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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

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Abstract: Survey study of the ectoparasitic mite *Varroa destructor* Anderson and Treuman infesting bee colonies was conducted to evaluate its infestation level for the first time in Qassim Region, Saudi Arabia. The infestation levels were variable according to the season and locality. Mite population parasitizing worker bees gradually increased from April and May and may reach its peak in June and July. Apiaries in Melida-1 presented the highest infestation level and declined significantly in Onayzah-2, Bakeriah and Melida-2 (18 to 13%), while Buraydah-1 and 2 and Onayzah-1 presented only 12% of the total annual mite population, respectively. The mites found on the bottom of bee hives started to increase in February and March and reached the peak during summer months (June-September). Apiaries in Melida-1 significantly recorded the highest level of infestation and followed by Buraydah-1, Onayzah-1 and 2, Bakeriah, Melida-2 (28 to 8%), while Buraydah-2 had the lowest infestation level with only 5% of the total annual mite population, respectively. For contamination of bee products purposes, certain local essential oil, safe to worker bees, including aloa, camphor, garlic, black seed and cloves were extracted in laboratory. Data showed that cloves was the most effective substance causing 62% mortality in *Varroa* mites, while garlic, camphor and black seed reduced mite infestation to 51, 47 and 43% 1 day after treatment, respectively. After 7 days, black seed was more effective than Garlic and camphor where they reduced mite infestation to 72, 66 and 56%, respectively. Aloe extract was the weakest extract causing reduction of only 34 and 45% for 1 and 7 days after treatment. Data showed that mite mortality percentage was positively correlated with time after treatment.

Key words: Survey, *Varroa destructor*, honey bee (*Apis mellifera*), botanical extracts, control, Qassim Region

INTRODUCTION

Oudemans (1904) was the first to describe *Varroa jacobsoni* from Java on *Apis cerana* Fabricus. In 1974, Delfinado and Baker (1974) illustrated and described *V. jacobsoni* and *Euvarroa sinhi* and initiated the new family Varroidae for the 2 genera. Delfinado and Aggrawal (1989) shown the new *Varroa* mite, *V. underwoodi* in bee hives in Nepal. Anderson and Treuman (2000) recently stated that *Varroa* mites is more than 1 specie. After studying mtDNA Co-I gene sequences and morphological characters of many populations of *V. jacobsoni* from around the world, it's been split into 2 species. *Varroa jacobsoni* infests *A. cerana* F. in the Malaysia-Indonesia Region, while *V. destructor* Anderson and Treuman infests its natural host *A. cerana* on mainland Asia and also *A. mellifera* L. worldwide

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(Zhang *et al*., 2007). *Varrod destructor*, now occurs nearly worldwide, is well known as an obligate ectoparasitic mite species and has a catastrophic effect on honeybees, bee hives, beekeepers and the beekeeping industry as a whole. Finley *et al*. (1996) mentioned that a 35 to 50% nationwide loss of beekeepers, 25% reduction in beehives and up to 50% increase in pollination services cost in some regions in USA. Generally, mite individuals suck tissue fluids or haemolymph of adults honey bee *A. mellifera* and may also feed on the regurgitated content of the honey sac. The mite parasitizes and complete its life cycle feeding on worker and drone brood (Delfinado and Baker, 1974; Martin, 1994). Moreover, Shen *et al*. (2005) reviewed that *Varroa* mites transmit Kashmire Bee Virus (KBV) and Deformed Wing Virus (DWV) in honey bees. They demonstrated that parasitization by *Varroa* mites suppress the immunity of honey bees, leading to activation of persistent and latent viral infection. Also, Chantawannakul *et al*. (2006) proved that there is co-existence of several bee viruses in a single *Varroa* mite individual for the first time in Thailand.

Chemical control using acaricides against *Varroa* mites infesting honey bee are still used to reduce *Varroa* mite populations in bee hives, including Coumaphos, Apistan, Amitraz, Folbex, Folbex-Forte, Apitol and Sinecar (Martin, 1994; Gregorc and Poklukar, 2003). Intensive utilization of many chemical substances had many disadvantages where they accumulation in bee products (honey and wax) due to their lipophilic and persistent nature (Wallner, 1999). Moreover, repeating chemical utilization resulted in the development of mite resistance that resulted to a reduction in their efficacy against mite infestation (Geregorc Poklukar, 2003; Batisha *et al*., 2008). Many studies had been carried out using some extracts of natural essential oil of various plants such as rosemary, lemongrass, camphor, thyme, majoram, sag, mint, clove, ginger roots, santonica seeds, fennel and eucalyptus (Fathy and Fouly, 1997; Gregorc and Poklukar, 2003: Batish *et al*., 2008). Other scientists investigated the effectiveness of rotenone and some organic acids such as formic and oxalic acids with low toxicity in addition to certain apicultural techniques such as screened bottom board, freezing drone brood, drone brood excision, swarming queen-arrest method and hygienic behavior (Gregorc and Poklukar, 2003; Bacandritos *et al*., 2007).

Because, there is no earlier published data with regard to mite infestation level in Qassim Region, the present study was conducted to assess the infestation levels of *V. destructor* infesting bee colonies in different districts for the first time in Qassim Region. During the last decades, Saudi beekeepers utilize chemical acaricides against *Varroa* mite, it was felt necessary to evaluate the effectiveness of certain local essential oil plants in controlling *Varroa* mites was also investigated to minimize the contamination with chemical control substances that may harm bees, honey and environment.

MATERIALS AND METHODS

Survey Studies and Assessment of Infestation Levels of *Varroa destructor* in Different Sites during 12 Months Period in Qassim Region

Seven apiaries in 4 districts (3 bee hives each, hybrid craniolian and identical in strength) were randomly chosen to survey the infestation levels of *Varroa* mite in bee hives during the period from January 2006 to January 2007. Apiaries included in this study were two in Buraydah, Melida and Onayzah and one apiary in Bakeriah. The apiary of Agricultural Technology College (Buraydah-1) and Al-Mezini apiary (Buraydah-2); the apiary of Agricultural Experimental Station of the Faculty of Agriculture and Veterinary Medicine, Qassim University (Melida-1) and Al-Shwehi apiary (Melida-2); Al-Qounasi apiary (Onayzah-1) and Al-Garbouh apiary (Onayzah-2); Al-Bazehi apiary (Bakeriah). Survey studies dealt with mite individuals living on worker bees and those found on the bottom of bee hives.

Assessment of Mite Infestation Levels on the Bottom of Bee Hives

A strong white sheet, coated with vaseline, was monthly inserted on hive's floor. After 1 week, the sheet was removed and the fallen or dead mites were visually inspected and counted. The earlier technique was repeated for 12 months.

Assessment of Infestation Levels of Mites Living on Worker Bees

Three samples of 30 worker bees per sample were monthly collected (for 12 months) from each tested apiary by removing a frame from the center of each colony. Worker bees were swept with a brush into a beaker containing water and soap as a washing liquid. Each beaker was gently shacked for 30 sec then the bees were collected using a wire net. *Varroa* mites, which fell from bees during washing, were collected and counted for further data analysis (Ritter, 1981).

Control of *Varroa* Mites by Essential Oil

Plant Materials and Isolation of Essential Oil

- Essential oil of camphor, garlic, aloe, cumine (black seeds) and cloves were tested against the ectoparasitic mite *V. destructor*
- The tested essential oil was extracted by water steam distillation using a Clevenger apparatus. After extraction, the essential oil was dried over anhydrous sodium sulphate and stored in a refrigerator at 5°C (Nazrul Islam *et al*., 2002)
- Various concentrations of the tested essential oil ranging from 3.125 to 75% were prepared with 1:1 sugar solution (50 g sucrose/50 mL distilled water) containing Tween 40 as an emulsifier. The prepared solutions were carefully mixed to obtain homogenous solution

Preparing Solutions of Essential Oil

Serial dilutions ranging from 0.0 to 750 g L^{-1} from all extracted oil was prepared using sugar solution (1:1) in the presence of Tween 40 as an emulsifier. After that, the prepared oil were carefully mixed to obtain homogenous solutions. In all cases, the volume of each concentration was completed to 5 m L^{-1} with sugar solution (1:1).

Effect of Essential Oil on Worker Bees

A. mellifera worker bees were collected from the apiary of the College of Agriculture and Veterinary Medicine, Qassim University. Three replicates of 10 worker bees for each concentration, was kept separately in plastic cups covered with cloth netting and secured with a rubber band to prevent bees from escaping. Groups of untreated bees were subsequently treated with sugar solution plus Tween 40 and used as a check. Different concentrations of each oil were immersed in small piece of cotton and placed on cloth netting for bee feeding. Dead and survived bees were recorded 24 h after treatments to exclude any concentrations that been recorded to have fatal effects on bees. From the earlier test, the following concentrations of tested extracts shown in Table 1 were examined against *Varroa* mites under laboratory conditions.

Table 1: Names and concentrations of the tested essential oil, safe to worker bees, used against *Varroa* mites

Concentrations % (g L^{-1})	Families	Scientific names	English names
62.5	Myrtaceae	*Eucalyptus camaldulensis* Denh.	Camphor
62.5	Aloeaceae	*Aloe vera* L.	Aloe
31.25	Myrtaceae	*Syzygium aromaticum L.*	Cloves
125.0	Ranomalaceae	*Nigella sativa*	Black seed
31.25	Alliaceae	*Allium sativum* L.	Garlic

Effect of Essential Oil on *Varroa* Mites

Ten worker bees were collected and subjected to be infested with 10 adult females of *Varroa* mite and kept in a plastic cup as previously mentioned. Bees in this treatment were fed on sugar solution containing 6.25% of camphor extract and replicated for 5 times. Five other groups of infested worker bees were fed on 6.25, 3.125, 12.5 and 3.125% of aloe, cloves and black seed while the fifth group was fed on sugar solution only and used as a check. In all cases, numbers of dead mites were counted 1 and 7 days after treatments.

Data Analysis

Analysis of Variance test (ANOVA) one way for the completely randomized design was used to compare means of *Varroa* mite individuals collected from beehive's bottom and those living on worker bees during 12 months in seven apiaries of four sites in Qassim Region. In all cases, means were compared using Student-Newman-Keuls Test (LSD) ($p = 0.005$) (Costat Statistical Software, 1990).

With regard to the effect of essential oil extracts on worker bees and *Varroa* mites, numbers of living and dead worker bees and mites were counted and the mortality percentages were corrected according to Abbott's (1925) formula.

RESULTS

Survey Study

Survey generally showed that infestation levels of the ectoparasitic mite *V. destructor* significantly fluctuated during the 12 month experiment and significantly affected by sites.

Infestation Levels of *V. destructor* Found on the Bottom of Bee Hives

Data in Table 2 showed that mite population found on the bottom of beehives started from low numbers in February (48.7 mite hive^{-1}) and March (63.86 mite hive^{-1}), which presented 5 and 7% of the total annual mite population (Fig. 1). Mite population significantly increased by an average of 121.14 and 147.71 mite hive^{-1} in June and July, respectively, which presented 14%, while mite populations during August and September were sub-equal (146.80 and 120.85 mite hive^{-1}, respectively) and presented 12% of the annual mite population during 12 months. The infestation level was significantly declined in October, November and December which presented only 7, 5 and 3% of the total annual mite population, respectively (Table 2, Fig. 1).

Table 2: Average numbers of *Varroa* mite individuals found on the bottom of bee hives and those living on worker bees during 12 months (in different sites)

	No. of *Varroa* mites	
Months	On hive bottom	Living on worker bees
January	30.85b	7.28a
February	48.70c	9.42b
March	63.86e	12.00d
April	94.71g	15.28f
May	108.57h	16.00g
June	121.14i	22.00j
July	147.71k	24.57k
August	146.80j	19.57i
September	120.85i	18.06h
October	68.57f	13.85e
November	51.14d	10.71c
December	29.00a	10.28c
Mean±SD	85.98±43.12	14.91±6.24
LSD ($p>0.05$)	0.610	0.609

Mean values within each column followed by the same letter(s) aren't significantly different

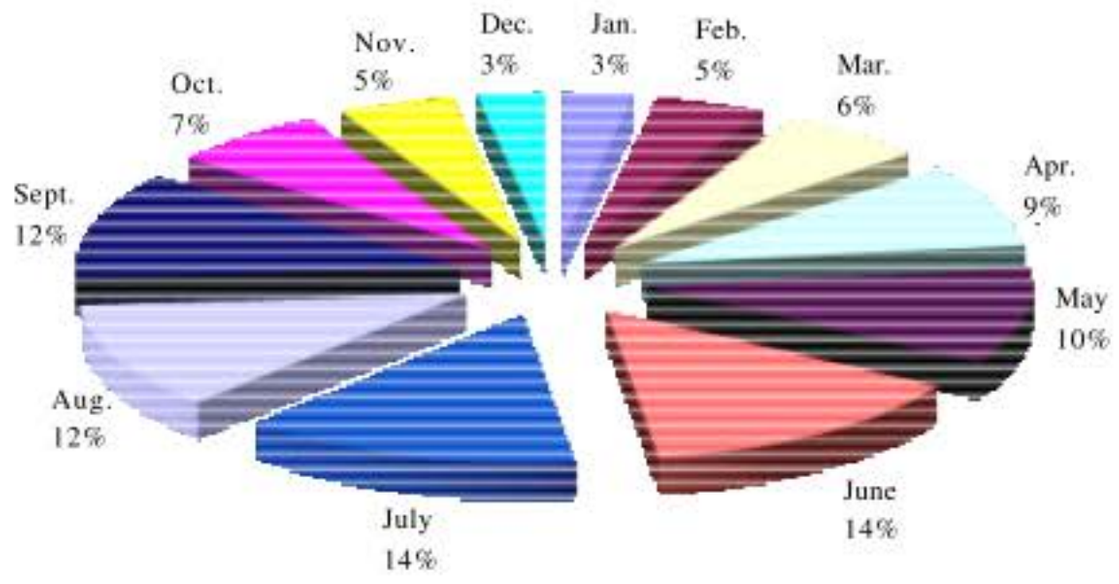

Fig. 1: Infestation percentage of *Varroa* mites found on the bottom of bee hives during 12 months (in different sites)

Table 3: Average numbers of *Varroa* mite individuals found on the bottom of bee hives and those living on worker bees in different sites (during 12 months)

	No. of *Varroa* mites	
Location	On hive bottom	Living on worker bees
Melida-1	167.40g	18.91f
Buraydah-1	140.80f	12.16a
Onayzah-1	91.16e	12.41ab
Onayzah-2	63.00c	17.50e
Bakeriah	64.60d	17.00d
Melida-2	47.50b	13.58c
Buraydah-2	27.00a	12.83b
Mean±SD	85.99±50.99	14.91±6.68
LSD (p>0.05)	0.466	0.465

Mean values within each column followed by the same letter(s) aren't significantly different

On the other hand, collecting mite individuals during the whole period of experiment showed that apiaries in Melida-1 significantly recorded the highest level of infestation by an average of 167.4 mite hive^{-1} (Table 3). The infestation levels significantly decreased in Buraydah-1 followed by Onayzah-1, Bakeriah, Onayzah-2, Melida-2, Buraydah-2 by an average of 140.8, 91.16, 64.6, 63.0, 47.5 and 27.0 mite hive^{-1}, respectively. From the earlier results, it was noticed that mite infestation level in Melida-1 apiary presented about 28%, while the infestation levels declined in apiaries of Buraydah-1, Onayzah-1, Bakeriah, Onayzah-2, Melida-2, Buraydah-2 by 23, 15, 10, 11, 8 and 8% of the total annual infestation (LSD = 0.466) (Table 3, Fig. 2).

Infestation Level of *V. destructor* Living on Worker Bees

Data presented in Table 2 and Fig. 3 showed that population of *V. jacobsoni* living on worker bees was low in winter time and gradually increased from April and May with an average of 15.28 and 16.0 mite bee^{-1} (presented 8 and 9% of the total annual number of mites), respectively. In July, the average number of mites parasitizing worker bees reached its peak of an average of 24.57 mite bee^{-1} which is almost 14% of the total infestation (Fig. 3). Reduction in infestation level was significant in later months (from August till October) where each bee was infested by an average of 19.57, 18.06 and 13.85 mites, respectively (Table 2). These values presented 11, 10 and 8% of the total mite infestation, respectively (Fig. 3).

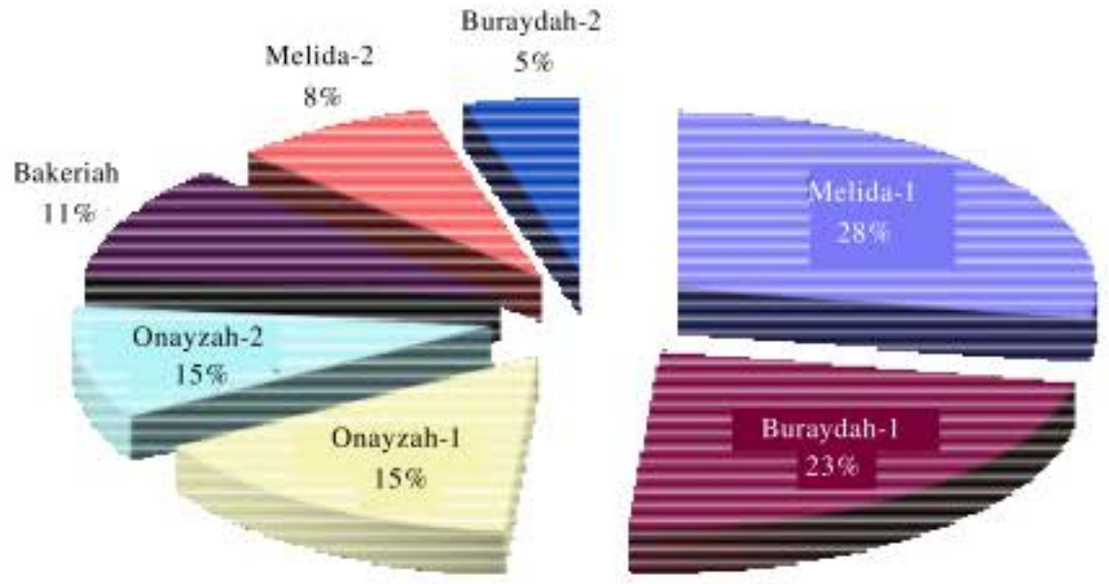

Fig. 2: Infestation percentage of *Varroa* mites found on the bottom of bee hives different sites (during 12 months)

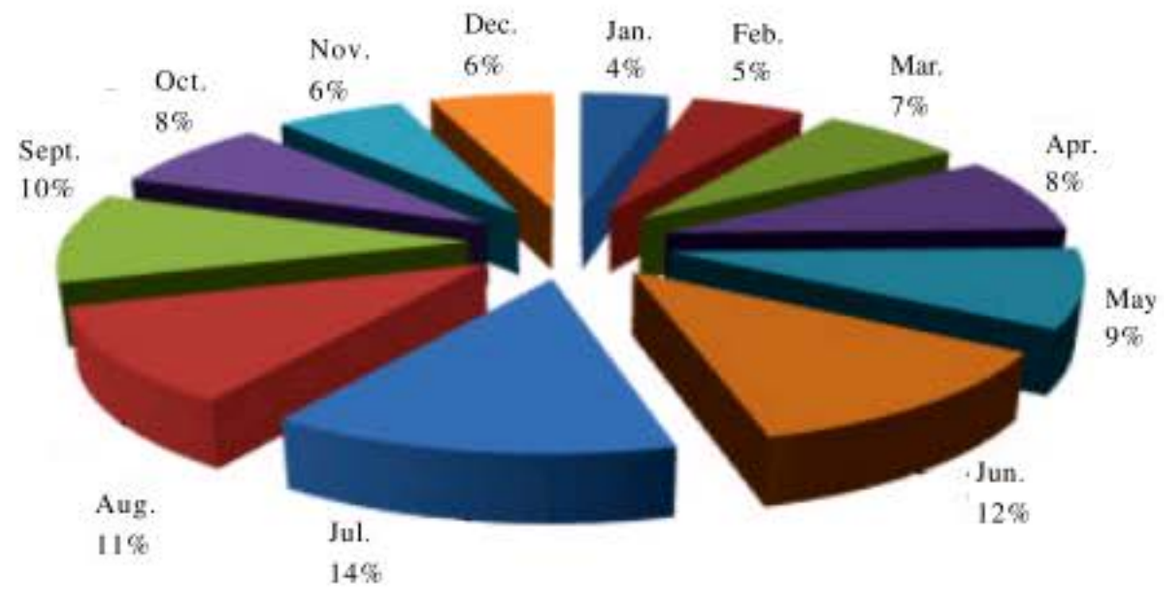

Fig. 3: Infestation percentage of *Varroa* mites found/30 worker bees during 12 months (in different sites)

On the other hand, data in Table 3 obviously showed that there were no significant differences between infestation levels in Buraydah-1; Buraydah-2 and Onayzah-1 where each worker bee was infested by an average of 12.16, 12.83 and 12.41 mite individuals presented about 12% of the total mite infestation on worker bees during 12 months, respectively (LSD = 0.465). The infestation level significantly increased in Melida-2, Bakeriah, Onayzah-2 and reached its highest level in Melida-1. In the earlier sites, the infestation percentage averaged 13, 16, 17 and 18% where each worker bee was noticed to be infested by an average of 13.58, 17.0, 17.5 and 18.91 mite individuals, respectively (LSD = 0.465) (Fig. 4).

Effect of Essential Oil Extracts on *Varroa* Mite Infestation Affecting Honey Bees

The effectiveness of local essential oil (safe to worker bees), aloa, camphor, garlic, black seed and cloves were tested against *Varroa* mite individuals living and feeding on worker bees hymolymph in laboratory. Data in Table 4 and Fig. 5 showed that cloves (3.125%) was the most effective essential oil causing 62 to 77.4% *Varroa* mite mortality at 1 and 7 days after treatment. Extracts of garlic (3.125%), camphor (6.25%) and black seed (12.5%) came second, third and fourth in mite infestation reduction percentages with 51, 47 and 43% at 1 day after treatment, respectively.

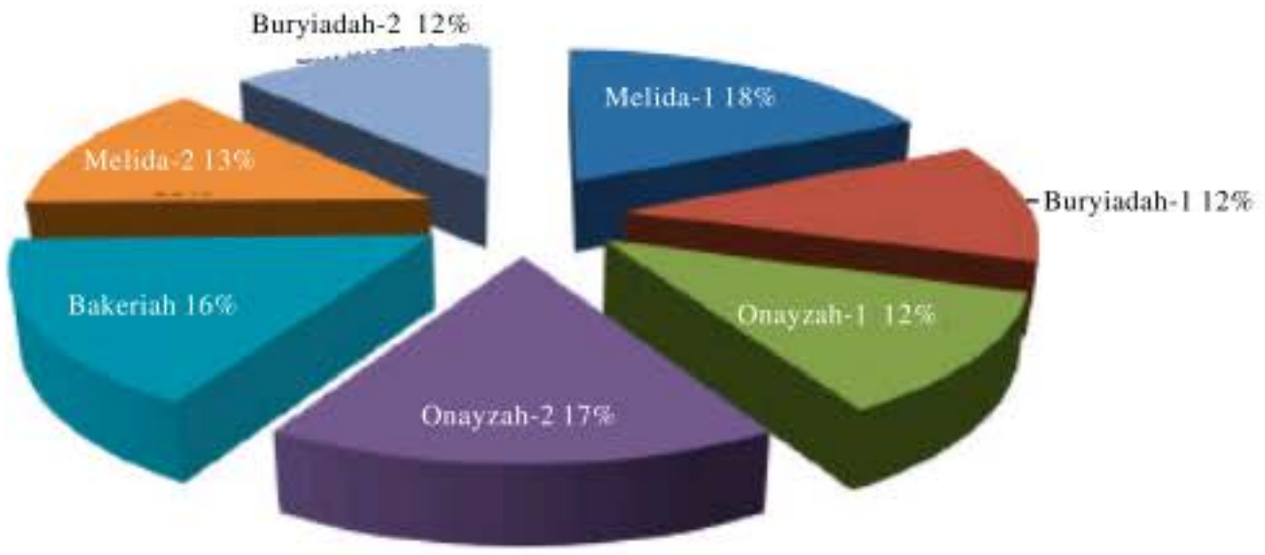

Fig. 4: Infestation percentage of *Varroa* mites/30 worker bees in different sites (during 12 months)

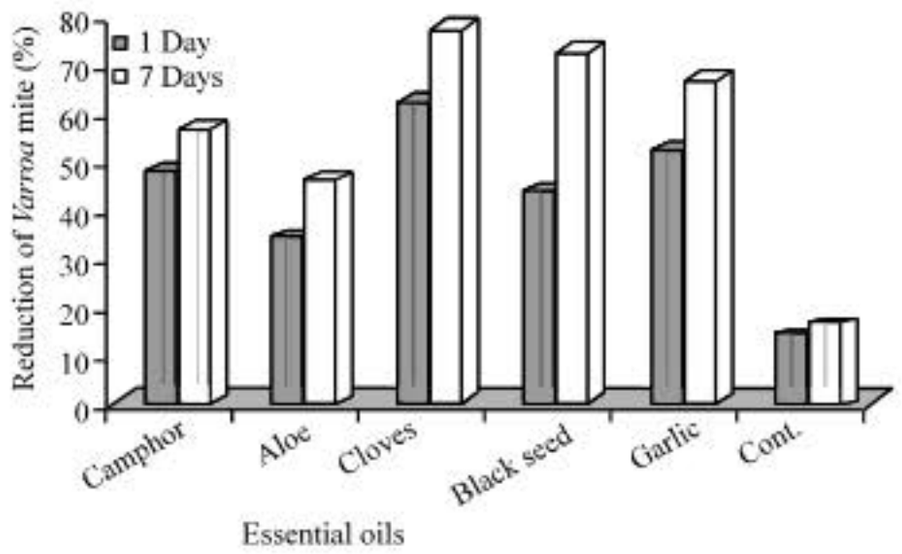

Fig. 5: Reduction percentages of *Varroa* infestation on worker bees 1 and 7 days after treatment with essential oil

Table 4: Corrected mortality (%) of *V. destructor* parasitizing worker bees 1 and 7 days after treatment in laboratory

		Mortality percentage after	
Essential oil	Conc. % (g L^{-1})	1 day	7 day
Camphor	62.50	47c	56b
Aloe	62.50	34a	45a
Cloves	31.25	62e	77e
Black seed	125.00	43b	72d
Garlic	31.25	51d	66c
Mean	---	47.40	63.20
SD	---	9.22	11.47
LSD (p>0.05)	---	1.776	1.778

Mean values within each column followed by the same letter(s) aren't significantly different

Seven days after treatment, black seed was significantly more effective than garlic and camphor where they reduced mite infestation to 72, 66 and 56%, respectively. Aloe extract was the weakest extract with reduction in mite infestation by only 34 and 45% at 1 and 7 days after treatment, respectively. In general, it was noticed that mortality percentage was positively correlated with time after treatment ($r = 0.93$). Therefore, it can be recommended to repeat the application for two or three times as needed according to the infestation level.

DISCUSSION

For the first time in Qassim Region, survey studies showed that the infestation levels, which were represented by numbers of *Varroa* mites collected from the bottom of beehives or parasitizing worker bees, affected either by season or locality were differed significantly. The highest level of mites was obtained during the period extending from June until September. Apiaries in Melida-1 recorded the highest level of mite infestation and significantly decreased in Buraydah-1 followed by Onayzah-1, Bakeriah, Onayzah-2, Melida-2, Buraydah-2. Concerning *Varroa* mites parasitizing worker bees, results showed that the infestation level of *V. destructor* infesting worker bees gradually increased from April and reached its peak in July. These findings may be due to adult female mites that find bee brood to start laying eggs and build up their population. Similar results were obtained by Ritter (1981), Cervanica and Aspiras (1987) and Contzen *et al*. (2004), who stated that *Varroa* mites began to lay eggs in bee brood of *A. mellifera* during spring and summer time. Also, Fathy and Fouly (1997) found that *V. jacobsoni* was found in great numbers in June and July. Moreover, it was noticed that *Varroa* mite reached its highest rate of infestation to honeybees in New Zealand during April-June (Stevenson *et al*., 2005).

The use of essential oil plants of the tested substances as natural acaricides is of immense significance in view of the environmental and toxicological implications of the indiscriminate use of synthetic acaricides and overcoming or reducing the problem of increasing *Varroa* resistance. Accordingly, the essential oil tested against *Varroa* mite showed that cloves was the most effective essential oil followed by garlic, camphor and black seed. Black seed was significantly more effective than garlic and camphor especially seven days after treatment, while aloe extract was the weakest substance. Fathy and Fouly (1997) noticed that the aqueous sugar solution of rosemary, fennel and marjoram essential oil were more effective than extracts of lemongrass, sag and thymus, that means there are certain essential oil plants which are considered to be a promising source of natural acaricides against *Varroasis*. Therefore, the efficiency of an extract may mainly due to its contents. Batisha *et al*. (2008) found that *Eucalyptus* oil rich in cineole was very effective against *Varroa* mites in India.

From the present results, it can be also concluded that mortality percentage of *V. destructor* was positively correlated with time after treatment ($r = 0.93$). Therefore, it is highly recommended to repeat the application for two or three times as needed according to the infestation level especially in winter time when no brood in bee colonies. Similarly, Fathy and Fouly (1997) found that mite mortality positively increased by repeating the treatments in broodless bee hives. Contradictory, Gregorc and Poklukar (2003) found that treatments with rotenone and oxalic acid in aqueous sugar solution can be administrated during summer in July-August. However, they achieved 98.65% mite mortality by using three treatments of oxalic acid in broodless colonies and significantly decreased in colonies with capped brood. While, Wachendorfer *et al*. (1985) and Bacandritos *et al*. (2007) repeated the application of formic and oxalic acid against *V. destructor* for four successive times (approximately one every 16 days) in Greece. Bacandritsos *et al*. (2007) obtained 65.33% cumulative mite mortality in November after the first three treatments. In December, when no brood in hives, the mite mortality obtained by the fourth treatment increased to 77.3%.

In general, the effect of natural substances such as volatile or essential oil plants, even if the treatments were repeated, can't completely eliminate *Varroa* invasion from bee colonies especially in countries like Saudi Arabia where sealed brood cells are present almost all around the year. Further studies are highly needed with regard to the effect of natural substances on *Varroasis*.

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