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Evaluation of certain plant extracts for the control of parasitic bee mites, *Varroa jacobsoni*

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ABSTRACT

Toxicity of aqueous, methanolic and acetonic extracts of three plants, *Rhazya stricta* (Decaisne) *Azadirachta indica* (A. Juss) and *Heliotropium bacciferun* (Forsk) to parasitic bee mite, *Varroa jacobsoni* (Oudemans) was evaluated in the field and laboratory conditions. All extracts of the plants demonstrated remarkable toxicity to *Varroa* mite with varying degree. The greatest effect being due to acetone extracts followed by aqueous extract. Of the three plants, the acetonic extract from *R. stricta* has shown more toxic effect than others with LC_{50} of 123.4 ppm after 48 hours compared to 169.3 and 150.8 ppm for *A. indica* and *H. bacciferum*,respectively. Mortality percentage was 100 % for acetonic extract of *R. stricta* after 48 hours of treatment with LC_{50} of 500 ppm, while it was 90 and 93% for *A. indica* and *H. bacciferum,* respectively. Acetonic extract of *R.stricta* reduced infestation to 0.59 % under field conditions with 500 ppm after 12 days of treatment. These findings suggested that, the application of acetonic extracts of the used plants, specially extract of *R.stricta* may have a promising practical application against *Varroa* mites.

INTRODUCTION

The use of synthetic acaricides against *Varroa* mites at bee colonies raises the possibility of contamination of hives products (Ruijter 1995 and Wallner, 1995). There are considerable interests in the use of natural products for control of parasitic bee mites. There is a great awareness for a need for new pesticides with a new or different mode of action and/or improved effectiveness and safety over those currently in use.

Several trials of the use of natural products suggest this material may be useful in maintaining mite infestation rates below economic injury levels (Calderone *et al.*,1997) . Lactic acid is toxic to *Varroa* (Kraus and Bery 1994) and formic acid kills *Varroa* mites and another bee mites (Liu and Nasr 1993). Colin, 1990 revealed that essential oils of thyme and sage were effective against *Varroa.* Calderone and Spivak (1995) reported high efficacy of thymol based control strategies when there was little brood in the colonies. Many plant extracts have been shown to be effective against insects. Among these are extracts of neem tree, *Azadirachta indica* (Xie *et. al.,* 1995 and Jacobson 1983). *Rhazya stricta* and *Heliotropium bacciferum* are known to possess some biological activity against insects (Elhag. *et al.*, 1996) and rats (Adam, 1998), besides medical values as being used by local folk medicine practitioners (Al –Yahia *et. al* .,1990).

The objectives of this study were to evaluate the toxic effect of three extracts from three plants as possible sources of botanical pesticides against parasitic bee mite *Varroa jcobsoni* under laboratory and field conditions.

MATERIAL AND METHODS

Colonies : Evaluation of compounds for control of *Varroa* mites was conducted at the apiary of Faculty of Agriculture, Alexandia University. Colonies of *Apis mellifera* L. of carniolan hybrids were used in this study. Each colony consisted of 9 full depth combs of worker bees and each had sufficient amount of brood.

Extracts: Test materials were collected from the central parts of Saudi Arabia. Aerial parts of *Rhazya stricta* and *Heliotropium bacciferum*, neem seed kernels were air dried in the laboratory, ground to a fine powder and extracted by warm distilled water, methanol or acetone at ambient temperatures. A gentle warming to 35 – 40 °c was sometimes found necessary especially when the solvents were taken directly from the refrigerator. The powderd materials were mechanically stirred for 2 –3 hr. with the appropriate solvent and filtered. Solvents used in the extraction process were obtained from Winlab ltd. and BDH.

Test procedure: Stock solutions of the three plant material extracts were prepared by redissolving the extract in warm distilled water (0.5 g / 100 ml). Different concentrations of 100, 200, 400 and 500 ppm were prepared from the stock solutions. Spraying method was used in this study. Ten bees infested with *Varroa* were placed inside glass cups (200cc) coverded with muslin and sprayed with hand sprayer for each concentration of the tested extracts. Each concentration was replicated three times. Sugar syrup (30%) was supplied for each glass cup and changed every day to prevent fermentation. Control glass cups sprayed with water only. The mortality percentages were recorded 24 and 48 hr after treatment. Values of LC_{50} were calculated according to Finney (1971). Abbott's formula (1925) was used when necessary. All tests were carried out under laboratory conditions (temperature 25 °c ±2, R.H 60 ± 5).

Field application: Colonies of honey bees infested with *Varroa* mites were used in this study. Treatments were carried out using plastic sprayer (500 ml). Solutions of all extract's concentrations were sprinkled over the bees inside the colonies . Each treatment was replicated three times for different concentrations. Treatment and recording rate of infestation were done at intervals of four days and repeated three times for all extracts. For determining the degree of infestation, adult workers (about 150 bees) were obtained from brood nest area of each experimental colony. Samples were transferred to a beaker (250 cc) containing soap water. The mites were collected and counted.

RESULTS AND DISCUSSION

The mortality percentages of *Varroa* mites following different plant extracts treatment at 24 and 48 hours were listed in Table 1.The results showed that all test extracts from the three plants were toxic to *Varroa* mites in a dose dependent manner. Data indicated that the toxic action of extracts from *R. stricta* specially acetonic extract was higher against *Varroa* than extracts of *A. indica* and *H. bacciferum*. Its 500 ppm aqueous and acetonic extracts caused 90 and 100 % mortality after 48 hours, respectively . Aqueous and acetonic fractions of *A. indica* and *H. baeciferum* gave 80, 86, 90 and 93 % mortalitiies at 500 ppm after 48 hours respectively. Table (2) summarizes the LC_{50} values and 95 % confidence limits of the extracts of the three tested plants against *Varroa* mites, 24 and 48 hours following application.The results showed that acetonic extracts of the three plants were generally more toxic than their aqueous or methanolic extracts. Both aqueous and acetonic extracts LC_{50}s of *R. stricta* (48 hr) were 148.9 and 123.4 ppm, respectively. Its values were 195.5 , 180.6 , 169.3 and 150.8 for

Table 1. Percentage of mortalities of Varroa mites after applicatiam with extracts of three plants.

Plant	Conc. (ppm)	Aqueous extract		Methanolic extract		Acetonic extract	
		Mortality % after					
		12 hr	48 hr	12 hr	48 hr	12 hr	48 hr
Rhazya strictaa	100	16.7	36.7	16.7	26.3	23.3	43.3
	200	33.3	56.7	30.0	40.0	43.3	66.7
	400	63.3	86.7	46.7	53.3	73.3	96.7
	500	76.7	90.00	53.3	63.3	83.3	100.0
	control	0.0	0.0	0.0	0.0	0.0	0.0
Azadirachtaa indica	100	13.3	26.7	13.3	23.3	16.7	30.0
	200	30.0	50.0	20.0	33.3	33.3	53.3
	400	60.0	76.7	40.0	46.7	63.3	83.3
	500	66.7	80.0	46.7	53.3	70.0	90.0
	control	0.0	0.0	0.0	3.3	0.0	0.0
Heliotropium bacciferum	100	16.7	30.0	16.7	26.7	20.0	33.3
	200	30.0	46.7	26.7	36.7	40.0	60.0
	400	63.3	83.3	43.3	50.0	66.7	86.7
	500	70.0	86.7	50.0	60.0	73.3	93.3
	control	0.0	3.3	0.0	0.0	0.0	0.0

A. indica and *H. bacciferum,* respectively, indicating that the acetonic extracts were more toxic than either aqueous or methanolic extracts. It seems logical to conclude that the acetonic extract of *R. stricta* is good candidate as botanical acaricide against *Varroa* mites , where it can serve as biodegradable natural plant product.

Field application results of extracts from the three tested plants are shown in Tables 3, 4 and 5. The average degree of infestation and reduction of infestation after treatment with different extracts from the above mentioned plants are given in

Table 2. Response of *Varroa jacobsoni* to three plants extracts

Plant	Time (hours)	LC $_{50}$ (95% confidence limits)		
		Aqueous extract	Methanolic extract	Acetonic extract
Rhazya stricta	24	274.43 (217.6 –346.1) (Y = -5.8 + 2.4X)*	447.0 (279.3 – 718.2) (Y = -3.9 + 1.5X)	214.6 (168.8 – 272.7) (Y = -5.6 + 2.4X)
	48	148.9 (111.4 – 198.9) (Y = -5.2 + 2.3X)	307.2 (202.0 – 468.0) (Y = -3.3 + 1.3X)	123.4 (97.0 – 156.8) (Y = -7.0 + 3.4X)
Azadirachta indica	24	320.7 (249.2 – 413.0) (Y = -5.7 + 2.3X)	592.7 (331.1 – 1067.4) (Y = -4.3 + 1.5X)	290.0 (225.2 – 373.5) (Y = -5.4 +2.2X)
	48	195.5 (148.7 - 256.6) (Y = -4.9 + 2.2X)	450.5 (243.5 – 839.6) (Y = -3.0 + 1.1X)	169.3 (131.6 – 217.5) (Y = -5.7 + 2.5X)
Heliotropium bacciferum	24	296.8 (231.4 – 381.0) (Y = -5.6 + 2.3X)	519.9 (293.8 – 925.7) (Y = -3.8 + 1.4X)	254.6 (196.6 – 329.8) (Y = -5.1 + 2.1X)
	48	180.6 (140.4 – 232.2) (Y = -5.6 + 2.4X)	354.3 (216.6 – 581.5) (Y = -3.1 +1.2X)	150.8 (116.3 – 195.2) (Y = -5.8 + 2.7X)

* Regression of equation normal equivalent devides of response (Y) on log dose (X) lines.

Table 3. Average rate of infestation (%) in adult worker bees after treatment with aqueous extract from three plants.

Plant	Conc. (ppm)	Before treatment	Days after treatment		
			4	8	12
Rhazya strictaa	100	17.61	14.79	10.59	8.45
	200	18.75	13.13	8.75	6.88
	400	18.71	10.47	5.61	3.22
	500	19.56	7.25	2.90	1.45
Azadirachtaa indica	100	19.22	15.97	12.24	10.11
	200	16.71	13.17	10.05	8.26
	400	17.94	13.07	8.19	5.44
	500	17.82	10.27	5.33	3.41
Heliotropium bacciferum	100	19.35	15.48	11.61	4.03
	200	18.79	14.09	9.39	7.38
	400	16.55	12.31	7.97	4.34
	500	17.93	9.65	4.13	2.51
	Control	19.11	18.59	19. 88	20.21

these Tables.The results in Table (5) indicated that the infestation percentage in the colonies treated with 500 ppm acetonic extract of *R . stricta* was reduced to 0.59 % after 12 days. While it was 1.45 Table (3) and 3.89 % Table (4) for aqueous and methanolic extracts, respectively. The infestation percentages of acetonic, aqueous and methanolic extracts of *A. indica* and *H. bacciferum* were reduced to 2.64 , 3.41 , 6.34 , 1.95 , 2.51 and 5.16 % after 12 days of treatment, respectively. It means that the acetonic extract of *R. stricta* is more toxic to *Varroa* inside colonies of honeybees than other extracts. The effect of extracts of the plants on worker bees indicated that all test extracts were less or no effect on honeybee at different tested

Table 4. Average rate of infestation (%) in adult worker bees after treatment with methanolic extract from three plants.

Plant	Conc. (ppm)	Before treatment	Days after treatment		
			4	8	12
Rhazya stricta	100	19.85	15.44	12.50	8.8
	200	15.89	11.92	9.93	7.94
	400	17.72	12.65	8.86	5.06
	500	17.53	4.74	5.84	3.89
Azadirachta indica	100	18.51	17.01	14.25	10.93
	200	17.47	14.81	12.14	9.89
	400	18.72	13.99	10.76	8.14
	500	17.66	12.10	8.20	6.34
Heliotropium bacciferum	100	18.93	16.90	13.92	10.83
	200	17.08	14.11	11.73	4.31
	400	17.69	13.74	10.11	7.66
	500	19.22	11.04	7.92	5.16
	Control	17.23	18.74	19.57	19.36

concentrations. *R. stricta* was previously reported to be toxic to mosquito larvae (Elhag *et al* ., 1996) and rats (Adam , 1998). Elhag *et al* (1996) reported that *R. stricta* have alkaloids and flavonoids as major constituents. Also, aerial parts of *H. bacciferum* contain alkaloids, flavoniods, tannins and volatile oils (Al – Yahya *et. al.*, 1990). Finally it can be concluded that the materials investigated in this study may have a practical application in protection of bees against *Varroa* mites. Also, acetonic extract of *R. stricta* and other plant extracts may considerd promising as natural botanical products against *Varroa* mites.

Table 5. Average rate of infestation (%) in adult worker bees after treatment with acetonic extract from three plants.

Plant	Conc. (ppm)	Before treatment	Days after treatment		
			4	8	12
Rhazya stricta	100	16.02	12.17	10.25	7.05
	200	16.55	10.07	7.19	4.31
	400	15.60	8.51	4.97	2.12
	500	18.75	7.64	2.08	0.59
Azadirachta indica	100	17.19	13.85	10.99	8.32
	200	18.35	11.94	9.23	6.48
	400	17.44	10.55	6.96	4.48
	500	16.63	4.26	4.81	2.64
Heliotropium bacciferum	100	17.35	13.18	11.38	8.44
	200	17.42	10.87	8.26	5.61
	400	18.07	4.46	5.88	3.33
	500	18.19	8.71	3.41	1.95
	Control	189.56	17.39	18.41	18.76

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الملخص العربى

تقييم كفاءة بعض المستخلصات النباتية فى مكافحة طفيل الفاروا الذى يصيب نحل العسل

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تم دراسة سميه بعض المستخلصات النباتية لكل من الحرمل *R. stricta* والنيم *A. indica* والرمرام *H. bacciferum* وهذه المستخلصات هى المائى والميثانولى والمستخلص الاسيتونى وذلك لمكافحة طفيل الفاروا الذى يصيب نحل العسل وذلك تحت الظروف المعملية والحقلية . وقد وجد من النتائج أن جميع هذه المستخلصات كانت ذات سميه عل الاكاروس بدرجات مختلفة .

وكان التأثير الأكبر للمستخلص الأسيتونى لكل النباتات مقارنة مع المستخلص المائى و الميثانولى لكل النباتات. وظهر أن المستخلص الأسيتونى لنبات الحرمل له سميه عالية فى مكافحة الفاروا عن المستخلصات الاخرى . كانت قيم LC_{50} بعد ٤٨ ساعة من المعاملة بتركيز ٥٠٠ جزء فى المليون هى ١٢٣,٤ مقارنة مع ١٦٩,٣ و ١٥٠,٨ لكل من المستخلص الأسيتونى لنبات النيم والحرمل على التوالى . كانت نسبة الموت ١٠٠% للمستخلص الأسيتونى لنبات الحرمل بعد ٤٨ ساعة من المعاملة بتركيز ٥٠٠ جزء فى المليون وهذا أعلى من النتائج المتحصل عليها لكل من النيم والرمرام حيث كانت ٩٠ ، ٩٣،٣% على التوالى وفى نفس الوقت عند المعاملة الحقلية وجد أن المستخلص الأسيتونى للحرمل قلل من الإصابة بالفاروا إلى ٠,٥٩% بعد ١٢ يوم من المعاملة مقارنة بجميع المستخلصات الاخرى .

ويمكن القول أن هذه المستخلصات خصوصا المستخلص الأسيتونى للحرمل أو المستخلص الأسيتونى للرمرام أو النيم يمكن إستخدامها كبديل آمن فى مكافحة الفاروا فى نحل العسل .يليها المستخلص المائى ثم المستخلص الميثانولى