J.Pest Cont. & Environ. Sci. 9(3): 27-38 (2001)

Toxicity of certain plant extracts to the parasitic bee mite *Varroa jacobsoni* in colonies of *Apis mellifera.*

By

Ahmed A. Zaitoon and Mohamed A. S. AL-Eryan
Dept. of Economic Entomology, Faculty of Agriculture, EL-Shatby, Alexandria University.

Received 15/7/2001, Accepted 18/8/2001

ABSTRACT

Aqueous extracts of three plants were evaluated in laboratory and field trials as safe and natural botanical products against the parasitic bee mite, *Varroa jacobsoni* (Oudemans), in colonies of the honeybee, *Apis mellifera* L. The tested plants were *Rhazya stricta* Decaisne, *Azadirachta indica* A. Juss and *Heliotropium bacciferum* (Forsk.). The results indicated that all the tested extracts were toxic to *Varroa* mites. The most potent product was *R. stricta* extract followed by *H. bacciferum* and *A. indica. R. stricta* extract had significantly lower with LC_{50} of 132.6 ppm than both of *A. ndica* and *H. bacciferum* extracts (181.7 and 177.7 ppm, respectively). *R. stricta* caused 96.64 % mortality to mites while it was 90 and 93 % for *A. indica* and *H. bacciferum*, respectively. Field evaluation showed that the percentages of infestation with mites reduced to 0.63, 2.68 and 1.22 % after 12 days of application (600 ppm) with *R. stricta, A. indica* and *H. bacciferum*, respectively. The results suggested that the tested aqueous extracts, particularly *R.stricta* are promising safe products for control of *V. jacobsoni* .

INTRODUCTION

The *Varroa* mite, an ectoparasite of honeybees has presented serious problems for beekeeping industry. It is nearly world wide distributed (Nixon, 1983). Adaptation ability to a new host (*Apis mellifera*), importation of queen bees from infested areas, and movement of infested bee colonies for pollination led to the rapid spread of this mite all over Egypt. The first record of this mite was in 1983 (Wienands, 1988). The parasitic mite *Varroa jacobsoni* (Oud) is considered the most serious global threat to beekeeping because of the widespread use of the western honeybee *Apis millifera* L. for honey production and crop pollination (Dejong 1990). Efforts to the control of *Varroa* mites have focused on the evaluation of both synthetic and natural compounds. However, the use of synthetic acaricides raises the possibility of contamination of hive products (Ruijter 1995, Wallner 1995). This concern has generated considerable interest in the use of natural products for the control of *Varroa* mites.

Several natural products proved to be useful in maintaining mite infestation rates below economic injury level (Calderone *et. al,* 1997). Thymol fumigant, thyme and sage oils and origanum oil were proved to be effective against *Varroa* (Imdorf *et al.,*1995; Colin, 1990 and Gal *et al.,* 1992). On the other hand, extracts from seeds and leaves of neem tree were found to inhibit feeding, metamorphosis, fecundity and oviposition in many insect species (Chen *et al.* 1996). Koul (1992), referred to use of neem as being both environmentally and economically desirable. *Rhazya stricta* Decaisne (Apocyanaceae) and *Heliotropium bacciferum* (Forsk.), (Boraginaceae), are herbaceous plant widely distributed in the kingdom of Saudi Arabia (Migahid, 1978). They are known

to possess some biological activity against insects (Elhag *et al.*,1996) and higher animals (Adam, 1998). The objective of this work is to evaluate the efficacy of three semi-arid adapted widely distributed plants on an agressive parasitic bee mites *V. jacobsoni.*

MATERIALS AND METHODS

Plant extracts: Tested plants were collected from the central regions of Saudi Arabia. Aerial parts of *R. stricta, H. bacciferum*, and *A. indica* seed kernels were taken, air dried, ground to a fine powder and extracted four times by warm distilled water at ambient temperature, The combined aqueous extracts were then freeze-dried using a labconco freeze drier-18 model 75018 for 48 hr.

Test procedure: Stock solutions of three plant material extracts were prepared by redissolving the extract in warm distilled water (0.5 g/100 ml). Concentrations of 100, 200, 400 and 600 ppm were prepared from each stock solution.

Insects: The noninfested and infestd brood and adult workers of honeybees, *Apis millifera* with *Varroa jacobsoni* were investigated in the apiary of Agriculture Researsh Station, Faculty of Agriculture, Alexandria University.

Laboratory experiment: Different concentrations of 100, 200, 400, and 600 of each aqueous plant extract were examined. Ten worker bees infested with *Varroa* mite were sprayed with each concentration using a hand sprayer. Insects put in glass cups (200 cc each) and covered with muslin texture. All experimental glass cups were supplied with sugar syrup 30% and changed

daily. The mortality percentages of *Varroa* mites were recorded 24 and 48 hours after treatment. Every concentration was triplicated. Values of LC_{50} and fiducial limites were calculated according to Finney (1971) and Aboutt's formula (1925) was used when nessesary.

Fjeld experiment: Colonies approximately of the same strength that were infested with *Varroa* mites were selected for the experiment. The same concentrations were tested under the field conditions using hand sprayer (500ml ca.). Different concentrations were sprinkled over the bees in the combs. Each concentration was replicated three times. The same treatments were repeated after 4 days.

To determine the degree of infestations, combs of sealed brood for each treatment were taken and about 150 cells worker brood were investigated. The infestation percentages were also calculated. Adult workers (Ca.150) were collected from brood nest area of each colony. Samples were transferred to a beaker (200 cc) containing soap water to separate the attached mites. The later were collected and counted.

RESULTS AND DISCUSSION

Mortality percentages of *Varroa* mites at 24 and 48 hr after treatment with different plant extracts using spraying method are presented in Table 1. The results indicated that all tested extracts were toxic to *Varroa* mites in a dose dependent manner. The toxic action was relatively less for *A. indica* and *H. bacciferum*.The extract of *R. stricta* was the most effective, where its 600 ppm caused 96.67 % mortality. While in the case of *H. bacciferum* and *A. indica*, it was 93 and 90 %, respectively. The LC_{50} values and confidence limits are shown in Table 2. The results indicated that *R. stricta* had significantly lower LC_{50}s

Table 2: Effect of the tested plant extracts on *Varroa* mites using spraying method under laboratory conditions.

Plant	Bioassay time	Regression of N.E.D response (r) on Log dose (X)	LC_{50}* (Conf. Limits) %	P
Rhazya stricta	24 hr	Y= 5.4 + 2.3 X	239.4 (186.3-307.6)	0.9
	48 hr	Y= 6.6 + 3.1 X	132.6 (103.8-169.2)	0.4
Azadirachta indica	24 hr	Y= 5.8 + 2.3 X	302.8 (237.8-385.6)	0.9
	48 hr	Y= 5.5 + 2.4 X	181.7 (140.7-234.5)	0.9
Heliotropium bacciferum	24 hr	Y= 5.6 + 2.3 X	294.9 (230.4-377.5)	0.8
	48 hr	Y= 6.4 + 2.8 X	177.7 (141.5-222.9)	0.7

plant extracts at different times were determined. The data of Table 3 indicated that the infestation percentages in colonies treated with *R. stricta* extracts ranged from 19.41 to 22.62 before treatment. After treatment with different concentrations of *R. stricta,* the rate of infestation percent with *Varroa* decreased to 1.01% after 12 days following application with 600 ppm. Rate of infestation in the case of treatments with *A. indica* and *H. bacciferum* was decreased to 4.44 and 3.11 %, 12 days following first application with 600 ppm, respectively. The same trend was noticed for all concentrations among various periods. The average rate of infestation percent with *Varroa* mite in adult workers is shown in Table 4. The data decleared that the rate of infestation decreased with the increasing of the concentrations of all plant extracts. The lowest infestation percent was observed for *R. stricta* extract followed by *H. bacciferum* and *A. indica*. They were 0.63, 1.22 and 2.68 % at 600 ppm, respectively, after 12 day following application. The side effect of the tested plant extracts on honeybees is shown

in Table 5. All the applied concentrations of the tested plantextracts showed low or no effect on honeybees at at different bioassay times.

The toxic effect of *R. stricta* was reported on mosquito larvae (ELhag *et al.*, 1996), *Agrotis ipsion* Hufn (ELhag *et al.*, 1999) and wistar rats (Adam, 1998). It might be due to its high content of active alkaloids (Rahman and Fatima, 1982). *H. bacciferim* also showed toxic effects against *Varroa.* Its aerial

Table 3: Average infestation percent of worker brood with *Varroa jacobsoni* after treatment with aqueous plant extracts under field conditions.

Plant	Concentration (ppm)	Before treatment	Days after first treatment		
			4	8	12
Rhazya stricta	100	21.32	16.45	13.16	10.22
	200	19.41	13.72	10.21	8.51
	400	22.62	13.71	8.63	3.60
	600	20.73	13.33	6.46	1.01
Azadirachta indica	100	24.15	20.83	16.04	12.11
	200	22.12	15.03	13.16	10.37
	400	18.43	13.98	10.21	7.00
	600	21.68	13.85	9.56	4.44
Heliotropium bacciferum	100	23.66	19.41	16.10	11.53
	200	21.90	18.54	11.67	9.12
	400	22.14	16.55	10.13	6.33
	600	24.57	19.23	8.44	3.11
Control	-	23.41	22.69	21.15	23.11

parts contain alkaloids, flavoniods, tannis and volatile oils (AL-Yahia *et al.*, 1990). The major active constituent in neem is the limonoid and azadirachtin (Xie et al., 1985). *A. indica* extract revealed toxic effect to *varroa* mites, but less than *R. stricta* and *H. bacciferim*. Also, Rembold (1989) reported that neem is toxic to the Mexican bean beetle. Findings of the present study indicated that investigated plant extracts particularly *R. stricta* are promising as safe natural products for the control of *Varroa* mites.

Table 4: Average infestation percent of adult worker with *Varroa jacbsoni* after treatment with aqueous plant extracts under field conditions.

Plant	Concentration (ppm)	Before treatment	Days after first treatment		
			4	8	12
Rhazya stricta	100	18.67	15.33	12.00	10.00
	200	20.95	12.22	10.18	6.08
	400	19.11	9.55	5.10	1.27
	600	16.46	4.43	2.53	0.63
Azadirachta indica	100	16.56	14.72	13.49	11.64
	200	16.13	13.55	11.61	7.74
	400	21.33	12.00	6.67	5.33
	600	17.44	10.07	5.37	2.68
Heliotropium bacciferum	100	19.12	15.73	12.66	11.25
	200	17.33	12.67	10.66	6.67
	400	18.18	11.03	6.44	4.52
	600	17.10	8.11	4.27	1.22
Control	-	19.54	18.91	19.26	17.89

Table 5: Effect of the aqueous plant extracts on the worker of honeybee following spraying method.

Plant	Concentration (ppm)	Percentage of mortality after	
		24 hr	48 hr
Rhazya stricta	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	600	0.0	3.3
Azadirachta indica	100	0.0	0.0
	200	0.0	0.0
	400	3.3	3.3
	600	6.7	10.0
Heliotropium bacciferum	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	600	3.3	3.3
Control	-	0.0	3.3

REFERENCES

Abbott, W.S. (1925). A method of comparing the effectiveness of an insecticide. J. Econ. Entomol., **18**: 265-267.

Adam, S.E.L. (1998) Toxicity to sheep of *Rhazya strictia*. Vet. Hum. Toxicol., **40:** 68-70.

AL-Yahia, M.A., I. A. Meshal, J.S. Mossa, A.A. AL-Badr and T. Mohammed (1990), Saudi plants. A phytochemical and

biological approach. KASCT No. 39. King Saud University Press.

Calderone, N.W., W.T. Wilson and M.A. Spivak (1997) plant extracts used for control of the parasitic mites *Varroa jacobsoni*(Acari: Varroidae) and *Acavapis woodi* (Acari: Tarsonemidoe) in colonies of *Apis mellifera* (Hymenoptera: Apidae) J. Econ. Entomol., **90** (5): 1080-1086.

Chen, CC., Y. J. Dong, L.L. Cheng and R.F. Huo. (1996). Deterrent effect of neem seed kernel extracts on oviposition of the oriental fruit fly (Diptera: Tephritidae) in Guava. J. Econ. Entomol., **89:** 462-466.

Colin, M.E. (1990) Essential oils of Labiatae for controlling honey bee Varrosis. J. Appl. Entomol., **110:** 19-25.

Dejong, D (1990). Mites: *Varroa* and other parasites of brood. pp. 200-218. In R.A. Morse and R. Nowogrodziki [eds.], Honeybee pests, predators and diseases, 2nd ed. Cornell University press, Ithaca, NY.

Elhag, E.A., F.M. Harraz, A.A. Zaitoon and A.K. Salama (1996). Evaluation of some wild herb extracts for control of mosquitoes (Diptera: Culiciade). J. King Saud University, Agric. Sci., **8:** 135-145.

Elhag, E.A., A.H. ELNadi and A.A. Zaitoon (1999). Effect of some plant extracts on two agricultural pesta *Agrotis ipsilon* (Lepidoptera: Noctuidae) and *Hypera brunneipennis* (Coleoptera: curculionidae) Alex J. pharm. Sci., **13** (1): 15-19.

Finney, D.J. (1971). Probit analysis. 3rd ed. Cambridge University press Cambridge, England.

Gal, H., Y. Slabezki and Y. Lensky (1992). A Preliminary report on the effect of origanum oil and thymol applications in honeybee (*Apis mellifera* L.) colonies in a subtropical climate on population level of *Varroa jacobsoni*. Bee Sci., **2:** 175-180.

Imdrof, A., V. Kilechenmann, S. Bogdavnov, B. Bachofen and C. Beretta (1995). Toxizitat von thymol, camphor, menthol and eucalyptol auf *Varroa jacobsoni* oud. Und *Apis mellifera* L. imboratest, Apidologie, **26:** 27-31.

Koul, O (1992). Neem allelochemicals and insect control. Chapman and Hall, London.

Migahid, A.M. (1978). Flora of Saudi Arabia. Riyadh, University Press, Riyadh Saudi Arabia.

Nixon, M. (1983). World maps of *Varroa jacobsoni* and *Tropilaeaps clareae* with additional records for honeybee diseases and parasites previously mapped. Bee world **64:** 125-131.

Rahman, A.U. and K. Fatima (1982). The alkaloids of *Rhazya stricta* and *Rhazya orientalis*. J. Chem.. Soc. Pak., **4:** 121-125.

Rembold, H. (1989), Isomeric azadirachtins an their mode of action. In focus on phytochemical pesticides. Vol. I. The neem tree. Ed. M. Jacobson, pp 47-67. CRC, Boca Raton, FL, USA.

Ruijter, A. de (1995). Issues in the control of *Varroa* infestation, pp. 24-26. In A. Matheson (ed.), New perspectives on *Varroa.* IBRA, Cardif, U.K.

Wallner, K. (1995). The use of varroacides and their influence on the quality of bee products. Am. Bee J., **135:** 817-821.

Wienands, A. (1988). The *Varroa* mite has spread over most of the world. Am. Bee J., **128:** (5): 358-359.

Xie, Y.S., P.G. Fields and M.B. Isman (1985). Repellency and toxicity of Azadirachtin and neem concentrates to three stored product beetles. J. Econ. Entomol., **88** (44): 1024-1031.

الملخص العربى

سمية بعض المستخلصات النباتية على طفيل الفاروا الذى يصيب نحل العسل

أحمدعلى زيتون- محمد عوض سليمان
قسم الحشرات الأقتصادية – كلية الزراعة (الشاطبى) – جامعة الأسكندريه

أجريت دراسة معملية وحقلية لمعرفة تأثير المستخلص المائى لثلاث من النباتات كبديل آمن على الإنسان والنحل والبيئة لمكافحة طفيل الفاروا والذى يسبب خسائر كبيرة فى طوائف نحل العسل. هذه النباتات هى الحرمل *Rhazya stricta*، النيم *Azadirachta indica* والرمرام *Heliotropium bacciferum* وقد أظهرت النتائج أن كل المستخلصات ذات كفاءة عالية فى مكافحة الفاروا وكان التاثير الأكبر من نصيب المستخلص المائى للحرمل ويأتى بعد ذلك الرمرام ثم النيم حيث كانت قيم LC_{50} أقل بالنسبة للحرمل(١٣٢,٦ جزء فى المليون) أما بالنسبة للنيم والرمرام فهى ١٨١,٧و ١٧٧,٧ على التوالي وذلك بعد ٤٨ساعة وكانت نسبة الموت فى الطفيل عند المعاملة بالمستخلصات النباتية وهى الحرمل والنيم والرمرام هى ٩٦,٦٠، ٩٠ ،٩٣,٩% على التوالى عند المعامله بتركيز ٦٠٠جزء فى المليون ، وعند معاملة الخلايا بالمستخلصات المائية للنباتات المذكورة وجد أن الإصابة بالفاروا قلت بعد ١٢ يوم إلى ٠,٦٣% بعد المعاملة بمستخلص الحرمل وإلى ٢,١٨ و ١,٢٢% بعد المعامله بكل من النيم والرمرام على الترتيب.

هذا وتشير النتائج السابقة إلى إمكانية استخدام هذه المستخلصات المائية للنباتات المذكورة كبديل آمن فى مكافحة طفيل الفاروا خاصة مستخلص نبات الحرمل.