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Research Article

Effect of Some Natural Compounds and Varroacides on Varooa Mites (*Varroa destructor*) In Honeybee (*Apis mellefra*) Colonies during Winter Season in El Gharbiya Governorate, Egypt

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Abstract:

The current study was carried out to assess the efficacy of some natural compounds (peppermint oil and camphor oil) and Varroacides (bayvarol, formic acid (65%), Varokiler and varofiga) for controlling Varroa mites in honey bee colonies during winter season in Gharbiya Governorate, Egypt. Results from this study found that, the total numbers of Varroa mites fallen on sticky boards were 2123, 2627, 1888, 2150, 1743, 1269, 1319 and 1208 (Varroa/colony), with an average was 163.30, 202.20, 145.23, 165.38, 134.07, 97.60, 101.46 and 92.9 (Varroa/colony) for honeybee colonies treated with mint oil, camphor oil, varo killer, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control), respectively. It also found that, the number of Varroa mites fallen on sticky boards per month was 497.10, 640.70, 459.25, 520.20, 421.45, 304.35, 322.10 and 294.00 (Varroa/colony/month) for aforementioned treatment during the season. From the current study it can summarized that mint oil, camphor oil, and Bayvarol treatments significantly surpassed other treatments in total and average numbers of varroa mites fallen on sticky boards without significant differences between them, followed by formic acid and Varro killer treatment without significant differences between them, meanwhile Varro viga significantly wasn't effect Varroa mites as compared with untreated check (control).

1. Introduction

Honey bees, *Apis mellifera*, are very valuable to the agricultural sector. They are the main pollinators to various plants and beekeeping is considered as source of income to many people (Morse and Calderone, 2000; Chazovachii et al., 2013; Qaiser et al., 2013) Honey bees are the main target to many pests and parasites including Varroa mites. Varroa mites transferred from its originally host, *Apis cerana*, to the Western honey bees, *Apis mellifera*, in the 1950_s and 1960_s. This was a direct result of human-assisted colony movement between Asia and Europe. The mite quickly spread westward into Europe and were transferred from there to North Africa. It was also introduced from Japan into Paraguay, establishing a foothold in the America. It was first detected in North America in 1987 and since then spread to most regions except for isolated areas, such as the Prairie Provinces of Canada (Sanford, 2000).

These mites can cause severe damages to the bees. The most significant event affecting 20th century apiculture has been human-assisted spread of the parasitic bee mite, *Varroa destructor* (Anderson and Trueman, 2000) (Acari: Parasitiformes: Varroidae) are external parasites that attack both honey bees and brood. They suck the blood from both3 the adults and developing brood, especially drone brood. This weakens and shortens the bee's life. *Varroa destructor* mite has become a serious pest of *Apis mellifera* all over the world (Baker and Peng, 1995; Rashid et al., 2014). It causes serious losses in apiculture of *A. mellifera*. It feeds on haemolymph of larvae, pupa and adult bees during all life (Anderson and Trueman 2000). It also, decreases brood, colony ability to pollinate plants (De Jong et al., 1984). Varroa infestation in honey bee colonies increases the sensitivity of bees to other pathogens including viruses and bacteria. This requires appropriate measures against Varroa mites. The most widely applied Varroa control method is the application of chemicals by beekeepers (Adnan ayan et al., 2019). Varroa mites need to be controlled because untreated infestations honeybee colonies of varroa mites will increase and may kill colonies (Ali, 2001). If the colonies are not examined for mites, losses may be mistaken for winter mortality or queenlessness. Mahmoud et al., (2019) tested the efficacy of two chemical compounds, Bayvarol and VarroKiller acaricides against varroa mite, and found that, both compounds showed their qualitative superiority in the

treatment of the colonies under study and reduce the infection of parasite on the adult bees below the minimum levels compared with the untreated colonies, and Bayvarol was a more superior than VarroKiller compound.

Resistance issues with currently registered acaricides support the need to develop alternative strategies for managing varroa mite. Beyond chemicals, other solutions rest on finding or importing varroa-tolerant bees (Rinderer et al., 1997) and selecting for hygienic behavior or breeding for tolerance (Harbo and Hoop ingarner, 1997). Mechanical control measures can also help keep varroa population at bay. These include drone brood trapping and specially designed varroa trapping bottom boards Controlling mite populations by integrated pest management (IPM) using a combination of measures (Chemical, cultural, and genetic) is probably the best strategy to pursue (Sanford, 2000).

The objective of the current study was to evaluate some natural and chemical compounds for controlling Varroa mites and to study their effects for decreasing the infestation levels of varroa mites in honeybee colonies during winter season under study area.

2. Materials and Methods

The current study was carried out to assess the efficacy of some natural compounds (peppermint oil and camphor oil) and Varroacides (bayvarol, formic acid (65%),Varrokiller and varofiga) for controlling Varroa mites (*Varroa destructor* Anderson and Trueman 2000) in honey bee colonies (*Apis mellifera* L.) during winter season, which extended from16th November 2019 until 8th August 2020. in the apiary of Plant Protection Department, Faculty of Agriculture, Tanta University, Gharbiya Governorate. Thirty-one honeybee colonies headed by Carnelian hybrid honeybee queens were used for this study, the experimental honeybee colonies were equal in colony strength, and each colony contained seven frames covered with adult honeybee workers and three frames of brood to evaluate some varrocides and natural compounds for Varroa control and to study their effect for decreasing varroa infestation levels on honeybees. The apiary was divided into seven different groups and one group was left to serves as untreated check (control) for comparison.

2.1. Experimental honeybee colonies:

Thirty-one honeybee colonies headed by Carnelian hybrid honeybee queens were used for this study, the experimental honeybee colonies were equal in colony strength, and each colony had seven frames covered with adult honeybee workers and three frames of brood. They were divided into eight different groups.

2.2. Varroacides and natural compounds used for Varroa control:

2.2.1. Varroacides used

Bayvarol, Varoviga and Formic acid were purchased from honeybee equipment stores in Egyptian markets.

2.2.2. Natural compounds used

Peppermint oil and Camphor oil were purchased from perfume shops in Egyptian markets.

2.2.3. Vaseline and sticky boards

Vaseline, which used to cover sticky boards (to catch varroa fallen), was purchased from the Republic Pharmaceutical and chemical company in Tanta City.

Stickyboards, thick plastic was purchased from the book stores, and they were cut the same measured of bottom boards in Langstroth hive.

2.3. Application methods

2.3.1. Application methods of natural compounds

Two ml of peppermint oil and camphor oil were applied to a piece of cotton for each experiment honeybee colony and put on top of the honeybee combs, the technique was repeated every week during the experiment period.

2.3.2. Application methods of Varroacides

Bayvarol strips one strip per honeybee colony, they were hanged between the brood frames inside the bee hives, they were left inside experimental honeybee colonies for 35 days, and then they were replacing with another new strips during the experiment period for twelve months.

Varrokiller strips one strip per honeybee colony, they were hanged between the brood frames inside the bee hives, they were left inside experimental honeybee colonies for a month, and then they were replacing with another new strips during the experiment period for twelve months.

Formic acid: 5 ml of formic acid (65%) was applied to a cotton strip and sprayed on one comb and the comb was situated between the brood nests of each tested honeybee colony for days and repeated every day during the experiment period.

Varofiga: was applied in two ways:

1- Mixed with sugar syrup for feeding honeybees: about 400g of sugar was added to warm water and stirred constantly to make sugar syrup, then about 1.5g of Varoviga was added and mixed with syrup, then the mixed was put in a plastic bag with a hole on it and situated inside the honeybee colonies day's intervals during the experiment period.

2- Dusting on honeybees: about 1.5g of Varoviga was mixed with 10g of powdered sugar then they dusted experimental honeybee colonies days intervals, during the experiment period.

3. Results and Discussion

Results in Table (1) show total and average numbers of Varroa mites fallen on sticky boards during winter season (December, January and February), 2019 and 2020.

3.1. Varroa mites fallen on sticky boards during December, 2019

Results indicated that, during Dec.7th, the total number of Varroa mites fallen on sticky boards was 195, 277, 135, 212, 169, 54, 255 and 89 (Varroa/colony), where their average numbers were 65.00, 69.25, 33.75, 53.00, 42.25, 13.50, 63.75 and 22.25 (Varroa /colony) for honeybee colonies treated with mint oil, camphor oil ,varokiller, bayvarol, formic acid, varoviga (with sugar syrap), varoviga (with sugar powder) and untreated check (control), respectively.

During December, 14th, the number of Varroa mites fallen on sticky boards for aforementioned treatments was 107, 299, 111, 233, 93, 105, 191 and 92 while their average was 35.67, 74.75, 27.75, 58.25, 23.25, 26.25, 63.67 and 23.00 (Varroa/ colony).

In December, 21th, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrap), varoviga (with sugar powder) and untreated check (control)was 167, 291, 259, 436, 233, 142, 111 and 139 (Varroa/colony), respectively, while their average was 55.67, 72.75, 64.75, 109.00, 58.25, 35.5, 37.00 and 34.75 (Varroa / colony).

In December, 28th, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrap), varoviga (with sugar powder) and untreated check (control) was 118, 344, 264, 276, 231, 212, 121 and 170 (Varroa/colony), where their average number was 39.33, 114.67, 66, 69, 77, 53, 40.33and42.5 (varroa/colony), respectively.

At the end of December month, the total number of Varroa fallen on sticky board was 587, 1211, 769, 1157, 726, 513, 678 and 490 (Varroa/colony/month) with an average of 146.75, 302.50, 192.25, 289.25, 181.50, 128.25, 169.50 and 122.50 (Varroa/colony/month).

During December month, results summarized that camphor oil and Bayvarol treatments surpassed other treatments in total and average numbers of varroa mites mortality and fallen on sticky boards, followed by Varro killer, formic acid and Varroviga (with sugar powder) treatments, meanwhile mint oil and Varro viga (with sugar syrap) wasn't effect varroa mites as compared with untreated check (control) significantly (Table 1 and Fig. 1).

Table 1. Total and average numbers of Varooa mites fallen on sticky boards during winter season, (December, 2019) (Mean ± S.E.).

Treatment	December				Total	Mean
	28 Dec	21 Dec	14 Dec	7 Dec		
Mint oil	(118) 39.33±13.70	(167) 55.67±10.00	(107) 35.67±11.30	(195) 65±14.90	(587)	146.75
Camphor oil	(344) 114.67±24.09	(291) 72.75±17.26	(299) 74.75±25.29	(277) 69.25±20.81	(1211)	302.5
Varro killer	(264) 66±22.4	(259) 64.75±20.8	(111) 27.75±12.90	(135) 33.75±8.70	(769)	192.25
Bayvarol	(276) 69±24.3	(436) 109±55.9	(233) 58.25±26.3	(212) 53±8.10	(1157)	289.25
Formic acid	(231) 77±38.5	(233) 58.25±18.6	(93) 23.25±7.20	(169) 42.25±12.40	(726)	181.5
Varroviga (with sugar syrup)	(212) 53±20.9	(142) 35.5±16.1	(105) 26.25±7.90	(54) 13.5±7.40	(513)	128.25
Varroviga (with sugar powder)	(121) 40.33±18.7	(111) 37±14.6	(191) 63.67±12.10	(255) 63.75±9.90	(678)	169.5
Untreated check (Control)	(170) 42.5±15.00	(139) 34.75±18.61	(92) 23±11.09	(89) 22.25±9.68	(490)	122.5

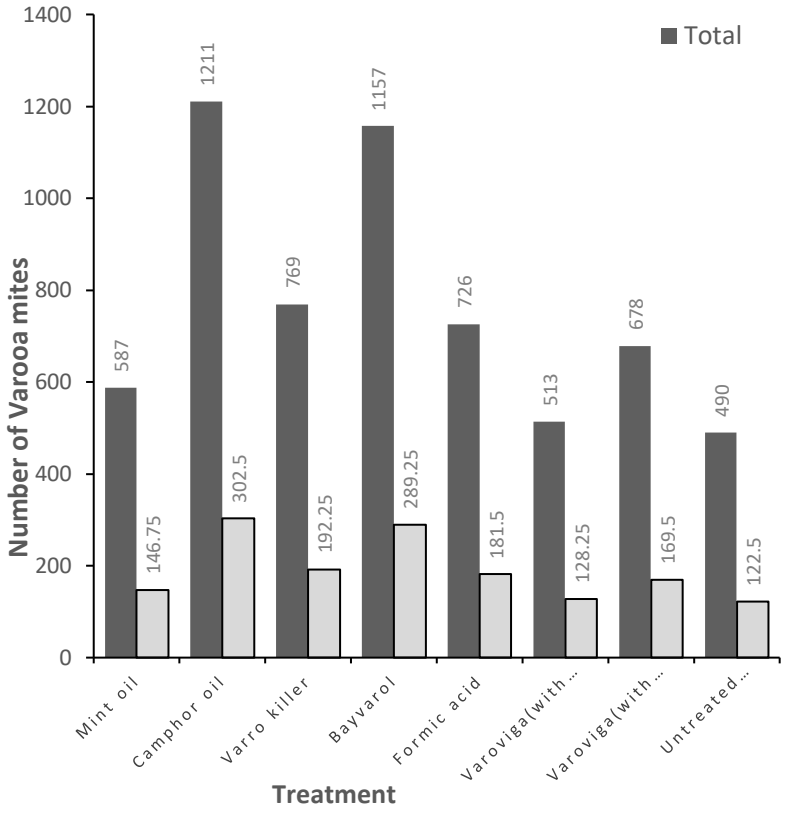

Figure 1. Relationship between Varooa mites fallen on sticky boards and treatments during December, 2019.

3.2. Varroa mites fallen on sticky boards during January month, 2020

Results in Table (2) and illustrated in Fig (2) indicated that, during Jan.4th, the total number of Varroa mites fallen on sticky boards was 222, 344, 273, 179, 198, 194, 127, and 154 (Varroa/colony), where their average number was 74, 114.7, 68.25, 44.75, 66, 48.50, 42.33 and 36.3 (Varroa/colony) for honeybee colonies treated with mint oil, camphor oil, varo killer, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control), respectively.

During January,11th, the total numbers of Varroa mites fallen on sticky boards for aforementioned treatments were 217, 292, 271, 159, 244, 133, 126 and 145 (Varroa/colony) with an average was 72.33, 97.3, 67.75, 39.75, 81.33, 33.25, 63.00 and 38.5(Varroa/colony). In January, 18th, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control) was 244, 323, 214, 180, 146, 175, 127 and 130 varroa/month, respectively, with an average was 81.33, 107.7, 71.33, 45, 73, 43.75, 63.5 and 32.5 (Varroa/colony).

In January, 25th, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control) was 180, 141, 106, 129, 143, 96, 108 and 128 varroa /month, where their average number was 90, 47, 35.33, 32.25, 71.5, 32, 54 and 32 (Varroa/colony).

At end of January month, the total number of Varroa fallen on sticky board was 863, 1100, 864, 647, 731, 498, 488 and 557(Varroa/colony/month) with an average 215.75, 275.00, 216.00, 161.75, 182.75, 124.50, 122.00 and 139.3 (Varroa /colony/month).

During January month, results summarized that mint oil, camphor oil, Varro killer, Bayvarol and formic acid treatments surpassed other treatments in total and average numbers of varroa mites mortality and fallen on sticky boards, meanwhile Varro viga wasn't effect varroa mites as compared with untreated check (control) (Table 2 and Fig. 2).

3.3. Varroa mites fallen on sticky boards during February month, 2020

Results in Table (3) and illustrated in Fig. (3) indicated that, during Feb.1st the total number of Varroa mites fallen on sticky boards was 161, 123, 62, 87,116,72, 79 and 44 (Varroa/colony), with an average number was 80.5, 41, 20.67, 21.75, 58, 24, 39.5 and 14.67 (Varroa/colony) for honeybee colonies treated with mint oil, camphor oil, varrokiller, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar power) and untreated check (control), respectively.

During February, 8th, the number of Varroa mites fallen on sticky boards for aforementioned treatments was 152, 86, 57, 86, 87, 72, 38 and 40 (Varroa/colony), while their average was 76, 43, 28.5, 21.5, 43.5, 24, 19 and 20 (Varroa/colony).

In February, 15th, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated

check (control) was 158, 35, 52, 74, 44, 36, 12 and 33 (varroa/month), respectively, while their average was 79, 17.5, 26, 18.5, 11, 12, 6 and 16.5 (Varroa/colony).

In February, 22nd, the total number of Varroa mites fallen on sticky boards for mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control) was 110, 40, 55, 63, 23, 33, 11 and 25(Varroa/colony), with an average number was 55.00, 20.00, 27.50, 15.75, 5.75, 11.00, 5.50 and 9.50 (Varroa/colony), respectively.

Table (2): Total and average numbers of Varooa mites fallen on sticky boards during winter season (January, 2019) (Mean ± S.E.).

Treatment	January				Total	Mean
	4 Jan	11 Jan	18 Jan	25 Jan		
Mint oil	(222) 74±18.90	(217) 72.33±32.90	(244) 81.33±42.60	(180) 90±4.10	(863)	215.75
Camphor oil	(344) 114.7±24.10	(292) 97.3±36.00	(323) 107.7±23.50	(141) 47±18.90	(1100)	275
Varro killer	(273) 68.25±18.60	(271) 67.75±22.50	(214) 71.33±20.30	(106) 35.33±7.30	(864)	216
Bayvarol	(179) 44.75±14.70	(159) 39.75±13.40	(180) 45±21.60	(129) 32.25±14.90	(647)	161.75
Formic acid	(198) 66±17.30	(244) 81.33±15.00	(146) 73±43.10	(143) 71.5±9.50	(731)	182.75
Varroviga (with sugar syrup)	(194) 48.50±21.80	(133) 33.25±15.60	(175) 43.75±13.90	(96) 32±17.80	(498)	124.5
Varroviga (with sugar powder)	(127) 42.33±13.30	(126) 63±24.70	(127) 63.5±0.40	(108) 54±9.90	(488)	122
Untreated check (Control)	(154) 36.3±31.30	(145) 38.5±22.50	(130) 32.5±45.50	(128) 32±26.40	(557)	139.3

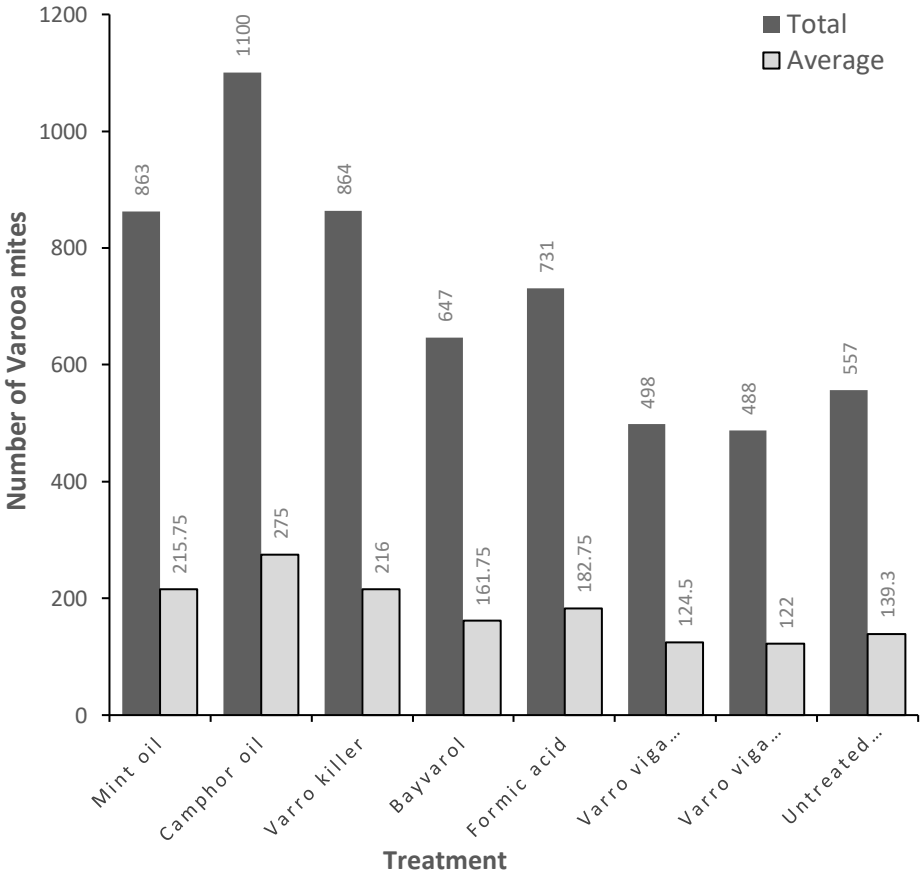

Figure 2. Relationship between Varroa mites fallen on sticky boards and treatments during January, 2019.

During February, 29th, the number of Varroa mites fallen on sticky boards for aforementioned treatments was 92, 32, 29, 36, 16, 45, 13 and 19 (Varroa/colony), while their average was 46.00, 16.00, 14.50, 9.00, 4.00, 15.00, 6.50 and 12.5 (Varroa/colony).

At the end of February month, the total number of Varroa fallen on sticky board was 673, 316, 255, 346, 286, 258, 153 and 161 (Varroa/colony/month) with an average 134.60, 63.5, 51.00, 69.20, 57.20, 51.60, 30.60 and 32.20(Varroa/ colony/season) (Table 3 and Fig. 3).

At the end of winter season, the total numbers of Varroa mites fallen on sticky boards were 2123, 2627, 1888, 2150, 1743, 1269, 1319 and 1208 (Varroa/ colony/ season), with an average was 163.30, 202.20, 145.23, 165.38, 134.07, 97.60, 101.46 and 92.9 (Varroa/colony/season) for honeybee colonies treated with mint oil, camphor oil, varo killer, bayvarol, formic acid, varoviga (with sugar syrup), varoviga (with sugar powder) and untreated check (control), respectively (Table, 3 and Fig.).

While the number of Varroa mites fallen on sticky boards per month was 497.10, 640.70, 459.25, 520.20, 421.45, 304.35, 322.10 and 294.00 (Varroa/ month) and the number of Varroa mites fallen on sticky boards per colony was 707.67, 585.65, 472.00, 537.50, 435.75, 317.25, 329.74 and 402.6 (Varroa/ month) for aforementioned treatment during the season respectively.

During winter season, results summarized that mint oil, camphor oil, and Bayvarol treatments significantly surpassed other treatments in total and average numbers of varroa mites mortality and fallen on sticky boards without significant differences between them, followed by formic acid and Varro killer treatment without significant differences between them, meanwhile Varroviga significantly wasn't effect Varroa mites as compared with untreated check (control).

Lastly, The current results in disagreement with data obtained by Al Ghamdi (2023) who evaluate the effectiveness of five acaricides; Apistan, Bayvarol, Apivar, Perizine and Bee Strips against *Varroa Destructor*. Who found that Bayvarol (4-Strips) showed the maximum efficacy level (96%), followed by Apivar (95%) and 2 Strips of Bayvarol (89%), while Apistan remained at minimum level (85%). Hýbl et al., (2021) used essential oils against *Varroa destructor* instead of acaricides. The results suggest that the most suitable oils are peppermint oil .

In the other hand, the current results in disagreement with data obtained by Aljedani (2021) tested Five essential oils (garlic oil, Peppermint oil, Cinnamon oil, thyme oil, Lavender oil) of plant natural products . Results summarized that, Garlic oil and thyme oil were found to be particularly efficient against Varroa Mites and honeybees as compared Peppermint oil. Nowar et al., (2018) whom treated honey bee colonies with oils of clove, peppermint, thymol, camphor, Varroazal, mavric and control (without treatment). Their data indicated that the most effective treatments were mavric and Varroazal, while the lowest effective one was the treatment of camphor oil, during the seasons of study. Morfin et al., (2023) conducted an experiment to determine the efficacy of three synthetic acaricides approved for use in Canada for control of V. destructor infestations, amitraz (Apivar), tau-fluvalinate (Apistan), and flumethrin (Bayvarol), whom found that, Amitraz caused a significantly higher mite mortality rate (92%) than flumethrin (78%) and tau-fluvalinate (72%) did. Amitraz was classified as “mostly effective” (90–97%), whereas flumethrin and tau-fluvalinate were classified as “minimally effective” (<80%) for V. destructor control in the mite populations studied. Sharaf El-Din et al., (2020) whom conducted an experiment to evaluate Varoviga and Bayvarol acaricides under the Egyptian conditions compared to formic acid. Their results showed that, formic acid had the major significant effect on varroa mites, followed by Bayvarol then Varoviga acaricides with no significant difference between them.

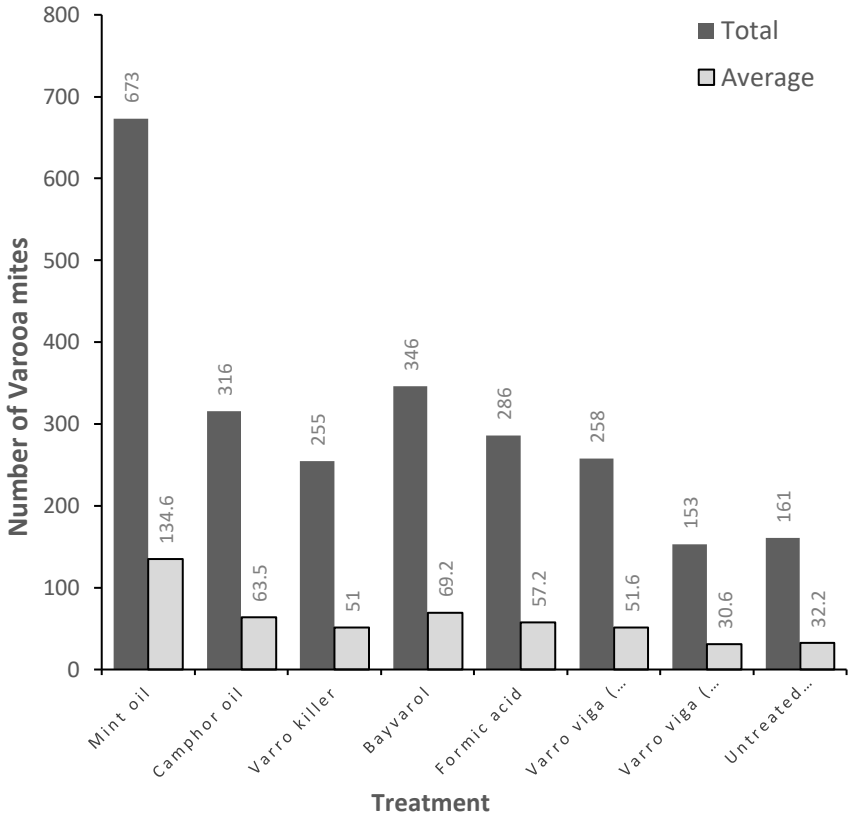

Figure 3. Relationship between Varooa mites fallen on sticky boards and treatments during February, 2020.

Table 3. Total and average numbers of Varooa mites fallen on sticky boards during winter season (February, 2019) (Mean ± S.E.).

Treatment	February					Total	Mean
	1 Feb	8 Feb	15 Feb	22 Feb	29 Feb		
Mint oil	(161) 80.5±17.70	(152) 76±54.30	(158) 79±25.50	(110) 55±18.90	(92) 46±6.60	(673)	134.6
Camphor oil	(123) 41±15.2	(86) 43±4.2	(35) 17.5±9.5	(40) 20±4.9	(32) 16±6.4	(316)	63.5
Varro killer	(62) 20.67±10.8	(57) 28.5±1.8	(52) 26±10.6	(55) 27.5±13.8	(29) 14.5±5.3	(255)	51.00
Bayvarol	(87) 21.75±9.9	(86) 21.5±12.2	(74) 18.5±9.5	(63) 15.75±8.0	(36) 9±4.4	(346)	69.2
Formic acid	(116) 58±7.8	(87) 43.5±11.0	(44) 11±11.0	(23) 5.75±5.8	(16) 4±4	(286)	57.2
Varroviga (with sugar syrup)	(72) 24±13.5	(72) 24±10.8	(36) 12±6.1	(33) 11±4.1	(45) 15±5.3	(258)	51.6
Varroviga (with sugar power)	(79) 39.5±9.5	(38) 19±2.1	(12) 6±1.4	(11) 5.5±0.4	(13) 6.5±1.1	(153)	30.6
Untreated check (Control)	(44) 14.67±5.53	(40) 20±4.2	(33) 16.5±0.35	(25) 9.5±0.35	(19) 12.5±7.42	(161)	32.2

Conclusion

The results of this study revealed that the total numbers of Varroa mites fallen on sticky boards were 2123, 2627, 1888, 2150, 1743, 1269, 1319, 1208 (Varroa/colony), with an average of 163.30, 202.20, 145.23, 165.38. , 134.07, 97.60, 101.46 and 92.9 (Varroa/colony) for honey bee colonies treated with mint oil, camphor oil, varokiller, bayvarol, formic acid, varoviga (with sugar syrap), varoviga (with sugar powder) and untreated check (control), respectively. It was also found that the number of Varroa mites fallen on sticky boards monthly was 497.10, 640.70, 459.25, 520.20, 421.45, 304.35, 322.10 and 294.00 (Varroa/colony/month) during the season. From the current study, it can be summarized that the treatments of mint oil, camphor oil, and bayvarol were significantly superior to the other treatments in of the total number and average number of Varroa mites fallen on sticky boards, without significant differences between them, followed by the formic acid and varokiller treatments, without significant differences. Among them, while varoviga (with sugar syrap and sugar powder) not a significant effect on Varroa mites compared to the untreated check (control).

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