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Acaricidal activity of *Swietenia mahogani* and *Swietenia macrophylla* ethanolic extracts against *Varroa destructor* in honeybee colonies

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ABSTRACT

The acaricidal (miticidal) activity of 90% ethanolic extracts of leaves and stem bark of *Swietenia mahogani* and *Swietenia macrophylla* were tested against *Varroa destructor* mite. Four concentrations were used over two different time intervals under laboratory and field conditions. In general, it was noticed that the acaricidal effect based on mortality and LC_{50} of all tested extracts against the *Varroa* mite was concentration and time dependant. The acaricidal action against *Varroa* mites was relatively the least for the *S. macrophylla* stem bark extract at 500 ppm concentration after 48 h while it reached 100% and 95% in case of *S. mahogani* bark and *S. macrophylla* leaves, respectively. The% infestation with *Varroa* in colonies treated with the different extracts at various time intervals showed that the rate of infestation decreased to 0.0% after 12 days from the beginning of treatments with 500 ppm of *S. mahogani* leaves extract compared to 0.79% decrease after treatment with Mitac, a reference drug (60 mg/colony). The rate of infestation in case of treatments with *S. mahogani* bark, *S. macrophylla* leaves and *S. macrophylla* bark was decreased to 0.11%, 2.41% and 1.08%, respectively. The highest reduction was observed with *S. mahogani* leaves extract followed by *S. mahogani* bark. All the tested extracts showed less or no effect on honey bees at the different concentrations and at different bioassay times. This study suggested that the use of natural plant extracts or their products as ecofriendly biodegradable agents could be of high value for the control of *Varroa* mite.

1. Introduction

Varroa destructor mite (Acari: Varroidae) has become a serious pest of the honey bee, *Apis mellifera* and *Apis cerana* (Hymenoptera: Apidae) worldwide. The disease caused by the mites is called "varroatosis". *Varroa* mites damage immature and adult bees by feeding on hemolymph, thus, greatly weakening or killing the bees (Weinberg and Madel, 1985). A significant mite infestation may lead to the death of an entire honey bee colony. The *Varroa* mite is thus considered as the parasite with the most pronounced economic impact on the beekeeping industry (Guzmán-Novoa et al., 2010).

The use of synthetic acaricides has been the major effective method against *Varroa* mites at bee colonies. But the intensive use of these acaricides resulted in the development of resistance and reduction of their efficacy (Milani, 1999). In addition, the remaining of these chemicals has numerous adverse effects on environment (Wallner, 1995; Kochanskg and Wilzer, 2001) and lead to contamination of hive products, especially wax and possibly honey, as well (Wallner, 1999). Therefore, there are urgent needs to search for new pesticides with a new or different mode of action and/or improved effectiveness against *Varroa* mites and safe to honey bees. In this respect, there are considerable interests, nowadays, in the use of natural products for controlling parasitic bee mites (Calderone et al., 1997; Hagigatian, 2000; Kraus and Berg, 1994; Liu and Nasr, 1993; Colin, 1990; Calderone and Spivak, 1995; Jacobson, 1983; Xie et al., 1995).

The application of some plant extracts or essential oils based products against infested apiaries were found able to maintain mite infestation rates below economic injury levels (Calderone et al., 1997; Hagigatian, 2000). Colin (1990) reported that the essential oils of thyme and sage were effective against *Varroa* mites. Later on, Calderone and Spivak (1995) proved the high efficacy of thymol-based control strategies when there was little brood in the colonies. In addition, a high insecticidal efficacy has been attributed to several plant extracts (Shaddel-Telli et al., 2008) and especially those of meliaceous species such as the highly reputed neem tree, *Azadirachta indica* and including certain members of the genus *Swietenia* (Omar et al., 2007; Nadal et al., 1973; Mikolajczak and Reed, 1987; Jimenez et al., 1997).

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The extracts derived from *Swietenia mahogani* bark and seeds showed insecticidal effect against several insects and were apparently non toxic to humans (Nadal et al., 1973). Four limonoids, humilinolides A–D from *Swietenia humilis* and cedrelanolide from *Cedrela salvadorensis* were evaluated for their insecticidal effect against the European corn borer, *Ostrinia nubialis* in comparison with toosendandin, a commercial insecticide derived from *Melia Azedarach*; they showed an activity comparable to that of toosendanin (Jimenez et al., 1997). Humilinolides C and D were also tested for insect antifeedant activity against the rice weevil, *Sitophilus oryzae* (L.) using a flow disk bioassay that requires only small amounts of compounds; they were found active at 0.50% (w/w) (Omar et al., 2007).

The objective of this study was the assessment of the miticidal effect of the leaves and stem bark extracts of *S. mahogani* and *S. macrophylla* against *V. destructor* mite and toxic effect on honey bees (*A. mellifera*) in laboratory and field experiments.

2. Materials and methods

2.1. Plant materials and extraction

The plant materials of *S. mahogani* (L.) Jacq. and *S. macrophylla* King. grown in Egypt were collected from plants cultivated in Zoo garden, Giza, June 2007. The identity of the plant materials was authenticated by Mrs. Teraiz Labib, plant taxonomist at El-Orman botanical garden, Giza, Egypt. The dried leaves and barks powder (100 g each) of both species were separately extracted with 90% ethanol on cold till exhaustion.

2.2. Preliminary phytochemical screening

The air-dried powdered leaves and stem barks of *S. mahogani* and *S. macrophylla* were subjected to preliminary phytochemical screening, applying chemical tests for identification of different plant constituents (Balbaa et al., 1981; Fieser and Fieser, 1959; Trease and Evans, 1983; Geissman, 1962).

2.3. HPLC quantitative determination of polyphenols

Polyphenols (flavonoids and catechins) were determined by HPLC in hydrolyzed samples of stem barks and leaves of *S. mahogani* and *S. macrophylla* following method described by Stricher (1993) and reported by Institute for Nutraceutical Advancement (INA, http://www.nsf.org/business/ina/ginkgo.asp). Identification of components was based on comparison of their retention times with those of available authentic samples and quantization on peak area computation. The external standard method was applied.

2.4. Insects

2.4.1. A. mellifera

Colonies of carnelian hybrids honeybees, *A. mellifera* L. were used in this study. Each colony consisted of 9 full depth combs of worker bees and each had sufficient amount of brood.

2.5. Bioassay method

Evaluation of compounds for control of *Varroa* mites was conducted at the apiary of Agricultural Experimental Station-Hada El-Elsham, Faculty of Meteorology, Environment and Arid Land Agriculture, King AbdulAziz University, Saudi Arabia, in October, 2008. The evaluation of the potentiality of the different extracts of the *Swietenia* species under investigation in controlling bee infection by *Varroa* mites was conducted on colonies of *A. mellifera* L. (each consisting of 9 full depth combs of worker bees with sufficient brood). Serial dilutions of the aqueous extractives of the 90% ethanol extracts (corresponding to 100, 200, 400 and 500 ppm of each extract) were applied by hand spraying (field application only). The effect of the extracts against *Varroa* mites and on honey bees was evaluated in comparison with that of the standard acaricide Mitac 20% EC (amitraz 20% w/v, NOR-AM Chemical Co, Wilmington, DE, USA).

2.6. Laboratory experiments

Filter papers were treated with 0.5 ml of the prepared concentrations in addition to the positive control (mitac 20% EC), then dried at room temperature for ten min. and placed inside glass cups. Groups of ten bees infested with *Varroa* mites were, separately, introduced inside glass cups (200 ml, capacity) covered with muslin and sugar sirup (30%) was supplied for each glass cups and changed every day to prevent fermentation. Triplicate experiments were performed for each concentration of the different extracts. The glass cups were regularly supplied with sugar sirup (30%), which changed every day to prevent its fermentation. The control glass cups were sprayed with pure water. All tests were carried out under laboratory conditions (25 ± 2 °C and 60 ± 5% relative humidity).

The mites which fallen from the adult bees and showed no movements were considered died. The percentages of mortality were recorded 24 and 48 h after treatment. Values of LC_{50} were calculated according to Finney (1971) and Abbott's formula (1925) being used when necessary. The effect of test materials on honey bees were done by the same of the above mentioned methods but with bees free from infestation by *Varroa*.

2.7. Field application

Colonies of honey bees (*A. mellifera* L.), infested with *Varroa* mites, were used in this study. Treatments were performed by means of plastic sprayer (500 ml). The different dilutions of the four plant extracts (100 ml each) were sprinkled over the bees inside the colonies (three colonies for each concentration). Every comb in the colony were exposed to test sample by raising up one by one and sprinkled over the bees which covered the comb. Mitac was applied on a polyethylene package of 5.5×30 cm, which was hanged between the middle combs for a treatment. The dose of mitac was 60 mg/ colony. Three colonies were used as a control. Each treatment was carried out in triplicate for the tested concentrations. Treatments and records of the rate of infestation were carried out at 4 days intervals (4, 8 and 12 days) and repeated three times for each of the investigated extracts. For determination of the degree of infestation, adult workers (about 150 bees) were obtained from brood nest area of each experimental colony; samples were transferred to beakers (250 ml) containing soap water. The mites were collected and counted.

2.8. Statistical analysis

All data are expressed as means ± SD. Unpaired Students' *t*-test was used for comparing two means. Multiple group comparisons for more than two means were carried out using one-way analysis of variance (ANOVA) followed by the Dunnett test for post hoc analysis. Statistical significance was accepted at a level of $p < 0.05$. All statistical analyses were performed using GraphPad InStat software, version 3.05 (GraphPad Software, Inc. La Jolla, CA, USA).

3. Results and discussion

The results of the preliminary phytochemical screening of both species showed a similarity in response to the applied tests of the same organs (leaves or stem barks). Carbohydrates and/or glycosides, free and/or combined flavonoids, sterols and/or triterpenes, as well as tannins of both condensed and hydrolysable types were identified in all the tested samples. Steam distilled volatiles were detected in the leaves and could not be traced in the stem barks. The leaves appeared free from oxidase enzymes which were detected in traces in the barks. Cardiac glycosides, alkaloids and/or nitrogeneous bases, saponins and anthraquinones could not be detected.

The HPLC analysis led to the following conclusions; catechin was prevailing among the polyphenols identified in the stem barks of the two species although detected in larger amounts in that of *S. macrophylla* (170.68 vs 135.70 mg/100 g dw); while in the leaves its amount was much higher in *S. mahogani* (71.50 vs 31.00 mg/100 g dw). The flavonol content (quercitin and kaempferol) of the leaves obviously exceeded that of the stem barks reaching 93.61 and 99.41 mg/100 g dw in the leaves *S. mahogani* and *S. macrophylla*, respectively. Quercetin being detected as major polyphenol aglycone in the leaves of *S. macrophylla* (68.72 mg/100 g dw).

The antifeeding and insecticidal effects of several members of family Meliaceae and humilinolides isolated thereof were reported by several research groups (Nadal et al., 1973; Mikolajczak and Reed, 1987; Jimenez et al., 1997; Abdelgaleil and El-Aswad, 2005; Omar et al., 2007). Nothing was reported regarding the miticidal activity of both species of *S. mahogani* and *S. macrophylla* against *V. destructor*, an external parasitic mite which infest honey bees and may contribute to the collapse of entire colony.

This showed that the ethanol extracts exerted a pronounced miticidal activity without almost affecting bees.

Different dilutions of the aqueous extractives of the 90% ethanol extracts were applied by residual film method to *Varroa* mites under laboratory conditions. The percentages of mortality of *Varroa* mites after treatment with the different extracts are presented in Table 1, and the LC_{50} values and confidence limits were determined and recorded in Table 2. The average rate of infestation in adult worker bees was studied through field application of the extracts on colonies of honey bees infested with *Varroa* mites by spraying method. The results of field application of the four tested extracts and mitac are displayed in Table 3 and their respective effects on honey bees are recorded in Table 4.

The results obtained indicated that all the tested extracts were toxic to *Varroa* mites in a dose dependent manner and did not affect bees. The extract of the leaves of *S. mahogani* being the most effective, since its 400 ppm concentration caused 100% mortality after 48 h. It was more effective than mitac 20% EC as positive control which caused 96.7% mortality at the same period. The toxic action was relatively the least for the extract of the stem bark of *S. macrophylla* that produced 90% mortality at the highest concentration 500 ppm, after 48 h; while those of *S. mahogani* stem bark and *S. macrophylla* leaves reached 100% and 95%, respectively. Moreover, it is obvious that the extracts of the stem barks and leaves of *S. mahogani* have significantly lower LC_{50}s after 48 h than their analogs obtained from of *S. macrophylla*. The values of LC_{50}s for the four extracts were 60.6, 85.4, 121.6 and 143.2, respectively.

The average degree of infestation% in adult worker before and after treatment with different concentration of extracts and Mitac 20% EC (60 mg/colony) at various time intervals was determined. The data of table (3) indicated that the average range of infestation% in colonies treated with the extracts and Mitac 20% EC were 14.32 to 18.75 before treatment. After treatment with different concentrations of the extracts and Mitac, the rate of infestation with *Varroa* was decreased to 0.0% after 12 days from the beginning of treatments with *S. mahogani* leaves extract (500 ppm) compared to 0.79% decrease after treatment with 60 mg/colony of Mitac (20%). This means that *S. mahogani* leaves ethanol extract is better than Mitac. Meanwhile in case of treatments with extracts of *S. mahogani* stem bark, *S. macrophylla* leaves and stem bark, the rate of infestation was decreased to 0.11, 2.41 and 1.08%, respectively, 12 days after treatment with the same concentration (500 ppm). The same observation was noticed for all the other concentrations among the different time intervals. Also, results indicated that there were significant differences among tested extracts. No significant difference was observed between Mitac as positive control and *S. mahogami* stem bark. The previous find-

Table 1
Mortality percentages of *Varroa* mites treated with the ethanol extracts of *S. mahogani* and *S. macrophylla*.

Ethanol extract	Concentration (ppm)	Mean percent mortality after	
		24 h	48 h
S. mahogani leaves	100	37.3 + 10.5	59.3* + 14.4
	200	59.0 + 13.2	78.5* + 17.1
	400	77.3 + 15.8	100.0* + 5.2
	500	94.3 + 20.7	100.0 + 4.4
S. mahogani stem bark	100	33.0 + 9.7	50.3*+12.2
	200	51.0 + 17.1	69.3 + 17.3
	400	70.7 + 22.1	93.5 + 29.7
	500	90.67 + 29.0	100.0 + 3.3
S. macrophylla leaves	100	27.7 + 5.1	38.0 + 11.3
	200	42.3 + 9.5	58.3 + 18.0
	400	67.3 + 17,3	90.0 + 27.4
	500	85.0 + 23.3	95.5 + 28.8
S. macrophylla stem bark	100	22.7 + 4.7	30.7 + 7.1
	200	36.7 + 5.7	50.0* + 13.3
	400	55.0 + 13.9	81.7* + 22.4
	500	68.7 + 19.1	90.0 + 24.1
Control (water)	–	00.00	3.3 + 0.8
Mitac	20% EC	80.0 + 21.1	96.7 + 11.3

Values are presented as M + SD, $N = 6$.
* Significantly different from corresponding 24 h value at $p < 0.05$ using unpaired Student's *t*-test.

Table 2
LC_{50} values and confidence limits of the ethanol extracts of *S. mahogani* and *S. macrophylla* applied on *Varroa* mites.

Ethanol extract	Bioassay time	Regression of N.E.D.* Response(Y) on log dose(X)	LC_{50} (Conf. Limits)%	*p*
S. mahogani leaves	24 h	$Y = -6.3 + 3.3X$	91.2 (76.5–103.4)	0.9
	48 h	$Y = -6.4 + 3.2X$	60.5 (41.5–81.1)	0.9
S. mahogani stem bark	24 h	$Y = -5.7 + 2.4X$	103.7 (88.9–149.6)	0.7
	48 h	$Y = -5.3–1.8X$	85.4 (69.4–129.1)	0.8
S. macrophylla leaves	24 h	$Y = -6.1–2.5X$	143.3 (94.3–176.4)	0.6
	48 h	$Y = -6.1 + 2.7X$	121.6 (96.8–169.9)	0.7
S. macrophylla stem bark	24 h	$Y = -5.5 + 3.1X$	158.5 (103–201.2)	0.7
	48 h	$Y = -5.4–3.2X$	143.2 (99.6–191.4)	0.8

* N.E.D. = Normal equivalent divide; Conf. Limits = confidence limits *p* = probability.

Table 3
Average rate of infestation (%) in adult worker bees after treatment with the ethanol extracts of *S. mahogani* and *S. macrophylla*.

Ethanol extract	Conc. (ppm)	Before treatment	Days after treatment		
			4	8	12
S. mahogani leaves	100	16.02 + 3.3	12.17 + 4.1	10.25* + 2.7	7.05* + 1.7
	200	16.55 + 2.9	9.07 * + 3.5	5.19* + 1.1	3.31* + 0.7
	400	15.60 + 2.5	6.51* + 1.9	3.11* + 0.8	0.31* + 0.1
	500	18.75 + 3.4	5.78* + 1.3	1.08* + 0.4	0.0
S. mahogani stem bark	100	17.19 + 2.4	13.85 + 3.8	10.99* + 2.9	8.32* + 1.2
	200	18.35 + 2.7	11.94 * + 3.9	6.23* + 1.8	4.08* + 0.8
	400	17.44 + 3.1	9.55* + 3.5	4.96* + 1.1	1.15* + 0.3
	500	16.63 + 2.8	5.26* + 1.3	2.09* + 0.5	0.11* + 0.03
S. macrophylla leaves	100	17.35 + 2.5	13.18 + 4.3	11.38* + 2.7	8.44* + 2.3
	200	17.42 + 2.1	10.87* + 3.1	7.26* + 1.7	5.61* + 1.7
	400	18.07 + 3.4	10.46* + 2.2	5.88* + 1.3	2.33* + 0.6
	500	18.19 + 3.2	8.71* + 2.3	3.09* + 0.9	1.08* + 0.3
S. macrophylla stem bark	100	16.13 + 2.4	14.21 + 2.9	11.98* + 2.6	9.11* + 3.0
	200	14.32 + 2.7	9.77* + 2.1	7.90* + 1.5	5.99* + 1.8
	400	16.22 + 3.2	8.79*+2.4	6.41* + 1.7	3.26* + 1.1
	500	17.06 + 4.1	9.22* + 2.7	5.99* + 1.1	2.41* + 0.5
Control	Water only	18.56 + 3.7	17.39 + 4.7	18.41 + 4.1	18.76 + 4.1
Mitac 20%EC	60 mg/colony	17.05 + 3.9	7.44* + 1.9	3.21* + 0.8	0.79* + 0.2

Data are presented as M ± SD, $N = 6$.
* Significantly different from corresponding before treatment value at $p < 0.05$ using one way ANOVA followed by Dunnett as a post hoc test.

Table 4
Effect of the ethanol extracts of *S. mahogani* and *S. macrophylla* on honey bees.

Ethanol extract	Concentration (ppm)	Percentage of mortality after	
		24 h	48 h
S. mahogani leaves	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	500	0.0	3.3
S. mahogani stem bark	100	0.0	0.0
	200	0.0	0.0
	400	3.3 + 0.6	3.3 + 0.5
	500	6.7 + 2.1	10.0 + 3.8
S. macrophylla leaves	100	0.0	3.3 + 0.8
	200	0.0	0.0
	400	0.0	0.0
	500	0.0	0.0
S. macrophylla stem bark	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	500	3.3	3.3
Mitac	60 mg/colony	3.3 + 1.0	6.7 + 2.8

Data are presented as M ± SD, $N = 6$.

ings demonstrated that the rate of infestation decreased with the increase in concentration for all the tested extracts. The highest reduction being observed for the extract of the leaves of *S. mahogani* followed by that of its stem bark. It means that the extracts of *S. mahogany* leaves and stem bark is more toxic to *Varroa* inside colonies of honeybees than Mitac which is recommended for controlling of *Varroa* mites. Shaddel-Telli et al., 2008 found that many medicinal plants can be used for control of *Varroa* mites.

In addition, all the tested extracts appeared non-toxic to honey bees, since insignificant effects were observed for the different concentrations and at different bioassay times (Table 4).

In conclusion, the findings of the present study indicated that the investigated natural products particularly *S. mahogani* leaves and stem bark are promising as safe natural products for control of *Varroa* mites. Also, these materials proved to be harmless to the bees and quite safe to the environment. Our results confirmed by the previous studies reported by Nadal et al., 1973 and the isolation of four limonoids, humilinolides A-D from *S. humilis* (Jimenez et al., 1997; Omar et al., 2007).

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