hormone (ecdysone) and inhibit transcription of ecdysone receptor-dependent genes (EcR) (Oberdorster *et al.*, 2001). Some types of flavonoids have been shown to have an ovicidal effect, oviposition, fecundity, mortality, weight decrease, and adult emergence on agricultural pests (Salunka *et al.*, 2005). Flavonoids have shown efficacy against nymphs and adults of the aphid *Eriosoma lanigerum* Hausmann (Ateyyat, 2012). Saponin exerts a rapid and potent effect against a wide variety of insect pests, which can exert a rapid and potent effect against a broad range of insect pests, as opposed to neurotoxicity. De Geyter *et al.* (2007) report increased mortality, decreased food intake, weight loss, retarded development, decreased reproduction, and repellent deterrent action. *Nicotiana spp*. is a frequently cited effective miticide of Varroa mites, the activity is generally attributed to nicotine (Mahmood *et al.*, 2014): when tobacco extract was used with clove oil and at a lower concentration of 5% for 24 hours, it was found to be significantly effective. Nicotine, the psychoactive component of tobacco leaves, is a non-systemic pesticide that binds to the cholinergic acetylcholine nicotinic receptor (nAch) in insect nerve cells, causing it to fire continuously (Gonzales *et al.*, 2010).

Cymbopogon citratus (lemon grass) tested positive only for phytosterol. Its insecticidal efficacy was 30.54% LC_{50} and had a miticidal potency of 27.73% LC_{50}. However, at the concentrations of 10-25% of the extract acaricidal activity was consistently low or non-significant requiring perhaps a higher concentration and longer contact time to have a killing effect.

Conclusion

Pollinator insects should not be harmed by acaricides because they are essential for the reproduction of a vast array of cultivated and native plants. Both chemical and biological methods of pest control necessitate the use of selective products, which are crucial in integrated pest management programs. With the presence of secondary metabolites, the most effective concentration plant extract as miticide are as follows: tobacco at 10, 15 and 20%, citronella and lagundi at 15%, kakawate and biday at 20% and citronella, herbaka, oregano and sambong at 25%. However, based on the selectivity index and LC_{50} of the botanical extracts, *O. basilicum (Biday)*, *V. negundo (Lagundi)*, *C. citratus* (Lemon grass)*G. sepium* (Kakawate), with LC_{50} values of 15.08, 23.79, 27.73, and 28.73 respectively, were the most effective miticides against Varroa while being less harmful to honeybees.

Recommendations

Ethanolic extracts of Kakawate, Biday, Lemon grass, Sambong and Lagundi are recommended to be developed into formulations as miticides against Varroa in honey bees. Further study to standardize extracts of Kakawate, Biday, Lemon grass, Sambong and Lagundi for use in miticide formulations to complement chemically synthesized acaricides in controlling *V. destructor*. Determine the performance of the selected plant extracts in field setting.

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