Pakistan J. Zool., vol. 46(3), pp. 609-615, 2014.

Control of *Varroa destructor* (Acari : Varroidae) in *Apis mellifera* (Hymenoptera : Apidae) by using Plant Oils and Extract

Rashid Mahmood,[1] Saima Asad,[2] Shazia Raja,[1] Atta ul Moshin,[2] Elizabeth Stephen Wagchoure,[1] Ghulam Sarwar,[1] Noor Islam[1] and Waqar Ahmad[2]

[1]*Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan*
[2]*PMAS University of Arid Agriculture, Rawalpindi, Pakistan*

Abstract.- To evaluate the acaricidal effects of some plant oils and tobacco extract on *Varroa destructor* mites, a study was conducted in laboratory as well as in bee hives at Honeybee Research Institute, NARC, Islamabad. In the lab experiments, clove oil and tobacco extract both proved to be equally effective against mites. The treatments were significantly effective when applied in 5% as compared to 10 and 15% concentrations. The most effective combination was clove oil and tobacco extract when used in 5% concentration for 24 h. In the field experiment all the oils/extract individually and in combinations confirmed clove oil + tobacco extract (T14) the best combination with 96.48±0.52 efficacy. The honey produced in kg was found maximum 20.5±0.29 in treatment T14. The highest number of fallen *Varroa* mites 381.67±24.31 was also occurred in T14 treatment and difference between the treatments was highly significant. No queens were lost and there was no adult honeybee mortality in any of the colonies during the experiment. A Completely Randomized Design (CRD) was applied to all laboratory and field experiments. It was concluded that clove oil + tobacco extract (T14) can play an important role in an integrated pest management program to control *Varroa* mite in honeybee colonies.

Key words: Plant oil, exhoneybees, mites, honey, acaricide, tobacco extract.

INTRODUCTION

In Pakistan, beekeeping is a profitable business. It is reported that there are more than 4,000 beekeepers rearing *Apis mellifera* in the beehives, about 400,000 colonies of *A. mellifera* has been producing 10,000 MT honey annually and 27000 families are being benefited from beekeeping (PARC, 2010-11).

Just after the introduction of *A. mellifera* in Pakistan during 1977-78, *Varroa destructor* mite became a serious pest of *A. mellifera* and attacked over a large number of honeybee colonies (Ahmad, 1988). Like other animals honeybees are also affected by various pests and diseases. One of the most common pests is *V. destructor* that provokes big losses in apiculture. It feeds on haemolymph of larva, pupa and adult bees during the whole life. (Anderson and Trueman, 2000).

3.2% oxalic acid alone and with 4g thymol was very effectively control *Varroa* mite and can be used without any side effect during broodless condition. No queens were lost, and there was no adult honeybee mortality in any of the colonies (Rashid *et al*., 2012a,b,c). The powdered form of thymol (4g) and the formic acid 65% (20ml) were applied 4 times at weekly interval in honeybee colonies with brood. It was found out that formic acid killed significantly higher number of mites as compared to thymol (Rashid *et al*., 2011). Formic acid and thymol are effective for the control of *T. clareae* without any side effect (Raffique *et al*., 2012).

Worm wood flowers, clove oil and peppermint oil can be considered promising agents for the control of *V. destructor*. Good results can be obtained when the above materials are used two to three times each month during the infestation period. Effective time of application depends on the mite level of infestation and the life cycle of the mite. The natural control agents used seemed to have no adverse effects on bees (Al-Abbadi and Nazer, 2003).

The control of *Varroa* is especially difficult as the majority of mites stay in the sealed brood for reproduction and are therefore well protected from different forms of treatment (Hoppe *et al*., 1989). The control of *Varroa* mite is based mainly on the

* Corresponding author: rashid_ento1@ yahoo.com
0030-9923/2014/0003-0609 $ 8.00/0

use of synthetic acaricides especially pyethroids. Nevertheless, the repeated use and in many cases sub lethal doses of these substances throughout years has caused problems, such as their application within the hives tends to contaminate the wax and honey. Furthermore, the mites can develop resistance against these chemicals (Milani, 1995).

In this context, the present work was carried out to evaluate the efficacy of plant oils *i.e.* clove, neem, garlic and olive and tobacco extract used alone and in combinations for the control of *Varroa* mite on honeybees in lab and field. These natural materials generally have no side effects on honeybees and are less hazardous to beekeepers.

MATERIALS AND METHODS

The experiment was conducted at HBRI of NARC, Islamabad. About 100 honeybee colonies were examined for the selection of forty eight experimental bee colonies having *Varroa* mite infestation. To collect the sample (250 bees/colony) of mite infestations of adult bee population, the alcohol wash technique was used (De Jong *et al.*, 1982). The mite infestation in brood was evaluated by opening 100 cells of sealed brood before treatment (Burgett and Burikam, 1985) while for the assessment of mite population in debris mite collection trays were kept for 24 h in the bottom of the bee colony. Mites fell in debris were counted (Devlin, 2001). The plants oils tested for their efficacies were neem oil (*Azadirachta indica*), garlic oil (*Allium sativum*), clove oil (*Syzygium aromaticum*), olive oil (*Olea europaea*) and tobacco extract (*Nicotiana tabacum*). Plant oil extractions were done by using Soxhlet Apparatus (Benthin *et al*., 1999) and preparation of tobacco extracts as mentioned by Gerard *et al.* (1997).

Laboratory bioassay

The compounds were assessed for their efficacy as miticides by exposing adult mites to volatile fumes of the oils. Adult mites were removed from sealed brood cells of worker and drones and placed in glass petri dish with five mites/ petri dish. A damp tissue paper was placed on the bottom of Petri dishes and covered with a sheet of Para film 'M' laboratory film. Small holes were punched in the film to allow evaporation of the water from the tissue paper below. A piece of filter paper equal in size to the diameter of the dish (9.0 cm) was placed on top of the Parafilm sheet. Forty micro liters of 5, 10 and 15% solution of each plant essential oil diluted in methanol was placed on the second piece of filter paper (4.25 cm diameter) attached to the lid of the Petri dish. In control dishes only methanol was poured on the filter paper. Each of five treatments *i.e.* neem oil (*A. indica*), garlic oil (*A. sativum*), Clove oil (*S. aromaticum*), olive oil (*O. europaea*) and tobacco (*N. tabacum*) extract were replicated 4 times, whereas methanol was used as base in formulations.

Dead and alive mites were counted after 24 and 48 h interval treatment. Bee mortality count was done by separating each mite sophisticatedly irresponsive mite towards stimulus considered an indication of increase in death tool. All bees were independently checked for the existence of mites.

Field tests in bee hives

Forty eight queen right honeybee colonies in Langstroth hives were used on mite infestation levels. The hives were placed at a distance of 5 meters from each other. Colonies were divided into 16 groups of 3 colonies each by using Complete Randomized Design (CRD). Each group of three colonies received the most effective concentration as determined previously alone (T1, garlic; T2, neem; T3, clove; T4, olive oil; T5, tobacco extract) and in combination (T6, garlic + neem; T7, garlic + clove; T8, garlic + olive; T9, garlic + tobacco, T10; neem + clove, T11, neem + olive; T12, neem + tobacco; T13, clove + olive; T14, clove + tobacco; T15, olive + tobacco; T16, control).

Treatments were applied every evening when the honeybees were present in the hives. 15 ml of each test material was sprayed by using a simple plastic sprayer (500 ml). Solutions of all extract concentration were sprinkled over the bees inside the colonies (Zaitoon, 2001). Mite and worker bee mortalities were recorded every day at 10 am and the mite collection trays were removed and transmitted to the lab for counting the mortalities.

Total 60 ml plant oil/ extract were applied at 5 days interval for 20 days. Each honeybee colony was equipped with a modified bottom board for

placing mite collection trays (mite excluders), through the back side of the hive. The mite fall were counted on the mite collection trays. Mite mortality was examined after five day in debris collected in mite collection trays placed under the screen. In order to evaluate total mite population an Apistan (Fluvalinate) strip was applied to the colonies. Apistan strip was removed from the colonies after 30 days and dropped dead mites were counted (Marcangeli and Garcia, 2004).Treatment efficacy was calculated for each colony by using following formula (Higes *et al.*, 1997):

$$E\ (\%) = \frac{V_{D+5}+V_{D+10}+V_{D+15}+V_{D+20}}{V_T} \times 100$$

Where, E, efficacy of plant oil/extract, V D+n , mites collected on different days, and VT, Total number of mites collected

Honey yield

Honey was harvested after all experiments with the help of manual honey harvester and compared honey yield of treated and control honeybee colonies. Honey production was measured by taking the weight of each hive body used for honey collection before and after the honey extraction process. The weight difference was considered as the amount of harvestable honey (Rashid *et al.*, 2012b).

Statistical analysis

A Completely Randomized Design (CRD) was applied to all laboratory and field experiments. Mean mite mortality, efficacy and honey yield were compared between treatments by Analysis of Variance techniques were applied to test the significance of data using least significance difference test (LSD) at 5% probability level (Montgomery, 2001). All data collected by performing experiments were analyzed through MSTAT C statistical computer based software (Freed and Eisensmith, 1986).

RESULTS AND DISCUSSION

To help beekeepers select the safe mite control alternatives, we evaluated different essential oils/extract at several concentrations for different intervals of time. The results obtained from our lab experiment showed that the essential oils/extract had a significant effect on the mite mortality (Three Way ANOVA, $F_{(5, 108)} = 33.86$, $P < 0.005$). The clove oil and tobacco extract both proved to be most effective against mites (Fig 1), followed by garlic, olive and neem oil, respectively. The percentage concentrations (Three Way ANOVA, $F_{(2, 108)} = 21.96$, $P < 0.005$), and timing of treatment (Three Way ANOVA, $F_{(1, 108)} = 73.97$, $P < 0.005$) were also found to be significantly different.

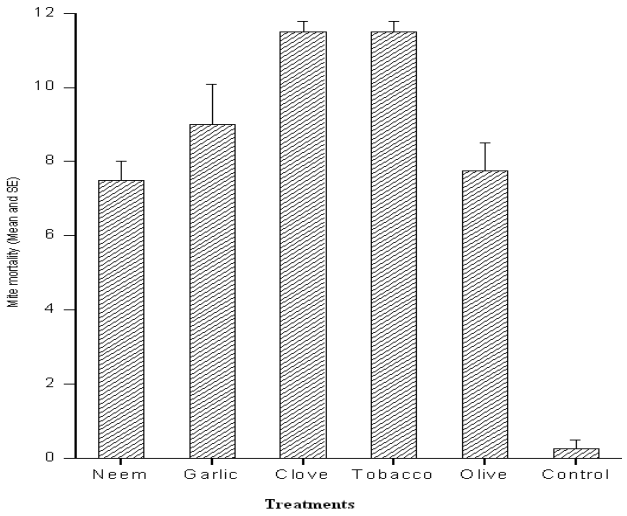

Fig. 1. *Varroa* mite mortality by different types of plant oils/tobacco extract in laboratory.

About 150 essential oils or any of their components have been tested to control the mite *V. destructor*, with different results. Their toxicity, repellent or attractive effect towards the mite and their influence on the mite's reproduction have been evaluated, when essential oils have been locally administered, or applied in pulverization or in a passive evaporation form (Imdorf *et al.*, 1999).

The results/findings of the experiment shows that the overall mean mortality for the number of mites for different concentrations *i.e.* 5%, 10% and 15% of essential oils/extract were 4.15±0.23, 2.95±0.16 and 2.35±0.18, respectively (Fig. 2), which clearly showed that 5% is the most effective as compared to 10 and 15% concentrations, which is

against the findings of Abdel Rahman and Rateb (2008) who found that the highest concentrations of lemon juice (10, 25, 50, 75 and 100%) caused high number of fallen dead mites and Zaitoon (2001) who found that the highest concentrations (500 ppm) of *Rhazya stricta* caused 100% mite mortality. Results are confirmed by Abd El-Wahab and Ebada (2006) who recorded significant differences between the sour orange, lemon grass and citronella oils in different concentrations.

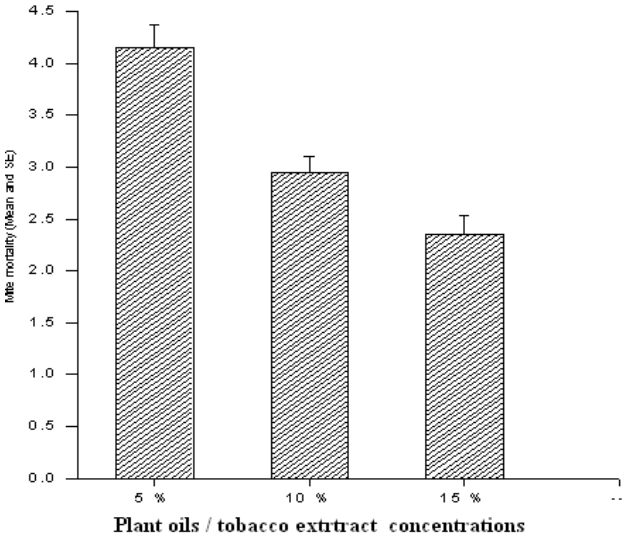

Fig. 2. *Varroa* mite mortality by different concentrations of plant oils/tobacco extract.

The 5% concentrations of different oils/extracts were applied in various combinations following the same procedure and it was found that even the combinations also killed mites in a significantly different manner (at 5% level of significance). The most effective combination was clove oil and tobacco extract with the mean mortality of 5.00±1.01 and the least effective treatment after control was garlic and tobacco extract (0.25±0.21). The results are presented in Figure 1.

As far as the timings of application are concerned (Fig. 3) the number of dead fallen mites were higher after 24h, than 48 h. These results are supported by the findings of Shoreit and Hussein (1994), who found that the maximum mean number of dead mites was observed after the first treatment with coriander extract after that it was gradually decreased. Abdel Rahman and Rateb (2008) resulted that the numbers of dead fallen *Varroa* mites were comparatively higher after 24 h, than after 48 h and after 72 h which exhibited the lowest number. This is contrary to the findings of Calderone and Spivak (1995) and El-Zemity *et al*. (2006) who found that essential oils showed good result against *Varroa* mite after 48 h of exposure.

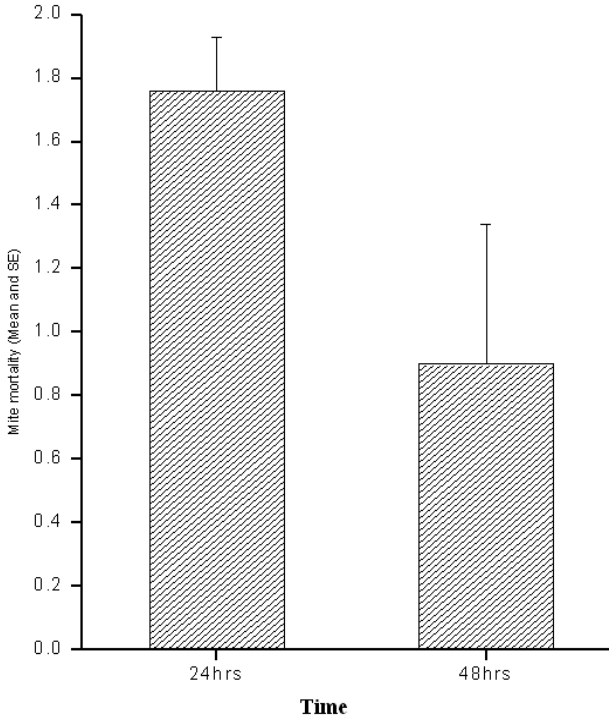

Fig. 3. Mean number of *Varroa* mite mortality as affected by different concentrations of all plant oils/extracts.

In field experiment using only 5% concentration alone and in combination all oils/ extract for the control of *Varroa* mites. The range of efficacy in colonies treated with different oils/ extract and their combinations were 71.58% to 97.24%. The percentages were arcsine square root transformed and when compared between different treatments the results were significantly different (One Way ANOVA, $F_{(15, 47)} = 2.312$, $P<0.05$). All tested combinations confirmed as clove oil + tobacco extract (T14) the best combination with mean value of efficacy (%) 96.48±0.52 (Mean±SE).

Table I.- Multiple Comparisons of plant oils/ extracts with control against Varroa mite mortality.

Treatment	Varroa (#)	Difference	Efficacy (%)	Difference	Honey yield (Kg)	Difference
Neem oil	278.33	204.00*	85.367	61.387*	12.300	6.067*
Garlic oil	201.33	127.00*	87.830	63.850*	14.833	8.600*
Clove oil	199.67	125.33*	88.010	64.030*	16.533	10.300*
Tobacco extract	180.33	106.00*	85.680	61.700*	14.567	8.333*
Olive oil	220.33	146.00*	87.113	63.133*	13.233	7.000*
Neem + Garlic oil	167.67	93.33*	86.857	62.877*	15.233	9.000*
Neem + Clove oil	167.00	92.67*	81.380	57.400*	15.133	8.900*
Neem Oil + Tobacco extract	187.33	113.00*	87.897	63.917*	17.800	11.567*
Neem + Olive oil	222.67	148.33*	85.783	61.803*	13.133	6.900*
Garlic + Clove oil	202.00	127.67*	84.070	60.090*	12.533	6.300*
Garlic Oil + Tobacco extract	169.00	94.67*	80.047	56.067*	12.333	6.100*
Garlic + Olive oil	197.33	123.00*	83.937	59.957*	13.300	6.767*
Clove Oil + Tobacco extract	381.67	307.33*	96.483	72.503*	20.500	14.267*
Clove + Olive oil	227.00	152.67*	87.190	63.210*	16.133	9.900*
Tobacco + Olive oil	216.33	142.00*	86.707	62.727*	14.500	8.267*
Control	74.33	-	23.980	-	6.233	-
LSD(0.05)	-	77.408		11.271		1.512
F-test	12.1**	-	36.3**	-	74.4**	-
C.V.	15.32	-	5.57	-	4.32	-

**Highly significant at 0.1%.
*Pairs that are significantly different are flagged with an asterisk.

The percentages were compared between different treatments the results were significantly different (at 5% level of significance) (Table I). From the results obtained from this study, it can be concluded that T14, 381.67±24.31 at 5% concentrations can be considered a promising agent for the control of *V. destructor* (Table I).

The honey yield from hives when treated with different essential oils/extracts was also weighed at the end of experiment. The mean honey yield (kg) compared between the treatments were higher 20.50±0.29 in treatment (T14) and lower in honey yield in control group (T16) *i.e.* 6.23±0.39 at 5% level of significance (Table I).

The results are in agreement with Allam-Sally (1999) and El-Zemity *et al*. (2006) who stated that the clove oil gave good results in controlling mites and Fouly and Al-Dehhairi (2009) who found clove killed 62% *Varroa* mites. Rashid *et al*. (2012a,b,c) found that highest mean efficacy (%) of 99±1.24 and honey yield (kg) 12.08± 0.86 was found in 3.2% oxalic acid treatment. Hussein *et al.* (2001) used six plant oils to control *Varroa* mites in honeybee colonies and rated clove oil best among the other essential oils. Similar results were also observed by Rajiter (1983), who found 50-79% mite mortality when applied different amounts of tobacco as fumigant. Abdol-Ahad *et al.* (2008) who concluded that tobacco extract without harmful effect against honeybees and decreased *Varroa* mite population. There are different researchers that reported a positive effect of tobacco on *Varroa* mite mortalities (Rijiter, 1982, 1983; Rijiter and Eijnd, 1984). The conclusion was the effective substances in neem are not volatile. Keeping in view the work of previous researchers the aim was to bring the essential oils in different concentrations into contact with mites. The results of experiment were also found in confirmation with Melathopoulos *et al.* (2000) who observed that when bees and mites were brought into contact with neem oil that it was spread on a surface it resulted in a 95% success. Hassan *et al.* (2008) indicated that neem oil efficacy rate is only 4.95% against *Varroa* mite and not suitable for *Varroa* control.

Botanical extracts obtained from different plant species have also shown a broad spectrum of biological activity in relation with mite population management (Banchio *et al.*, 2003, 2005; Jbilou *et al.*, 2006).

CONCLUSIONS

From the results obtained from this work, it can be concluded that the clove oil alone or in combination with tobacco extract at 5% concentrations can be considered promising agents for the control of *Varroa destructor*. It was also concluded that clove oil + tobacco extract can play an important role in an integrated pest management program to control *Varroa* mite in honeybee colonies. Good results can be obtained when the above materials are used 3-4 times each month during the infestation period. Also these materials proved to be harmless to the bee and quite safe to the environment.

ACKNOWLEDGMENTS

We acknowledge Mr. Qurban, Senior Scientific Assistant and Mr. Umar Daraz Bee Attendant for collecting mite collection trays and PMAS-Arid University for providing of oils extraction facility.

REFERENCES

ABD EL-WAHAB, T.E. AND EBADA, M.A., 2006. Evaluation of some volatile plant oils and mavrik against *Varroa destuctor* in honeybee colonies. *J. appl. Sci. Res.*, **2**: 514-521.

ABDEL RAHMAN, M.F. AND RATEB, S.H., 2008. Evaluation of lemon juice for controlling *Varroa destructor* in honeybee colonies. *Assiut J. Agric. Sci*., **2**: 195-206.

ABDOL-AHAD, S.T., NASER, M.S., ABOLFAZL, A.G., ABOLFAZAL, A.D., HOSEIN, C. AND MOZHGAN, M., 2008. Using medicinal plants for controlling Varroa mite in honey bee colonies. *J. Anim. Vet. Advan.*, **7**: 328-330.

AHMAD, R., 1988. Honeybee parasitic mites and their control in Pakistan. *Progr. Farm.*, **8**: 34-36.

AL-ABBADI, A. AND NAZER, I.K., 2003. Control of *Varroa* mite (*Varroa destructor*) on honeybees by aromatic oils and plants materials. *Agric. Mar. Sci.*, **8**: 15-20.

ALLAM SALLY, F.M., 1999. *Studies on the honey bee parasite* Varroa jacobsoni *Oudemans (Acari: Gamasida: Varroidae) in Egypt*. Ph.D. thesis, Acarology, Fac. Agric., Cairo Uni., Egypt.

ANDERSON, D.L. AND TRUEMAN, J.W.H., 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp. appl. Acarol*., **24**: 165-189.

BANCHIO, E., VALLADARES, G., DEFAGO, M., PALACIOS, S. AND CARPINELLA, C., 2003. Effects of *Melia azedarach* (Meliaceae) fruit extract on the leaf miner *Liriomyza huidobrensis* (Diptera, Agromyzidae): assessment in laboratory and field experiments. *Ann. appl. Biol.*, **143**: 187-193.

BANCHIO, E., ZYGADLO, J. AND VALLADARES, G.R., 2005. Quantitative variations in the essential oil of Minthostachys mollis (Kunth.) Griseb. In response to insects with different feeding habits. *J Agric. Fd. Chem.*, **53**: 6903-6906.

BENTHIN, B., DANZ, H. AND HAMBURGER, M., 1999. Pressurized liquid extraction of medicinal plants. *J. Chromat. A.*, **837**:211-219.

BURGETT, D. M. AND BURIKAM, I., 1985. Number of adult honeybees (Hymenoptera: Apidae) occupying a comb: A standard for estimating colony populations. *J. econ. Ent.*, **78**: 1154-1156.

CALDERONE, N.W. AND SPIVAK, M., 1995. Plant extracts for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) in colonies of the Western honeybee (Hymenoptera: Apidea). *J. econ. Ent.*, **88**: 1211-1215.

DE JONG, D., ANDREA, D. D. AND GONCALVES, L.S., 1982. A comparative analysis of shaking solution for the detection of *Varroa jacobsoni* on adult honeybees. *Apidologie*, **13**: 297-306.

DEVLIN, S., 2001. *Comparative Analyses of sampling methods for Varroa mite (*Varroa destructor *Anderson and Trueman) in honey bees (*Apis mellifera *L.)*. MPM, thesis, Simon Fraser Univ., Burnaby BC, Canada.

EL-ZEMITY, S., REZK, R.H.A. AND ZAITOON, A.A., 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**: 1032-1036.

FOULY, A.H. AND AL-DEHHAIRI, M.A., 2009. Evaluation of infestation levels of the ectoparasitic mite *Varroa destructor* infesting honeybee *Apis mellifera* and its control using essential oil in Qassim Region, Saudi Arabia. *J. Ent*., **6**:135-144.

FREED, R.D. AND EISENSMITH, S.P., 1986. MSTAT *Microcomputer statistical program.* Michigan State University of Agriculture and Applied Science, Michigan, USA.

GERARD, P.J., RUF, L. D., LORIMER, S. D. AND HEATH, A. C. G., 1997. Activity of extracts and compounds from New Zealand gymnosperms against larvae of *Lucilia cuprina* (Diptera: Calliphoridae). *N.Z. J. agric. Res*., **40**: 261-267.

HASSAN, M.F., SALLY, F.A., MARGARET A.R. AND ZAKI, A.Y., 2008. Utilization of essential oils and chemical substance against Varroa mite, *Varroa destructor* Anderson and Trueman on two stocks of *Apis mellifera lamerkii* in Egypt. *Acarines*, **2**:3-8.

HIGES, M., SUAREZ, M. AND LLORENTE, J., 1997.

Comparative field trials of *Varroa* mite control with different components of essential oils (Thymol, Menthol and Camphor). *Res. Rev. Parasit.*, **57**:21-24.

HOPPE, H., RITTER, W. AND STEPHEN, E.W.S., 1989. The control of parasitic bee mites: *Varroa jacobsoni, Acarapis woodi*, and *Tropilaelaps clarae* with formic acid. *Am. Bee J.*, **129**:739–742.

HUSSEIN, M.H., ALI, A.M., MORSI, M.A. AND RATEB, S.H., 2001. Using of some plant oils for controlling of some parasites, diseases and pests of honeybee colonies in Assiut region, Egypt. Safe Alternatives of Pesticides for Pest Management Conference, Assiut Univ., Egypt, pp. 349-355.

IMDORF, A., BOGDANOV, S., OCHOA, R.I. AND CALDERONE, N.W., 1999. Use of essential oils for control of *Varroa jacobsoni* Oud. in honeybee colonies. *Apdiologie*, **30**: 209-228.

JBILOU, R., ENNABILI, A. AND SAYAH, F., 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Afr. J. Biotechnol*., **5**: 936-940.

MARCANGELI, J. AND GARCIA, M. D. C., 2004. Effect of *Apis mellifera* (Apidae) honeybee brood amount on Oxavar® acaricide efficacy against the mite *Varroa destructor* (Varroidae). *Rev. Soc. Ent. Agr.*, **63**:35-38.

MELATHOPOULOS, A.P., WINSTON, M.L., WHITTINGTON, R., HIGGO, H. AND LEDOUX, M., 2000. Field evaluation of neem and canola oil for the selective control of the honey bee mite parasites *Varroa jacobsoni* and *Acarapis woodi*. *J. econ. Ent*., **93**:559-567.

MILANI, N., 1995. The resistance of *Varroa jacobsoni* Oud to pyrethroids: a laboratory assay. *Apidologie*, **26**: 415-429.

MONTGOMERY, D.C., 2001. *Design and analysis of experiments*. 5th Ed. John Willy and Sons, New York. pp. 64-65.

PARC, 2010-11. *Honeybee*. Ann. Rep. PARC. P. 98.

RAFFIQUE, M.K., MAHMOOD, R., ASLAM, M. AND SARWAR, G., 2012. Control of *Tropilaelaps clareae* mite by using formic acid and thymol in honey bee *Apis mellifera* L. colonies. *Pakistan J. Zool*., **44**:1129-1135.

RAJITER, A., 1982. Tobacco smoke can kill varroa mites. *Bee World*, **63**:138-148.

RAJITER, A., 1983. Preliminary results on treatment of varroa infected honey bee colonies with tobacco smoke. *Bee World*, **64**: 63-65.

RAJITER, A. AND EIJND, J.U.D., 1984. Detection of varroa mites in the Netherlands using tobacco smoke. *Bee World*, **65**: 151-154.

RASHID, M., WAGCHOURE, E.S., RAJA, S., SARWAR, G. AND ASLAM, M., 2011. Effect of thymol and formic acid against ectoparasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies. *Pakistan J. Zool.*, **43**: 91-95.

RASHID, M., WAGCHOURE, E.S., MOHSIN, A. U., RAJA, S. AND SARWAR, G., 2012a. Control of ectoparasitic mite *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies by using different concentrations of oxalic acid**.** *J. Anim. Pl. Sci*., **22**: 72-76.

RASHID, M., WAGCHOURE, E.S., MOHSIN, A.U. RAJA, S. AND SARWAR, G., 2012b. Control of ectoparasitic mites in honeybee (*Apis mellifera* L.) colonies by using thymol and oxalic acid. *Pakistan J. Zool*., **44**: 985-989.

RASHID, M., WAGCHOURE, E.S. MOHSIN, A.U., RAJA, S. AND SARWAR, G., 2012c. Control of *Varroa destructor* using oxalic acid, formic acid and Bayvarol strip in *Apis mellifera* (Hymenoptera: Apidae) colonies. *Pakistan J. Zool*., **44**: 1473-1477.

SHOREIT, M.N. AND HUSSEIN, M.H., 1994. Field trials for the control of varroa disease of honeybees by using coriander seeds extract. *Zagazig J. agric. Res.*, **21**: 279-288.

ZAITOON, A.A., 2001. Evaluation of certain plant extracts for the control of parasitic bee mites, *Varroa jacobsoni*. *J. Pest. environ. Sci*., **9**:77-88.

(Received 6 April 2013, revised 19 March 2014)