Research Article

Evaluation of Essential Oils for the Management of Parasitic Bee Mites, *Varroa destructor* (Acari: Varroidae) *In Vitro*

Muhammad Abu Bakar[1], Muhammad Anjum Aqueel[1], Abu Bakar Muhammad Raza[1], Rashid Mahmood[2], Ziyad Abdul Qadir[2], Muhammad Arshad[1] and Mubasshir Sohail[3]

[1]Department of Entomology, University of Sargodha, 40100, Sargodha, Pakistan; [2]Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakitan; [3]Nuclear Institute of Agriculture, Tando Jam-71000, Pakistan.

Abstract | The experiment was conducted at University College of Agriculture, University of Sargodha, Sargodha during 2015. The apiary had 20 Langstroth standard colonies of *Apis mellifera* naturally infested with *Varroa destructor. Varroa destructor* (Anderson and Trueman) is the most destructive pest of honeybees and a major threat to beekeepers in different areas of the world. Many control measures including application of chemicals, are adopted to control the infestation of mites in honey bee colonies. Chemical are still the most effective option; however, they pose different threats; due to which they should be alternated with non chemical options. Natural acaricides are considered important alternative control measure for *Varroa* mites. This study was planned to check the efficacy of different essential oils (Lemon oil, Eucalyptus oil, Wintergreen oil, Orange oil and Neem oil) in different concentrations against percent mortality of *Varroa* mites. The essential oils were applied at different concentrations (100 ppm, 200 ppm and 400 ppm) and percent mortality data were recorded after 12, 24, 48 and 72 hrs of the application. The results of present study were showed that lemon oil and eucalyptus oils were best compounds to manage *Varroa* mites at 200 ppm concentration. Percent mortality of *Varroa* mites was 59.2% and 58.3%, respectively after 72 hrs. When selected essential oils with different concentrations were applied against *Varroa*, no mortality of bees was observed. Neem oil gave (90%) significant control at 400 ppm concentration. There was no significant mortality observed in winter green oil. So, the neem oil, eucalyptus oil and lemon oil can be used against *Varroa* mites to improve the production of honey.

Received | May 03, 2019; **Accepted** | July 21, 2019; **Published** | October 21, 2019
***Correspondence** | Rashid Mahmood, Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan; **Email:** rashid_ento1@yahoo.com
Citation | Bakar, M.A., M.A. Aqueel, A.B.M. Raza, R. Mahmood, Z.A. Qadir, M. Arshad and M. Sohail. 2019. Evaluation of essential oils for the management of parasitic bee mites, *Varroa destructor* (Acari: Varroidae) *in vitro*. *Pakistan Journal of Agricultural Research,* 32(4): 566-571.
DOI | http://dx.doi.org/10.17582/journal.pjar/2019/32.4.566.571
Keywords | Honeybee, *Varroa* mites, Essential oils, Percent mortality, Concentrations

Introduction

Honeybee *Apis mellifera* L. is the most important insect that have benefited mankind for medicinal and nutritional purposes for thousands of years. *A. mellifera* is of great economic importance to agriculture not only for honey production but also for crop pollination (Klein et al., 2007). A number of insect-pests and microbes attack on honeybees. Among these insects pest, few mite species have been recorded as a parasite on the brood and adults of honeybees (Mahmood et al., 2011). Among these ecto-parasites, *Varroa destructor* is posing great threat to the beekeeping industry in Pakistan, since the last two decades (Saleem et al., 2008). The parasitic bee mite, *V. destructor* (Anderson and Truman) is causing

serious complications in beekeeping all over the world. The mites can easily be observed on adult bees, brood and in hive debris. Pupae cannot develop into adult form in case of heavy infestation (Shimanuki and Knox, 2000). Different pathogens such as acute bee paralysis virus, deformed wing virus, and fungi are probably transferred to bees by *Varroa* (Chen and Siede, 2007). It also reduces colony ability to pollinate plants (De Jong et al., 1984).

The repetition of pesticides exposure to mites caused severe problems in bees such as pesticide toxicity to bees and increased probability of disease-resistance (Watkins, 1997). Natural acaricides offer a desirable alternative to synthetic pesticides, tending to have low mammalian toxicity and little environmental effects. Organic acid and essential oils or essential oils compounds are the most known natural products with acaricidal activity (Eguaras et al., 1996).

Essential oils are defined as any volatile oil that have strong aromatic components and give distinctive odor, flavor or scent to a plant (Koul, 2008). These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. The aromatic characteristics of essential oils provide various functions for the plants including attracting or repelling insects (Koul et al., 2007).

It is well known that many essential oils and their components exhibit acaricidal activity. Different component of essential oils was tested for their activity against *Acarapis woodi* (Baggio et al., 2004). Because of the promising activity of the essential oils, against *Varroa* mite, many studies have been conducted to find out the effective monotepenoidal component in the essential oils (Calderone and Spivak, 1995). The eucalyptus oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones (Dehghani-Samani et al., 2015). Neem oil contains oleic acid, stearic acid, linoleic acid, nimbin and salannin (Mishra et al., 1995). The main chemical components of lemon oil are α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, linalool, β-bisabolene, limonene, trans-α-bergamotene, nerol and neral (Khani et al., 2012). Several synthetic acaricides have been applied to hives in order to control *Varroa* mites but, due to the widespread misuse of chemical treatments, several cases of resistance and contamination of hive products have been reported (Pettis, 2004).

Natural products such as essential oils offer a highly desirable alternative to synthetic products (Bakar et al., 2018). Products of botanical origin have shown a wide range of biological activities including toxicity, repellence, and antifeedant effects in parasites and growth regulatory properties (Damiani et al., 2011).

Keeping in view the serious threat of *Varroa* mite in the beekeeping and several constraints in its control, the present investigation was carried out to develop the safer and effective management option for the mite population by using some essential oils with their advantages of low cost and low health and environmental hazards for both consumers and bee keepers.

Materials and Methods

This experiment was conducted in the research laboratory of Department of Entomology, College of Agriculture, University of Sargodha, Sargodha. Five essential oils/ chemical were purchased from Musa Jee enterprise, Sargodha, Pakistan. (neem oil, lemon oil, eucalyptus oil, winter green oil and orange oil) and applied at dose rate of 0.25 ml (per treatment) with concentration of 100 ppm, 200 ppm and 400 ppm. One µl of essential was added in 9.99 ml of acetone by using micro liter pipette to make 10 ml volume of solution for 100 ppm concentration (El-Zemity et al., 2006). Similarly 200 ppm and 400 ppm concentration were prepared.

Ten worker bees of *A. mellifera* infested with *Varroa* mites were collected for each treatment from Apiary which contained 20 hives at College of Agriculture, University of Sargodha, Sargodha. The number of *Varroa* mites on the selected bees was also counted carefully by naked eye. The plastic jars (15 cm × 8 cm) were used for this experiment. The selected bees infested with *Varroa* were kept inside plastic jars for each treatment. A simple cardboard (2 × 2 cm) soaked in each concentration of the test substance was hanged by means of appropriate wire inside the jar. A screen mesh (8cm) was placed above 2 cm from bottom of each jar that allowed dead mites to fall through to a sticky paper placed below. Bees were served on sugar solution for 24 hrs.

Table 1: *Analysis of variance regarding percentage mortality of Varroa mites by using different essential oils.*

Source	DF	SS	MS	F Valve	P Valve
Essential oils	4	6426.1	1606.5	7.56	P<0.001
Time	3	43094.5	14364.8	67.55	P < 0.001
Dose	2	11040.0	5520.0	25.96	P < 0.001
Treatment × Time	12	2337.1	194.8	0.92	P > 0.1
Treatment × Dose	8	1400.2	175.0	0.82	P > 0.1
Time × Dose	6	4282.0	713.8	3.36	P < 0.01
Treatment × Time × Dose	24	2681.3	111.7	0.53	P > 0.1
Error	120	25517.0	212.6		
Total	179	96779.1			

0.01=Significant, 0.001=Highly significant.

Table 2: *Means ± SE of interaction between time intervals and concentrations against Varroa mites.*

Time	Dose	Mean
72 HAT*	100 ppm	37.71 ± 3.96^{bc}
	200 ppm	51.21 ± 3.09^{b}
	400 ppm	74.18 ± 3.9^{a}
48 HAT	100 ppm	29.16 ± 3.7^{cde}
	200 ppm	37.50 ± 3.1^{bc}
	400 ppm	50.73 ± 2.0^{b}
24 HAT	100 ppm	18.49 ± 2.5^{def}
	200 ppm	23.39 ± 2.9^{cdef}
	400 ppm	32.07 ± 2.5^{b}
12 HAT	100 ppm	10.66 ± 1.81^{f}
	200 ppm	13.62 ± 2.03^{ef}
	400 ppm	15.17 ± 1.91^{def}

** HAT= Hours after treatment application; Means sharing similar letters are not significantly different from each others.*

Evaluation of the tested materials and techniques were based on the mortality percentage and it was calculated through the modified (Abbott, 1925).

$$Percent\ mortality = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \times 100$$

Where;
n= Insect population; T= treated and Co = control.

The mites mortality data were recorded after 12, 24, 48 and 72 hours after treatment by counting the number of fallen mites on the bottom of the jars.

Data analysis

Percentage of mortality was calculated by Abbott's formula. Three factor factorial under Complete Randomized Design was used to analyzed the data of percent mortality of *Varroa* mites. Essential oils, time interval and concentrations were independent variables and percent mortality was considered as dependent variables. Means were separated by using Tukey HSD all pair wise comparison test. All the experimental analysis was performed using Minitab 16.1 software.

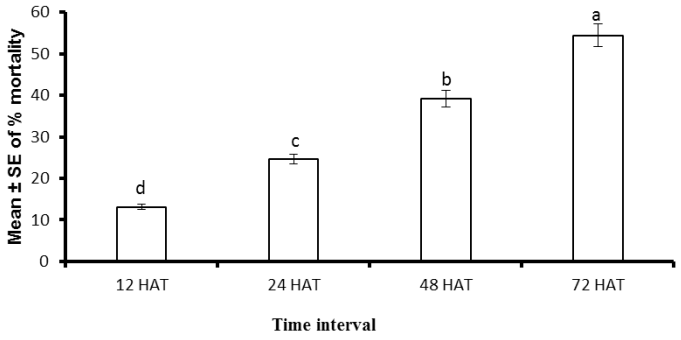

Figure 1: *Percent mortality of Varroa mites after application of essential oils at different time interval.*

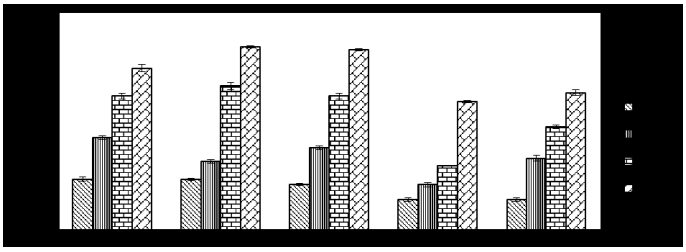

Figure 2: *Percent mortality of Varroa mites against different essential oils at 100 ppm concentration.*

Results and Discussion

Analysis of variance regarding percent mortality of different essential oils against *Varroa* mites showed that treatment (F=7.56, P<0.001) time (F=67.55, P<0.001) and dose (F=25.96, P<0.001) were significant. Similarly, the interaction between time and dose (F=3.36, P<0.01) showed significant results (Table 1). Maximum percent mortality was observed (74.18 %) with 400 ppm concentration after 72 hours for all treatments. Minimum mortality was observed (10.66 %) with 100 ppm concentration at 12 HAT (hours after treatment) (Table 2). Percent mortality increase with the passage of time in all selected essential oils (Figure 1). By using 100 ppm concentration the percent mortality (45 %) was recorded high in lemon oil after 72 hours which was significantly different from others. Minimum mortality (20.8 %) was observed in winter green oil at 100ppm after 72 hours. (Figure 2). At 200 ppm

concentration significant mortality percentage was observed (59.2 %, 58.9 %) in lemon oil and eucalyptus oil, respectively which was significantly different from other treatments after 72 hours (Figure 3). In case of 400 ppm concentration neem oil gave significant results (90 %) after 72 hours, but the results were not significantly differ from lemon oil and eucalyptus oils which also proved as effective with percent mortality 87.5% and 83.3%, respectively. Overall minimum mortality was observed in case of wintergreen oils (Figure 4). The percent mortality was observed maximum at higher concentrations of each treatment.

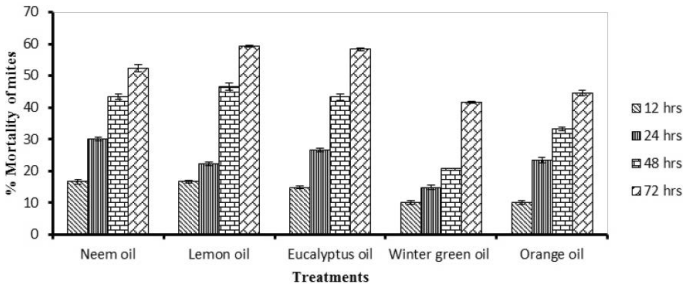

Figure 3: *Percent mortality of Varroa mites against different essential oils at 200 ppm concentration.*

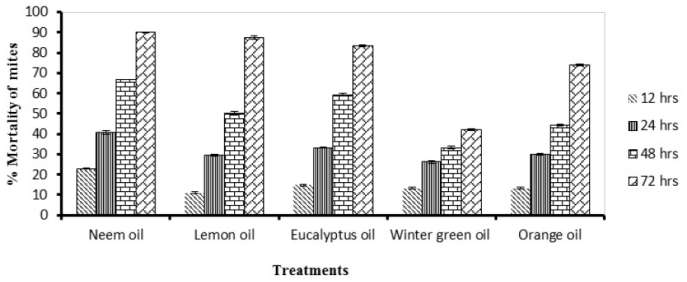

Figure 4: *Percent mortality of Varroa mites against different essential oils at 400 ppm concentration.*

Varroa mites have been controlled by using synthetic acaricides to infested colonies which were applied in formulated plastic strips. Recently, resistance to acaricides has become a major problem in the control of *Varroa* mite (Bakar et al., 2017). Use of synthetic lipophilic acaricides has led to the accumulation of residues in beewax, propolis and in honey, which is harmful for human health. But now a days, recommendation about chemical control are being changed to new products like essential oils (Mahmood et al., 2014). Because resistance developed in *Varroa* mites against chemicals therefore essential oils are good alternative to manage *Varroa* mites (Bakar et al., 2017). Superior better the results of our study showed that all essential oils except winter green oil proved best to control *Varroa* mites. At 100 ppm concentration the percentage mortality was maximum in lemon oil. Similarly, at 200 ppm concentration lemon oil and eucalyptus oil gave significant control of *Varroa* mites. Neem oil proved as the best to control *Varroa* mites at 400 ppm concentration. Essential oils proved to be a better control option for the control of *Varroa* mites as it being degradable in nature, target specific and less resistance as compared to synthetic pesticides (Gerard et al., 1997). Our results are also in favor of (Calderone et al., 1997) who suggested that essential oils are best compound to maintain infestation of mites below economic injury level. Essential oils ceased the natural intestine movement due to which insect can no longer feed (Blaney et al., 1990). According to (El-Zemity et al., 2006) eucalyptus and lemon oil were found to the best compound to manage *Varroa* mites. Similarly, according to (Bunsen, 1991) neem oil proved to be best compound against *Varroa* mites. Essential oils have several modes of action including repellency and anti-feedant activity, disruption of molting and cuticle, as well as retardation of growth and fecundity (Cosimi et al., 2009). Lemon oil and eucalyptus oils have fumigant activity against mites (Miresmailli et al., 2006). Among the various components of eucalyptus oil, 1, 8-cineole is the most important one. In fact, it is a characteristic compound of the genus Eucalyptus which is largely responsible for a variety of its pesticidal properties and insecticide effects (Batish et al., 2008). Neem oil provides repellant, anti-hormonal and anti-feedant properties. Salannin in the neem oil provides repellant properties (Blaney et al., 1990). General insect mortality is dose and exposure time dependent. In present study percentage mortality increased with increase in time interval. With the passage of time the toxicity of essential oils against *Varroa* mites increase and proved more potent against *Varroa* mites. Due to application of essential oils at different concentrations, a significant mortality of mites was observed. Furthermore, these oils did not affect the bees population.

Conclusions and Recommendations

The finding of present study showed that the investigated essential oils particularly neem, lemon and eucalyptus oils are optimistic as safe natural products against *Varroa* mites. These chemicals are available in the market and are cheap and safe to the environment.

Author's Contribution

Ziyad Abdul Qadir: Overall Management of the ar-

ticle.

Muhammad Abu Bakar: Conducted research, Data collection, Wrote abstract, Methodology.

Muhammad Anjum Aqueel: Coceived the idea.

Abu Bakar Muhammad Raza: Technical Input at every step.

Rashid Mahmood: Introduction, Conclusion, References.

Muhammad Arshad: Result and discussion, Introduction, References.

Mubasshir Sohail: Data entry in SPSS and analysis.

References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267. https://doi.org/10.1093/jee/18.2.265a

Baggio, A., P. Arculeo, A. Nanetti, E. Marinelli and F. Mutinelli. 2004. Field trials with different thymolbased products for the control of varrossis. Am. Bee J. 140: 898-900.

Batish, D.R., H.P. Singh, R.K. Kohli and S. Kaur. 2008. Eucalyptus essential oil as a natural pesticide. For. Ecol. Manage. 256(12): 2166-2174. https://doi.org/10.1016/j.foreco.2008.08.008

Blaney, W., M. Simmonds, S. Ley, J. Anderson and P. Toogood. 1990. Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae. Entomol. Exp. Appl. 55(2): 149-160. https://doi.org/10.1111/j.1570-7458.1990.tb01358.x

Bakar, M.A., M.A. Aqueel, A.B.M. Raza, M. Arshad, R. Mahmood and Z.A. Qadir. 2018. Comparative efficacy of five commercial synthetic acaricides against *Varroa destructor* (Anderson and Trueman) in *Apis mellifera* L. colonies. Pak. J. Zool. 50(3): 857-861. https://doi.org/10.17582/journal.pjz/2018.50.3.857.861

Bakar, M.A., M.A. Aqueel, A.B.M. Raza, M.I. Ullah, M. Arshad, M. Sohail and J. Molina-Ochoa. 2017. Evaluation of few essential oils for the management of parasitic bee mites, *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* L. Colonies. Pak. J. Zool. 49(6): 2005-2010. https://doi.org/10.17582/journal.pjz/2017.49.6.2005.2010

Bunsen, J.D. 1991. Experimentelle untersuchungen zur bekaempfung der milbe varroa jacobsoni oud: einem ektoparasit der honigbiene (Apis mellifera L.), mit Stoffen natuerlicher Herkunft: Justus-Liebig Universitaet.

Calderone, N.W. and M. Spivak. 1995. Plant extracts used for control of the parasitic mites Varroa jacobsoni in colonies of the western honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 88: 1211-1215. https://doi.org/10.1093/jee/88.5.1211

Calderone, N.W., W.T. Willson and M. Spivak. 1997. Plant extracts used for control of parasitic mite Varroa jacobsoni (Acari: Varroidae). J. Econ. Entom. 90: 1080-1086. https://doi.org/10.1093/jee/90.5.1080

Chen, Y.P. and R. Siede. 2007. Honey bee viruses. Adv. Virus Res. 70: 33-80. https://doi.org/10.1016/S0065-3527(07)70002-7

Cosimi, S., E. Rossi, P. Cioni and A. Canale. 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evaluation of repellency against Sitophilus zeamais Motschulsky, Cryptolestes ferrugineus (Stephens) and Tenebrio molitor (L.). J. Stored Prod. Res. 45(2): 125-132. https://doi.org/10.1016/j.jspr.2008.10.002

Damiani, N., L.B. Gende, M.D. Maggi, S. Palacios, J.A. Marcangeli and M.J. Eguaras. 2011. Repellent and acaricidal effects of botanical extracts on Varroa destructor. Parasitol. Res. 108(1): 79-86. https://doi.org/10.1007/s00436-010-2043-3

De Jong, D., L.S. Goncalves and R.A. Morse. 1984. Dependence on climate of the virulence of Varroa jacobsoni. Bee World. 65(3): 117-121. https://doi.org/10.1080/0005772X.1984.11098789

Dehghani-Samani, A., S. Madreseh-Ghahfarokhi, A. Dehghani-Samani and K. Pirali-Kheirabadi. 2015. Acaricidal and repellent activities of essential oil of Eucalyptus globulus against Dermanyssus gallinae (Acari: Mesostigmata). J. Herbmed Pharmacol.4(3): 81-84.

Eguaras, M., S. Quiroga and O. García. 1996. The control of Varroa jacobsoni Oud. (Acari: Gamasida) by means of Organic acids. Apiacta. 31: 51-54.

El-Zemity, S., H. Rezk and A. Zaitoon. 2006. Acaricidal activity of some essential oils and their monoterpenoidal constituents against the parasitic bee mites, Varroa destructor (Acari: Varroidae). J. Appl. Sci. Res. 2(11): 1032-1036.

Gerard, P.J., L.D. Ruf, S.D. Lorimer and A.C.G.

Heath. 1997. Activity of extracts and compounds from New Zealand gymnosperms against larvae of Lucilia cuprina (Diptera: Calliphoridae). NZ. J. Agric. Res. 40(2): 261-267. https://doi.org/10.1080/00288233.1997.9513246

Imdorf, A., S. Bogdanov, R.I. Ochoa and N.W. Calderone. 1999. Use of essential oils for the control of Varroa jacobsoni Oud. in honeybee colonies. Apidologie. 30: 209-228. https://doi.org/10.1051/apido:19990210

Khani, A., F. Basavand and E. Rakhshani. 2012. Chemical composition and insecticide activity of lemon verbena essential oil. J. Crop Prot. 1(4): 313-320. https://doi.org/10.5402/2012/263517

Klein, A., B.E. Vaissière, J.H. Cane, I. Steffan-Dewenter, S.A. Cunningham, C. Kremen and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Pro. R. Soc. London. B. Bio. Sci. 274: 303-313. https://doi.org/10.1098/rspb.2006.3721

Koul, O. 2008. Phytochemicals and insect control: an antifeedant approach. Crit. Rev. Plant Sci. 27: 1-24. https://doi.org/10.1080/07352680802053908

Koul, O., G. Singh, R. Singh and J. Singh. 2007. Mortality and reproductive performance of Tribolium castaneum exposed to anethole vapours at high temperature. Biopestic. Int. 3: 126-137.

Miresmailli, S., R. Bradbury and M.B. Isman. 2006. Comparative toxicity of Rosmarinus officinalis L. essential oil and blends of its major constituents against Tetranychus urticae Koch (Acari: Tetranychidae) on two different host plants. Pest Manage. Sci. 62(4): 366-371. https://doi.org/10.1002/ps.1157

Mahmood, R., E.S. Wagchoure, S. Raja, G. Sarwar and M. Aslam. 2011. Effect of thymol and formic acid against ectoparasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies. Pak. J. Zool. 43(1): 91-95.

Mahmood R., S. Asad, S. Raja, A.U. Mohsin, E.S. Wagchoure, G. Sarwar, N. Islam and W. Ahmad. 2014. Control of *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) by using plant oils and extract. Pak. J. Zool. 46(3): 609-615.

Mishra, A., N. Singh and V. Sharma. 1995. Use of neem oil as a mosquito repellent in tribal villages of Mandla district, Madhya Pradesh. India. J. Malariol. 32(3): 99-103.

Pettis, J.S. 2004. A scientific note on Varroa destructor resistance to coumaphos in the United States. Apidologie. 35(1): 91-92. https://doi.org/10.1051/apido:2003060

Saleem, M., M. Shafiq, M.I. Haq, A.M. Sabir and M. Hassan. 2008. Evaluation of some miticides aganist Varroa destructor (Varroidae: Acari) in colonies of honeybee Apis mellifera L. Pak. Entomol. 30(2): 133-136.

Shimanuki, H. and D.A. Knox. 2000. Diagnosis of honeybee diseases. Agric. Handbook no. AH-690. USDA, Washington, DC.

Watkins, M. 1997. Resistance and its relevance to beekeeping. Bee World. 78(1): 15-22. https://doi.org/10.1080/0005772X.1997.11099327