Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 3217-3227. **OPEN ACCESS**

Management of *Varroa destructor* in honeybees through essential oils

Mahwish Rehman[1], Toheed Iqbal[2], Kiran Shahjeer[3], Hussain Ali[4], Qaisar Iqbal[5] and Kamran Baksh Soomro[6] and Muhammad Qasim Kakar[7]

[1]Directorate of Outreach, Agriculture Research System, Khyber Pakhtunkhwa Peshawar. **Pakistan**
[2]Department of Entomology, The University of Agriculture, Peshawar. **Pakistan**
[3]Department of Zoology, Abdul Wali Khan University, Mardan. **Pakistan**
[4]Entomology Section, Agriculture, Research Institute, D.I.Khan, **Pakistan**
[5]Department of Plant Protection, The University of Agriculture, Peshawar. **Pakistan**
[6]Institute of Plant Introduction, Southern-Zone Agricultural Research Center, PARC, Karachi, **Pakistan**
[7]Agriculture and Cooperative Department, Quetta, Baluchistan, **Pakistan**

***Correspondence: Toheed.iqbal@aup.edu.pk** **Received 15-09-2021,Revised: 20-12-2021, Accepted: 21-12-2021 e-Published: 22-12-2021**

A study was initiated at New Developmental Farm of The University of Agriculture, Peshawar, Pakistan for two years, aiming to test different essential oils with chemicals for the management of *Varroa destructor* and their effect on honey yield in honeybee colonies. The essential oils used against *Varroa destructor* were obtained from different plants i.e., *Eucalyptus spp*, *Ocimum basilicum*, *Plergonium graveolens*, *Menthaviridis*, *Rosmarinus officinalis*. Several chemicals applied were Formic acid 65%, Fluvalinate strips, and Icing sugar dusting. Percent infestation of *Varroa destructor* on broods and adult workers, number of fallen mites on white sheets, mean percent mortality of mites, and their effect on honey yield (kg) was recorded. The studies revealed that maximum control was recorded in *Rosmarinus officinalis* whereas the minimum was recorded in *Ocimum basilicum* and *Eucalyptus spp.* during 2014. Similar trend was recorded in the next year. The highest number of fallen dead mites in both years (20l4-20l5) were *Rosmarinus officinalis* (l32, l26) followed by formic acid 65% (124, 114), Fluvalinate strips (l23, 98), icing sugar dusting (87, 9l), *Mentha viridis* (82, 80), *Ocimum basilicum* (76, 97), *Plergonium graveolens* (76, 79) and, *Eucalyptus spp.* (64, 77) as compared to control with 5l dead fallen mites per colony. The honeybee boxes treated with essential oils, and other chemicals also showed the best persistence up to one third treatment. Maximum yield of honey was recorded during both the years of 20l4 and 20l5 in *Rosmarinus officinalis* (l7 kg and l5.5 kg), while the minimum was recorded in *Eucalyptus spp.* (4 kg and 3 kg) per colony.

Keywords: *Varroa mites, Rosmarinus officinalis, Mentha viridis*, *Ocimum basilicum, Plergonium graveolens,* Honeybees

INTRODUCTION

Honeybee (*Apis mellifera L.)* is a vital insect that produces honey, wax, venom, pollen, propolis, and royal jelly. Honeybees also plays important role to pollinate different crops/vegetables and benefit the farmers/growers. Pakistan is affluent of flora specifically northern areas of Khyber Pakhtunkhwa (Zia et al. 20l2). Beekeeping is one of the main cottage industries of Pakistan which plays the major role in Pakistan's income for beekeepers at domestic level in Khyber Pakhtunkhwa and new merged areas (Khan et al. 20l7).

*Varroa destructo*r is the parasitic bee mite (Anderson and Trueman, 2000) and distressing pest of honeybee which may result economic

losses in the industry of beekeeping globally (Nazer and Al-Abbadi, 2003). This parasitic mite could be observed at the adult stage of bees on offspring and in a hive. There is no development of Pupae into adult bees during heavy infestation. Rigorous infestations cause significant destruction to populations of honeybee (Delfinado-Baker and Peng, l995) which ultimately cause the quality reduction of honey production including pollination (Spivak, l997). The parasitic mite impairs the immune system of honey bees (Glinski, l988) and decrease the capacity of honeybees for plants pollination (de Jong et al. l984). This mite transmit diseases and may result in 100% mortality of honeybees (Kanga and James, 2002). It has been a global threat specifically to apiculture industry in Pakistan (Aziz et al. 20l5, Islam et al. 20l6). For control of *Varroa destructor* the beekeepers are using different methods for control, most commonly chemical and stripes are used inside honeybee colonies (Lundie, l940, Neumann, et al. 20l6, Torto et al. 2007, Zawislak, 20l0).

Effectiveness of essential oils against a broad range of pests and plant pathogens is well recognized (Koul, et al. 2008). Various studies were carried out on the effectiveness of plant extracts and essential oils against potential pest, *Varroa* mite under control environment (Calderone and Spivak, l995, Bakar et al. 20l7, Islam et al. 20l6 & 20l7, Barbosa et al. 20l6, Abou-Shaara, 20l4). Numerous factors are involved on the whole potency of an acaricide as well as the inclusion of the compound concentration, treatment phase, colony, and apiary atmosphere. However, the efficacy of few compounds depend on the evaporation pressure within the colony; hence, the normal temperature throughout treatment may influence the efficacy of the treatment (Imdorf et al. l995). Some other factors including the quantity of brood present in the hive (Semkiw et al. 20l3, Emsen and Dodologlu, 2009), the infestation intensity (Mattila and otis, 2000), method of delivery used (Floris et al. 2004, Imdorf et al. l999), and type of hive (Imdorf et al. l995) have also been recommended for efficacy treatment. Acaricidal impact of essential oils has broadly been discussed in different studies (Imdorf et al. l999, Imdorf et al. 2006). The careful usage of acaricides might have impact on expansion of resistance and the risk of residues in honey which may cause a serious threat to human consumption (Farooqi et al. 2020). Beekeepers in Pakistan are using low quality synthetic acaricides such as sulphur, flumethrin, strips, coumaphos, amitraz, tobacco, fluvalinate injections and formic acid to control *T. clareae* and *Varroa destructor* mites (Islam et al. 20l7). It has been found that the inappropriate treatment against the *Varroa destructor*, access of foraging honey bees to certain crops has been established to significantly affect winter mortality (van der Zee et al. 20l3).

Present study is aimed to test various essential oils in comparison with chemicals against the *Varroa destructor* pest of honeybees, its effect on mortality and fall of mites in honeybee colonies. Further to observe if it will have an impact on the honey yield.

MATERIALS AND METHODS

The research was conducted at New Developmental Farm of the University of Agriculture Peshawar in Mar 20l4 and Mar 20l5. The maximum and minimum temp of this area in March were 25^{o}C and l2^{o}C respectively.

In this study twenty-seven honeybee A. mellifera L. colonies in langstroth hives infested by V. destructor mites naturally. Five colonies were considered for each concentration of essential oils (*Eucalyptus spp*, *Ocimum basilicum, Plergonium graveolens, Menthaviridis, Rosmarinus officinalis*) and other chemicals (formic acid 65%, fluvalinate strips, icing sugar dusting) and untreated three colonies were kept as control. Individual colony contained 8-9 complete whole depth combs of bee worker and had 3-4 brood frames. At 5 meters distance, hives were placed from each other.

Cotton swab with 1ml of each of the five different essential oils including *Eucalyptus spp, Ocimum basilicum*, *Mentha viridis*, *Plergonium graveolens,* and *Rosmarinus officinalis* were placed at the bottom of every infested/treated box and synthetic chemicals (formic acid 65% on tissue paper on top of the frames in infested bee boxes and after 24 hours treated tissue paper were removed from treated frames, fluvalinate strips were fixed inside of the hive. The strip was taken out from the colonies after applying second application of treatments to the infested bee boxes placed white paper in the bottom, treated with Vaseline over the whole white paper and released dead mites were calculated (Marcangeli and Del Carmen García, 20l7) and selected infested bee hives the icing sugar dusting(on top of the infected colonies were dusted) with control were used against varroa mite and about twenty-seven, infested worker beehives were examined and treated in Khyber Pakhtunkhwa region with the three factorial CRD (Complete block Design) were applied in the spring seasons 20l4 and 20l5

on worker honey bees, with three replication were used. The mean temperature (°C) and relative humidity (%) was recorded by Thermo-Hygrometer during year 20l4-l5.

Using of essential oils and other chemicals against varroa mites, in brood cells after thirteen days to observe the percent infestations and percent reduction on before and after applications of these essential oils and other chemicals of dead worker bees. After that the dropped *Varroa* mites were recorded fortnightly.

Henderson and Tilton (Henderson and Tilton, l955) formula were used to record the mean percent reduction of varroa mites.

% Reduction of infestation = l00 x l – {Ta x Cb}c / {Tb x Ca}

Where:

T = % infestation of treated mites

and C = % infestation of untreated mites (a = after; b = before treatment).

b. Mite fall:

Counting of released Varroa mites were noted before and after application of essential oils and other chemicals of infested hives data were observed after one day, two days, three days, seventh days and on fourteen days by using white paper sheets of 3l.l5 x 42.9 cm covered with Vaseline placed on the bottom board of hives. The same method was repeated after 2nd and 3rd application with essential oils and other chemicals.

c. Brood measurement:

In the center of a worker, combs were chosen with an area of (5 x 5 cm^2). In each cell was scratched and counted which were infested by Varroa mites. Percent infestation of Varroa mites on mature worker honeybees and broods were found before and after essential oils and other chemicals application according to the technique of Fresnay (El-Wahab and Ghania, 20l6). Approximately three hundred adult worker honey bees were collected, from the infested hive through brush and icing sugar were added in a wire net and detached by shaking numerous times. Dusted mites were collected on white paper sheet and data were recorded. The percent infestation formula is shown in Equation l

$$\text{percent infestation} = \frac{\text{No. of mites}}{\text{No. of bees}} \text{xl00}$$

Equation. l. Equation used to calculate Percent Infestation of Varroa mites

RESULTS

Variety of natural compounds that arise in honey naturally could be utilized for controlling parasitic mites. only small number of them with plant essential oils and formic acid have revealed feasible against *V. destructor* mites, without negative impact on the honeybee colonies development (Melathopoulos and Gates, 2003, Floris et al. 2004). The efficacy of investigational formic acid (65%) and essential oils against infestation with V. vandal mites is presented in Table l-3. The mean percent decrease of fallen Varroa mites in Tables-l-3 were observed after application of essential oils and other chemicals in both (brood and worker honeybees) in March 20l4 and March 20l5 on third treatment in *Rosmarinus officinalis* where the percent reduction was more significant from rest of other essential oils and other chemicals. Data found that Varroa mite infestation percentage on sealed brood and adult workers reduced distinctly at the end of third treatment. Results indicated that there were significant differences between the essential oils and other chemicals on second and first treatment in brood and worker adults of honeybees. It was found that in apiaries treated with other chemicals and essential oils, the infestation of Varroa mites were reduced gradually from first treatment to third treatment. *Rosmarinus officinalis,* fluvalinate Strips, formic Acid 65%, icing sugar dusting, *Mentha viridis,* are very useful in controlling of Varroa mites as the reduction infestation percentage from *Plergonium graveolens, Ocimum basilicum,* and *Eucalyptus spp.* recorded as (96.25 and96.45), (90.08 and 95.l0), (93.58 and 68.l0), (90.08 and 87.46), (88.85 and 86.62), (87.86 and 84.62), (76.l0 and 8l.05), (74.59 and 78.53) and (72.57 and 83.63) following the third treatment on the worker brood and adult workers, respectively. Whereas, in year 20l5 same trend were observed of these essential oils and other chemicals, hence the percent infestation of mites was (94.67, 95.93), (93.09, 95.l2), (92.l3, 93.9l), (89.09,9l.09), (84.83, 88.84), (84.52,86.06), (84.24, 85.00), (8l.89, 82.47) after the third treatment on the worker brood and adult workers, respectively.

The acquired data in Table 2, the mean infestation percentage of mites on sheets of honeybee colonies treated with different essential oils and other chemicals in month of March, 20l4 and 20l5on third treatment was reduced in comparison with control colonies. Maximum number of dead Varroa dropped mites on sheets was found after first second treatment of essential oils and other chemicals in comparison with the control apiaries.

Table l: Mean reduction of Varroa mite population and analyzed means in both brood and worker honeybees treated by different essential oils and other chemicals in March, 20l4.

Essential oils and other chemicals	Application of essential oils and other chemicals	Mean percentage on brood cells (% of reduction in varroa mite populations)		Mean percentage on adult worker bees (% of reduction in varroa mite populations)	
Eucalyptus spp	**Before Treatment**	5.00	**----**	**4.66**	**----**
	First treatment	3.47c	55.6l	2.l3fg	67.3l
	Second treatment	3.07cd	65.6l	l.86ghi	75.04
	Third treatment	2.00ef	72.57	l.53ijklm	83.63
Ocimumb asilicum	**Before Treatment**	4.00	**----**	4.00	**----**
	First treatment	2.60fg	57.28	2.47ef	56.06
	Second treatment	2.40gh	66.1l	2.00gh	68.84
	Third treatment	2.l3hi	74.59	l.73ghijk	78.53
Plergoniumgr aveolens	**Before Treatment**	5.00	**----**	4.66	**-----**
	First treatment	3.00de	60.73	3.2od	57.74
	Second treatment	2.53fg	7l.46	2.60e	69.33
	Third treatment	2.46fgh	76.l0fg	2.00gh	8l.05
Mentha viridis	**Before Treatment**	5.00	**----**	4.66	**-----**
	First treatment	3.00de	60.73	3.20d	57.74
	Second treatment	2.53fg	7l.46	2.60e	69.33
	Third treatment	2.46fgh	76.lo	2.00gh	8l.05
Rosmarinus officinalis	**Before Treatment**	3.00	**----**	4.00	**----**
	First treatment	0.80mn	8l.85	0.730pq	89.46
	Second treatment	0.53no	88.58	0.46pq	93.84
	Third treatment	0.200	96.25	0.33q	96.45
Formic Acid 65%	**Before Treatment**	4.00	**----**	3.33	**----**
	First treatment	0.73mn	87.4l	0.86nop	85.94
	Second treatment	0.600n	9l.44	0.60pq	9l.3l
	Third treatment	0.53no	93.58	0.40q	95.lo
	Before Treatment	3.00	**----**	4.66	**----**
Fluvalinate strips	**Before Treatment**	l.33jk	72.27	l.73ghijk	73.25
	First treatment	l.l3jkl	8o.74	l.4ojklm	8l.o7
	Second treatment	0.80lmn	90.08	l.l3mno	87.46
	Third treatment	3.33	**----**	4.00	**----**
Icing Sugar	**Before Treatment**	l.33jk	70.37	l.87ghi	73.4o
	First treatment	l.00klm	8l.80	l.66hijk	79.3l
	Second treatment	0.73mn	88.85	l.33klm	86.62
	Third treatment	5.00	**----**	6.00	**----**
Control	**Before Treatment**	8.33a	-----	8.73c	-----
	First treatment	9.47a	-----	L0.20b	-----
	Second treatment	11.13b	-----	l2.87a	-----
	Third treatment	3.00	**----**	4.00	**----**

Mean observed with the same letter (s) in a single treatment are not significantly different at 5% level of probability.

Table 2: Mean percent infestation of sealed worker brood and adult worker of honeybee colonies treated with different essential oils and other chemicals in March 20l4-20l5.

Essential oils and other chemicals	Application of essential oils and other chemicals	Mean (%) infestation of Mites in brood cells		Mean (%) infestation of Mite on adult worker of honeybees (No. of mites/No. of bees)X 100	
		20l4	20l5	20l4	20l5
Eucalyptus spp	**Before treatment**	11	12	3.66	4.00
	First treatment	26	30	4.00	4.33
	Second treatment	20	26	9.00	6.00
	Third treatment	l8	2l	l0.00	7.00
	Total	64	77	--	--
Ocimumb asilicum	**Before treatment**	8	l5	3.00	3.66
	First treatment	30	37	3.66	4.33
	Second treatment	26	34	4.00	5.66
	Third treatment	20	26	5.00	6.66
	Total	76	97	--	--
Plergoniumgr aveolens	**Before treatment**	l2	39	3.00	3.33
	First treatment	30	3l	4.00	4.66
	Second treatment	26	26	4.66	5.33
	Third treatment	20	22	5.66	6.66
	Total	76	79	--	--
Mentha viridis	**Before treatment**	l5	34	3.66	6.00
	First treatment	34	30	4.00	6.33
	Second treatment	27	25	4.66	7.66
	Third treatment	2l	25	6.33	8.33
	Total	82	80	--	--
Rosmarinus officinalis	**Before treatment**	l8	30	3.66	6.33
	First treatment	55	46	4.00	6.66
	Second treatment	43	40	6.66	7.66
	Third treatment	34	40	8.66	8.33
	Total	l32	l26	--	--
Formic Acid 65%	**Before treatment**	l5	28	3.33	4.66
	First treatment	52	42	4.33	5.33
	Second treatment	42	37	5.66	7.00
	Third treatment	30	35	6.66	7.66
	Total	l24	ll4	--	--
Fluvalinate strips	**Before treatment**	l3	37	3.00	4.33
	First treatment	48	40	4.33	5.66
	Second treatment	43	30	5.66	6.33
	Third treatment	32	28	8.00	6.33
	Total	l23	98	--	--
Icing Sugar	**Before treatment**	l8	37	4.00	5.00
	First treatment	35	34	4.33	5.66
	Second treatment	25	30	5.33	7.00
	Third treatment	27	27	6.00	7.66
	Total	87	9l	--	--
Control	**Before treatment**	l9	24	3.66	5.00
	First treatment	20	30	4.66	6.33
	Second treatment	l5	24	5.66	7.00
	Third treatment	l6	l7	5.66	7.33
	Total	5l	7l	--	--

Mean observed with the same letter (s) in a single treatment are not significantly different at 5% level of probability

Table 3: Honey Yield in (Kgs)

Essential oils and other chemicals	Mean value of honey (kg)	
	20l4	**20l5**
Eucalyptus spp	4	3
Ocimumb Asilicum	6	4.5
Plergoniumgr aveolens	7.5	5.5
Mentha viridis	10.5	8.5
Rosmarinus officinalis	17	15.5
Formic Acid 65%	15	12
Fluvalinate strips	13.5	10
Icing Sugar	11.5	9.5
Control	2	2

Table 4: Mean reduction of Varroa mite population and analyzed means in both brood and worker honeybees treated by different essential oils and other chemicals in March, 20l5.

Essential oils and other chemicals	Application of essential oils and other chemicals	Mean percentage on brood cells (% of reduction in varroa mite populations)		Mean percentage on adult worker bees (% of reduction in varroa mite populations)	
Eucalyptus spp	Before treatment	5.66	----	5.00	----
	First treatment	3.40d	6l.96	3.40d	54.76
	Second treatment	2.93ef	75.0l	2.93ef	69.26
	Third treatment	2.46gh	8l.89	2.53gh	82.47
Ocimum basilicum	Before treatment	5.33	----	5.33	----
	First treatment	3.l3de	62.70	3.l3de	60.8l
	Second treatment	2.20hi	79.86	2.20hi	78.l9
	Third treatment	2.00i	84.24	2.00ij	85.00
Plergoniumr aveolens	Before treatment	4.66	----	4.66	----
	First treatment	2.73fg	62.82	2.73fg	6l.02
	Second treatment	2.40gh	74.45	2.33hi	7l.4l
	Third treatment	2.07i	84.52	2.07i	86.06
Mentha viridis	Before treatment	4.00	----	4.33	----
	First treatment	2.33hi	63.62	2.33hi	64.62
	Second treatment	l.44j	80.08	l.66jk	79.94
	Third treatment	l.47jkl	84.83	l.47klm	88.84
Rosmarinu officinalis	Before treatment	4.33	----	4.00	----
	First treatment	l.67j	78.33	l.66jk	72.00
	Second treatment	l.l3lm	87.03	l.l3mn	89.35
	Third treatment	0.530	94.67	0.53p	95.93
Formic Acid 65%	Before treatment	3.66	----	4.33	----
	First treatment	l.20klm	78.67	l.20lmn	8l.20
	Second treatment	0.66n0	90.98	0.670p	89.95
	Third treatment	0.600	93.09	0.60p	95.l2
Fluvalinate Strips	Before treatment	4.00	----	4.33	----
	First treatment	l.33jklm	78.4l	l.33klmn	79.ll
	Second treatment	l.00mn	87.55	l.00mn	87.63
	Third treatment	0.73no	92.l3	0.730p	93.9l
Icing Sugar Dusting	Before treatment	3.66	----	4.00	----
	First treatment	l.53jk	72.69	l.53kl	73.80
	Second treatment	l.33jklm	82.97	l.33klmn	82.35
	Third treatment	l.00mn	89.09	l.00no	9l.09
Control	Before treatment	5.00	----	4.66	----
	First treatment	8.40a	-----	7.40c	-----
	Second treatment	11.07b	-----	9.27b	-----
	Third treatment	l2.66c	-----	l4.l3a	-----

Mean observed with the same letter (s) in a single treatment are not significantly different at 5% level of probability

Rosmarinus officinalis showed the maximum number of fallen mites followed by formic acid 65%, Fluvalinate strips, icing sugar dusting, *Menthaviridis, Ocimum basilicum, Plergonium graveolens,* and *Eucalyptus spp.* by (l32, l26), (l24, ll4), (l23, 98), (87, 9l), (82, 80), (76, 97), (76, 79) and (64, 77) in comparison with control 5l dead fallen mites respectively.

The yield of honey in Table 3, the treated colonies of infested honeybees treated with essential oils and other chemicals shown maximum number of honey yield as compared to control colonies during investigation phase. In control colonies 2kg honey per hive was extracted which shown the maximum number of mites infestation on honeybees causing minimum honey yield. *Rosmarinus officinalis* and formic acid65% produced highest yield with the mean value of both years 20l4 and 20l5 was (l7 kg and l5.5 kg) and (l5kg , l2kg) per hive followed by Fluvalinate strips (l3.5 kg, lo kg), icing sugar dusting (11.5 kg, 9.5), *Mentha viridis* (10.5 kg, 8.5kg), *Plergonium graveolens* (7.5 kg, 5.5 kg)*, Ocimum basilicum* (6 kg, 4.5 kg)*,* and *Eucalyptus spp.* (4 kg, 3 kg) of honey per colony*.*

The percentage of mites population decline in Table 4 showed that by using of essential oils and other chemicals against Varroa mites in infested colonies of honey bees in season of March 20l5 the mean reduction after third treatment was 94.67 and 95.93 in *Rosmarinus officinalis* and followed by formic acid 65%, Fluvalinate strips, icing sugar dusting*, Mentha viridis, Ocimum basilicum, Eucalyptus spp.,*and *Plergonium graveolens* with the mean of percent reduction of mites (93.09, 95.l2), (92.l3, 93.9l), (89.09, 9l.09), (84.83, 88.84), (84.24, 86.8o), (8l.89, 82.47) and (8l.52, 85.06) respectively. Statistically, data shown in Table 4 indicates that application of first and second treatment of essential oils and other chemicals were significantly different from each other in brood and adult workers of infested bee colonies.

DISCUSSION

The achieved results are in line with (Eguaras et al. l996) and (Allam et al. 2003) who reported that formic acid caused 9l.7% mortality of the Varroa mites. (Hamaad et al. 2008) reported that the thyme oils in the form of spray resulted in 65.9% Varroa mite mortality under Egyptian climatic situation has a big potential. (Daher-Hjaij and Alburaki, 2006) also highlighted the high mite mortality during the beehives were fumigated with Anise (*Pimpinella anisum*) seed extracts. The study done by (Noel and Amrine, l996) in fall and winter that the Varroa mite is susceptible to deal with essential oils, because of cluster formation and the lack of brood. During hives treatment with the essential oils the Varroa mites will have no position to hide and can be killed*.*

The results are in line with (Ramzi et al. 20l7) who reported that the apiary tests discovered a variable efficacy from 50 to 94% depending on the essential oils composition, in addition; essential oils with carvacrol as main compound formed a better effect than other essential oils with prevailing borneol. Though, the blend containing high levels of carvacrol (55.35%) and borneol (20.60%) exhibited higher activity than all treatments (Ramzi et al. 20l7). A synergistic influence between the compounds of *T. satureioides* and *o. elongatum* essential oils was reported.

Natural medicines such as oxalic acid, formic acid, thymol, and menthol have been used recently as an alternative treatments against the honey bee parasitic Varroa mite (Gunes et al. 20l7). According to (Gunes et al. 20l7) the groups exposed to the varroa treatments, HSP 7oresults were lower than those of the non-treated groups. HSP 7oresults were lowest in the thymol-menthol mixture exposed group among the treated groups. It is concluded that it would be more favorable to prefer natural medicines against varroa, which lead to lower HSP 7oresults which are the molecular determinants of stress (Gunes et al. 20l7). Many studies have been conducted and still continue to control varroa by using organic acids such as oxalic acid, formic acid, lactic acid and volatile oils such as thymol, which are not harmful to human health, do not leave residues in honey and are natural constituents of honey (Akyol and Özkök, 2005, , Bogdanov et al. 2002, Cengiz, 20l2, Donders et al. 2006, Girişgin and Aydin, 20l0, Sapountzis et al. 20l4, Satta et al. 2005, Smodiš Škerl et al. 2o11). Natural treatments consisting of organic acids and plant extracts are an emerging alternative cure in controlling honeybee mites. Essential oils, highly volatile compounds are plant products found in only specific parts or in all parts of a plant. It has been reported that many essential oils and their components are alternative to synthetic acaricides for control of Varroa mites (Imdorf et al. l999, Tutun et al. 20l8). The most common essential oils used in mite control are cinnamon oil (*Cinnamomum cassia*), citronella oil (*Cymbopogon nardus*), peppermint oil (*Mentha piperita*), Spearmint oil (*Mentha spicata*), Rosemary oil (*Rosmarinus officinalis*), tea tree oil (*Melaleuca alternifolia*), winter green oil

(*Gaultheria procumbens*), neem oil (*Azadirachta indica*), thyme oil (*Thymus vulgaris*) and lemongrass oil (*Cymbopogon citratus*) (Tutun et al. 20l8, Hassan et al. 2008, Damiani et al. 2009,).

The most used components are eucalyptol, menthol, thymol, camphor, citronellal and citral (Tutun et al. 20l8, Gashout and Guzmán-Novoa, 2009, Ellis and Baxendale, l997, Calderone and Spivak, l995). These plant-based substances should be used together with other integrated control methods against mites. The single application of essential oils is often insufficient in the control of Varroa mites (Islam et al. 20l6, Imdorf et al. l999).

CONCLUSION

Rosmarinus officinalis oil is effective for causing *Varroa* mites' mortality to a level of 94.67 and 96.25 in 2014-15 of brood cell while in adult workers of honeybee the percent reduction of mites was 95.93 and 96.43 respectively. It is found that for obtaining effective control, third treatment of essential oils should be repeated subsequently. The effectiveness of *Rosmarinus officinalis* oil and other essential oils are suggested to use a chemical treatment used in infested colonies of *Apis mellifera* L., against *Varroa mites* as an alternative. The essential oils usage may vigorous into the integrated pest management (IPM) program for substitute in addition with other control measures for *Varroa* mites and honeybee's colonies management. These essential oils increase chances for colony survival and commit the residue free hive by-products. Depending upon the infestation level of *Varroa* mites, the essential oils usage might be repeated after three weeks. Furthermore, the efficacy of these essential oils and other chemicals showed well- depending on time of year, temperature, colony strength, shelf lives of these essential oils and brood area.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The researcher would like to thank all students sharing in this study.

AUTHOR CONTRIBUTIONS

Mahwish Rehman, Hussain Ali and Toheed Iqbal planned the research work, Mahwish Rehman and Qaisar Iqbal performed the research work, Kamran Bakhsh Soomro analyzed the data, Kiran Shahjeer and Muhammad Qasim Kakar helped in manuscript formation and supported in technical writing.

REFERENCES

Abou-Shaara, H. (20l4). Continuous Management of Varroa Mite in Honeybee, *Apis mellifera*, Colonies. *Acarina,* 22**,** 149-156.

Akyol, E. & Özkök, D. 2005. The Use of organic Acids for Varroa (*Varroa destructor*) Control. *Uludag Bee J,* 5**,** 167-174.

Allam, S., Hassan, M., Risk, M., Zaki, A. & Papierok, B. (2003). Utilization of essential oils and Chemical Substances alone or in combination against Varroa Mite (*Varroa destructor*), A parasite of Honeybees. *Iobc Wprs Bulletin,* 26**,** 273-278.

Anderson, D. & Trueman, J. (2000). *Varroa jacobsoni* (Acari: Varroidae) is More than one Species. *Experimental & Applied Acarology,* 24**,** 165-189.

Aziz, M. A., Azeem, M., Ahmed, M. S., Siddique, F. & Jamal, M. (20l5). Control of Varroa Destructor Anderson and Trueman (Acari: Varroidae) on *Apis mellifera* Linguistica by Using Thymol and Formic Acid in Pothwar Region of Punjab, Pakistan. *Asian J. Agric. Biol,* 3**,** 150-154.

Bakar, M. A., Aqueel, M. A., Raza, A. B. M., Ullah, M. I., Arshad, M., Sohail, M. & Molina-ochoa, J. (20l7). Evaluation of Few Essential oils For the Management of Parasitic Bee Mites, *Varroa destructor* (Acari: Varroidae) in *Apis Mellifera* L. Colonies. *Pakistan Journal of Zoology,* 49.

Barbosa, L. C. A., Filomeno, C. A. & Teixeira, R. R. (20l6). Chemical Variability and Biological Activities of Eucalyptus Spp. Essential oils. *Molecules,* 21**,** 1671.

Bogdanov, S., Charrière, J.-D., Imdorf, A., Kilchenmann, V. & Fluri, P. (2002). Determination of Residues in Honey After Treatments with Formic And oxalic Acid Under Field Conditions. *Apidologie,* 33**,** 399-409.

Calderone, N. W. & Spivak, M. (l995). Plant Extracts for Control of The Parasitic Mite *Varroa jacobsoni* (Acari: Varroidae) in Colonies of The Western Honeybee (Hymenoptera: Apidae). *Journal of Economic Entomology,* 88**,** l211-1215.

Cengiz, M. M. (20l2). In Honeybee Colonies (*Apis mellifera* L.), Usage of Different organics Compounds and Their Effects to Colony Performance Against *Varroa destructor* Infestation. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi,* l8.

Daher-Hjaij, N. & Alburaki, A. (2006). Control of *Varroa jacobsoni* oud. By Fumigation With Natural Plant Substances. *Arab J. Pl Prot,* 24**,** 93-97.

Damiani, N., Gende, L. B., Bailac, P., Marcangeli, J. A. & Eguaras, M. J. (2009). Acaricidal And Insecticidal activity of essential oils on *Varroa Destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae). *Parasitology Research,* 106**,** 145-152.

De Jong, D., Gonçalves, L. S. & Morse, R. A. (l984). Dependence on Climate of The Virulence of *Varroa jacobsoni*. *Bee World,*65**:**117-121.

Delfinado-Baker, M. & Peng, C. Y. (l995). *Varroa jacobsoni* and *Tropilaelaps clareae*: A Perspective of life history and why Asian Bee-Mites Preferred European Honey Bees. *American Bee Journal (USA)*. FAO. 1-80

Donders, J., Cornelissen, B. & Blacquière, T. (2006). Varroa Control Preceeding Honey Flow; Thymol and Formic Acid Residue. Proceedings of The Netherlands Entomological Society Meeting, Ede, The Netherlands. 141-145.

Eguaras, M., Quiroga, S. & Garcia, C. (l996). organic Acid in The Control of *Varroa jacobsoni* oud. *Apiacta,* 31**,** 51-54.

El-Wahab, A. & Ghania, A. (20l6). Impact of Some Pollen Substitutes in Liquid Form on The Biological Activities of Honeybee Colonies. *J Agr Sci Tech,* 12**:** 1035-1041.

Ellis, M. D. & Baxendale, F. P. (1997). Toxicity of Seven Monoterpenoids to Tracheal Mites (Acari: Tarsonemidae) and Their Honeybee (Hymenoptera: Apidae) Hosts When Applied As Fumigants. *Journal of Economic Entomology,* 90**,** 1087-1091.

Emsen, B. & Dodologlu, A. (2009). The Effects of Using different organic Compounds Against Honeybee Mite (*Varroa destructor* Anderson and Trueman) on colony developments of Honeybee (*Apis mellifera* L.) And Residue Levels In Honey. *Journal of Animal and Veterinary Advances,* 8**,** 1004-1009.

Farooqi, M. A., Irsa, B., Ali, S., Sajjad, A., Hassan, M. W. & Akhtar, S. (2020). Impact of Selected Insecticides on *Apis mellifera* L. (Hymenoptera: Apidae) Under Controlled Conditions. *Pakistan J. Zool,* 52**,** l93-l98.

Floris, I., Satta, A., Cabras, P., Garau, V. L. & Angioni, A. (2004). Comparison Between Two Thymol Formulations in The Control of *Varroa destructor*: Effectiveness, Persistence, And Residues. *Journal of Economic Entomology,* 97**,** 187-191.

Gashout, H. A. & Guzmán-Novoa, E. (2009). Acute Toxicity of essential oils and other natural compounds to the parasitic Mite, *Varroa destructor*, and to larval and adult worker Honeybees (*Apis mellifera* L.). *Journal of Apicultural Research,* 48**,** 263-269.

Girişgin, A. O. & Aydin, L. (20l0). Efficacies of Formic, oxalic And Lactic Acids Against Varroa Destructor in Naturally Infested Honeybee (*Apis Mellifera* L.) Colonies in Turkey. *Kafkas Univ Vet Fak Derg,* 16**,** 941-945.

Glinski, Z. (l988). The Effect of Varroa Jacobsoni oud. on The Incidence and Course of Chalkbrood Disease In Apis mellifera L. Colonies. *Annales Universitatis Mariae Curie-Skłodowska. Sectio Dd: Medicina Veterinaria,* 43.

Gracia MJ, Moreno C, Ferrer M, Sanz A, Peribáñez MÁ, et al. (20l7). Field efficacy of acaricides against *Varroa destructor*. PLoS oNE 12(2): eol71633.

Gunes, N., Aydın, L., Belenli, D., Hranitz, J. M., Mengilig, S. & Selova, S. (20l7). Stress Responses of Honey Bees To organic acid and essential oil treatments Against Varroa Mites. *Journal of Apicultural Research,* 56**,** 175-181.

Hamaad, R., Eldoksch, H., Abdel-Samed, A. & Abdel-Moein, N. (2008). Effect of Essential oils And a Thymol Formulation for Controlling Varroa Destructor in Honeybee (*Apis mellifera*) Colonies. *Egypt Journal Agricultural Research,* 86**,** 951-961.

Hassan, M., Allam, S., Rizk, M. & Zaki, A. (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: Journal of The Egyptian Society of Acarology,* 2**.**1: 3-8.

Henderson, C. F. & Tilton, E. W. (l955). Tests with acaricides against the brown Wheat Mite. *Journal of Economic Entomology,* 48**,** l57-l6l.

Imdorf, A., Bogdanov, S., Kilchenmann, V. & Maquelin, C. (1995). Apilife Var: A New Varroacide with Thymol as The Main Ingredient. *Bee World,* 76**,** 77-83.

Imdorf, A., Bogdanov, S., ochoa, R. I. & Calderone, N. W. l999. Use of Essential oils For the Control of *Varroa jacobsoni* oud. In Honeybee Colonies. *Apidologie,* 30**,** 209-228.

Imdorf, A., Bogdanov, S., Kilchenmann, V. & Berger, T. (2006). Toxic effects of essential oils And Some of Their Components on *Varroa destructor* oud and *Apis mellifera* L Under Laboratory Conditions. *Alp Science (Switzerland)*.

Islam, N., Amjad, M., Ehsan-Ul-Haq, S. E. & Naz, F. (20l6). Management of *Varroa destructor* By Essential oils And Formic Acid in *Apis mellifera* Linn. Colonies. *Journal of Entomology and Zoology Studies,* 4**,** 97-104.

Islam, N., Amjad, M., Haq, E., Stephen, E. & Naz, F. (20l7). Efficacy of Essential oils And Formic Acid in the Management of *Tropilaelaps clareae* in *Apis mellifera* Linnaeus Colonies in Relation to Honey Production. *Pakistan Journal of Agricultural Research,* 30**,** l94-20l.

Kanga, L. H. & James, R. R. (2002). Varroa Control with Fungal Pathogens May Be an Option Soon. *American Bee Journal,* 142**,** 519-519.

Khan, W., Khan, N. & Naz, S. (20l7). Beekeeping In Federally Administered Tribal Areas of Pakistan: opportunities And Constraints. *Sarhad Journal of Agriculture,* 33 (3), 459-465.

Koul, o., Walia, S. & Dhaliwal, G. (2008). Essential oils As Green Pesticides: Potential and Constraints. *Biopesticides International,* 4**,** 63-84.

Lundie, A. (l940). The Small Hive Beetle, Aethina Tumida. *Science Bulletin. Department of Agriculture and Forestry, Union of South Africa*.

Marcangeli, J. & Del Carmen García, M. (20l7). Effect of *Apis mellifera* (Apidae) Honeybee Brood Amount on oxavar® Acaricide Efficacy Against the Mite *Varroa destructor* (Varroidae). *Revista De La Sociedad Entomológica Argentina,* 63.

Mattila, H. R. & otis, G. W. (2000). The Efficacy of Apiguard Against Varroa And Tracheal Mites, And Its Effect on Honey Production. *American Bee Journal,* 140**,** 969-973.

Melathopoulos, A. P. & Gates, J. (2003). Comparison of two Thymol-based Acaricides, Api Life Var (R) And Apiguard (Tm), for the control of Varroa Mites. *American Bee Journal,* 143**,** 489-493.

Nazer, I. & Al-Abbadi, A. (2003). Control of Varroa mite (*Varroa destructor)* on Honeybees By aromatic oils and Plant Materials. *Journal of Agricultural and Marine Sciences [Jams],* 8**,** 15-20.

Neumann, P., Pettis, J. S. & Schäfer, M. o. (20l6). Quo Vadis Aethina Tumida? Biology And Control of Small Hive Beetles. *Apidologie,* 47**,** 427-466.

Noel, B. & Amrine, J. (l996). More on Essential oils For Mite Control. *American Bee Journal (USA)*.

Ramzi, H., Ismaili, M. R., Aberchane, M. & Zaanoun, S. (2017). Chemical Characterization and acaricidal activity of *Thymus satureioides* C. & B. and *Origanum elongatum* E. & M. (Lamiaceae) Essential oils Against *Varroa destructor* Anderson & Trueman (Acari: Varroidae). *Industrial Crops and Products,* 108**,** 201-207.

Rosenkranz, P., Aumeier, P. & Ziegelmann, B. (2010). Biology and Control of *Varroa destructor*. *Journal of Invertebrate Pathology,* 103**:** 96-119.

Sapountzis, P., Duport, G., Balmand, S., Gaget, K., Jaubert-Possamai, S., Febvay, G., Charles, H., Rahbé, Y., Colella, S. & Calevro, F. (20l4). New Insight into The Rna Interference Response Against Cathepsin-L Gene in The Pea Aphid, Acyrthosiphon Pisum: Molting or Gut Phenotypes Specifically Induced by Injection or Feeding Treatments. *Insect Biochemistry and Molecular Biology,*51**,**20-32.

Satta, A., Floris, I., Eguaras, M., Cabras, P., Garau, V. L. & Melis, M. (2005). Formic Acid-Based Treatments for Control of *Varroa destructor*In A Mediterranean Area. *Journal of Economic Entomology,* 98**:** 267-273.

Semkiw, P., Skubida, P. & Pohorecka, K. (20l3). The Amitraz Strips Efficacy in Control of Varroa Destructor After Many Years Application of Amitraz in Apiaries. *Journal of Apicultural Science,* 57**,** 107-12l.

Smodiš Škerl, M. I., Nakrst, M., Žvokelj, L. & Gregorc, A. (2011). The acaricidal Effect of Flumethrin, oxalic Acid and Amitraz Against *Varroa destructor* in Honeybee (*Apis mellifera* Carnica) Colonies. *Acta Veterinaria Brno,* 80**,** 5l-56.

Spivak, M. I. (997). Honey Bee Hygienic Behavior as a defense Against *Varroa jacobsoni* Mites. *Resistant Pest Management,* 9**,** 22-24.

Torto, B., Boucias, D. G., Arbogast, R. T., Tumlinson, J. H. & Teal, P. E. (2007). Multitrophic Interaction Facilitates Parasite–Host Relationship Between An Invasive Beetle And The Honey Bee. *Proceedings of The National Academy of Sciences,* l04**,** 8374-8378.

Tutun, H., Koç, N. & Kart, A. (20l8). Plant Essential oils Used Against Some Bee Diseases. *Turkish Journal of Agriculture-Food Science and Technology,* 6**,** 34-45.

Van Der Zee, R., Gray, A., Holzmann, C., Pisa, L., Brodschneider, R., Chlebo, R., Coffey, M. F., Kence, A., Kristiansen, P. & Mutinelli, F. (20l3). Standard Survey Methods For Estimating Colony Losses And Explanatory Risk Factors In *Apis mellifera.Journal of Apicultural Research,* 52**,** 1-36.

Zawislak, J. (20l0). Managing small hive beetles. Cooperative Extension Service, University of Arkansas. *US Department of Agriculture, and county governments cooperating*.

Zia-Ul-Haq, M., Raza Shah, M., Qayum, M., & Ercisli, S. (2012). Biological screening of selected flora of Pakistan. *Biological research*, *45*(4), 375-379.