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Article

Study the effectiveness of essential oils (anise, clove) and Bacillus thuringienesis in controlling Varroa mites (Varroa destructor) on honey bees Apis mellifera.

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Abstract: This study was conducted at the A.L.- Musayyib Technical College / Department of Biological-Control Techniques from 1/10 /2021 to 1/4 /2022. The study aimed to determine the effectiveness of essential oils (anise, clove) and Bacillus thuringienesis in controlling Varroa mites (Varroa destructor) on honey bees Apis mellifera. The results revealed that the efficiency of the bacterial suspension with different concentrations was less effective in the average destruction of the adult Varroa mite in the field compared with the Epistan pesticide and the control treatment in which only distilled water was used and the natural fall of the Varroa mite. The highest mean of Varroa mites killed using the bacterial suspension spray at a concentration of (33 x 106) CFU/ml after 48 hours of treatment was (6.63), which indicates a significant difference with the control treatment, which amounted to (4.54), while the lowest mean of Varroa death was at a concentration of (33). × 104) CFU / ml after 24 hours of treatment, as it reached (4.25). As for the Epistan pesticide treatment, it was used to compare it with the rest of the results of the study, where the pesticide treatments were significantly superior in all periods on the concentrations of the bacterial suspension and the control treatment and the natural fall with a significant difference, the average of death was (34.25, 34.00, 31.46, 27.4, 42.25) after (24, 48, 72, 96, 142) hours, respectively. The results showed that treating bee hives with cotton soaked in essential oil (for cloves, anise) significantly affected the average mortality of Varroa mites. It was shown that clove essential oil was significantly superior to the essential oil of anise with two concentrations (1, 2) ml/cell in the periods (24,48) hours after treatment. Whereas, the average mortality of Varroa at a concentration of (1) ml/cell of clove oil (was 19.12, 23.17), while at a concentration of (2) ml/cell, it reached (27.17, 32.12) after (24, 48) hours, respectively. While the average mortality of Varroa mites was at a concentration of (1) ml/cell of anise oil (17.4, 21.00), while at a concentration (2) ml/cell, it reached (24.96, 28.83) after (24, 48) hours, respectively compared with the control treatment and the natural fall.

Keywords: Bacillus thuringienesis, Pimpinella anisum, Varroa destructor, Apis mellifera.

Introduction

Honey bee insect(Hymenoptera: Apidae) Apis mellifera L.considered as living social insect[1]. The importance of honey bees comes first in pollinating a third of the crops that feed the world[2]. The other important is the production of many honey bee products such as (honey, wax, royal jelly, bee venom, bee gum and pollen), which are considered as food, medical and industrial products[3]. Honey bee breeders are facing significant losses in their colonies in many countries due to the Varroa mite, V. destructor [4] and associated pathogens, which are the most devastating[5]. Varroa mite is considered one of the most dangerous pests and diseases that affect honey bees worldwide, as it leads to the destruction or weakening of many colonies and reduces their production [6]. The use of chemical pesticides in the control of the Varroa mite irregularly and continuously for several years leads to the contamination of the products of honey bee colonies, as it can be found in honey, pollen and beeswax more commonly, and the Varroa mite gains resistance to pesticides[7].This means that honey bee products are considered unsuitable for use, as these residues have recently become a global concern as they enter the human food cycle and cause diseases[8]. Therefore, it is necessary to rely on modern methods and new methods that include relatively cheap and safe materials for humans, honeybee colonies and the environment and biodegradable to control varroa mites, using biological control, which represents the global trend, such as control with plant essential oils (clove, anise) and B.thuringiensis (Bt). Which are considered one of the safest ways to control these pests[9]. Aims of the study: Study the effectiveness of Bacillus thuringienesis in controlling Varroa mites (Varroa destructor) on honey bees Apis mellifera. Study the effectiveness of essential oils (anise, clove)in controlling Varroa mites (Varroa destructor) on honey bees Apis mellifera. Study the effect of essential oils (anise, clove) and Bacillus thuringienesis on honey bees (Apis mellifera).

Materials and methods

This study was conducted in the Musayyib Technical College / Department of Biocontrol Techniques laboratories from 1/10/2021 to 1/ 4/ 2022. The field experiment was applied in the researcher apiary of the non-controlling withVarroa pesticides in the last year from 1/10/2021 to 1/12/2021. All beehives prepared for the experiment were from the Iraqi hybrid bees A. mellifera; experiments were conducted on it with three replications for each concentration in addition to the control treatment, where the study was conducted using (39) beehives, each containing (8) wax cells, all covered with bees and newly inoculated queens (2021). The queens are the same age, and the hive box and frames are of the same type of wood, shape and size. The bases of the hive boxes were provided with a movable chute that contains a wire clip resembling a sieve through which the Varroa parasite can pass without bees. A3 white paper greased with Vaseline® was placed in the trowel [10]. To facilitate collecting the fallen Varroa without opening the cell Bees and ensure that they do not return to the brood and worker bees again. Hives were examined weekly to follow up and calculate the severity of infection in the colony and the apiary. Collecting 100 worker bees from the brood area in a plastic box with a wire cover and then adding sugar powder [11]. And according to equation [12]. Varroa mite samples were diagnosed at the University of Baghdad \ College of Science \ Natural History Research Center and Museum \ Department of Insects and Invertebrates; it was diagnosed as V. destructor

Isolation of B. turgidness from soil and its identification.

Sample collection.

10 soil samples were taken from agricultural lands from Aldewania Governorate after removing the surface layer of the soil to a depth of 3-5 cm and from the different soil texture, taking care when taking samples from agricultural lands and making sure that B. thuringiensis preparations were not used in the land it by inquiring with the land owner. The following information was fixed on it (the name of the area - the nature of the land - the date of taking the samples), and then a code was given to each sample and kept in the refrigerator. Bacteria were cultured and purified in (N.A.) Petri dishes were then incubated upside down for 72 hours at a temperature of 30±2°C, and the growth of B. turgidness colonies was observed [13]. A bacterial suspension has been prepared with a concentration of (Colony-forming- unit) CFU/ml 107 × 33. According to equation [14].

Preparing the bacterial suspension.

Method [13] was used to prepare the bacterial suspension.

Culture media.

Nutrient agar, MR-VP, Ammonium salt sugars, Medium base T3 Agar.

Microscopic diagnosis.

The phenotypic traits were examined based on what was mentioned [15].

Dyes and reagents.

Gram stain, basic fuchsin dye, 0.5. sodium ascent solution

basal fuchsin dye.

The examination was carried out for colonies kept inside the incubator for a period of (3 days - a week) and positive colonies with Gram stain only to observe the presence of spores and crystals by preparing sterile and dry glass slides after they were marked with the number of the colony to be examined and a small drop of water was added in the middle of the slide, and a small part of the colony was taken by Flame sterilized metal lube, It was gently distributed and mixed with a drop of water and passed over a light flame several times to dry, left to dry at room temperature until it dried, then dipped in the basic fuchsin dye for 3-5 minutes, washed with distilled water, left to dry, and then examined with an oil lens 100x.

The method of extracting essential oils from the studied plants.

The reproductive parts of cloves and anise plants (flower buds of Syzygium aromaticum from the family Myrtaceae and seeds of the anise plant Pimpinella anisum from the family Apiaceae) were collected from local markets, and the samples were well-dried. Grind 100 gm of the reproductive parts of cloves and anise plants separately in an electric mill with a speed of 2500 r/min for a short period of time before extracting the essential oil from them. The extraction process was performed in the College of Agriculture - University of Al-Qadisiyah - Laboratory of the Department of Horticulture and Landscaping using a modified steam distillation device, Clevenger type Fine. 100 grams of the powder from which the essential oil is extracted were immersed in 700 ml of distilled water in the 1-liter glass beaker for the device. The extraction process was carried out according to the method [16]. tham It was sealed and kept in the refrigerator at 3°C and aqueous sodium sulfate was added to it to pull out the water droplets. The process of extracting

the essential oil was repeated several times to obtain an adequate amount for the study.

Study the effectiveness of different concentrations of the bacterial suspension B.thuringiensis in controlling the V. destructor in the field.

The study was field-applied to honey bee colonies with an autumn experiment and included the months of (October and November) with one treatment per week. The entire colonies were treated from the inside with wax, wood, open and closed brood and members of the colony, including the queen. Using a 50ml hand sprayer at a distance of 15cm. The concentrations used were ml/ CFU (33 x 102, 33 x 104 and 33 x 106) in addition to the control treatment (sterile distilled water only), where each concentration was repeated on three beehives containing 8 wax, all covered with bees. Then, the readings were taken with the number of Varroa falling on the moving galaxies and their leaves (A3) greased with Vaseline for all treatments and after (24, 48, 72, 96 and 144) hours of applying the treatment.

Study the effectiveness of essential oils soaked with cotton in controlling the V. destructor in the field.

The experiment was carried out by placing cotton soaked with clove and anise essential oils at a concentration of 100% each separately inside the cell on moving galaxies and with three replications for each concentration in addition to the control treatment, i.e., a total of 12 cells for the anise oil treatments and 12 cells for the clove oil treatments. This study was conducted according to the method [17].

Statistical analysis:

After collecting the data and classifying it for the studied traits, it was analyzed statistically by a program (Genstat), which uses Complete Randomized Blocks designed for field trials and a test (L.S.D.). To compare the averages at the probability level(0.05) [18].

Results

Study the effectiveness of different concentrations of the bacterial suspension B. Thuringiensis in the average mortality of V. destructor in the field.

The results in Table (1) showed that the efficiency of the bacterial suspension with different concentrations was less effective in the average controlling of the adult Varroa mite in the field, compared with Epistan pesticide and the control treatment in which only distilled water was used and the natural fall of the Varroa mite. The highest mean of Varroa mites killed using the bacterial suspension spray at a concentration of (33 x 106) CFU/ml after 48 hours of treatment was 6.63, which indicates a significant difference with the control treatment, which amounted to 4.54, while the lowest mean of Varroa death was at a concentration of (33). × 104) CFU / ml after 24 hours of treatment, as it reached (4.25). As for the Epistan pesticide treatment, it was used to compare it with the rest of the results of the study, where the pesticide treatments were significantly superior in all periods on the concentrations of the bacterial suspension, the control treatment and the natural fall with a significant difference, the average of death was (34.25, 34.00, 31.46, 27.4, 42.25) after (24, 48, 72, 96, 142) hours respectively. Epistan was used to compare it with the results obtained when applying the bacterial suspension B.thuringiensis.

Bacterial suspension treatment	concentration CFU/ml	Reading rate after 24 hours	Reading rate after 48 hours	Reading rate after 72 hours	Reading rate after 96 hours	Reading rate after 144 hours
distilled water control	0	4.25 c	4.54 c	4.33 c	4.37 d	7.17bc
Bacterial suspension	33× 102	5.33 b	6.25 b	6.33 b	5.71 b	7.83 b
Bacterial suspension	33× 104	4.92 b	6.04 b	6.29 b	5.46 c	7.96 b
Bacterial suspension	33× 106	5.83 b	6.63 b	6.38 b	6.46 b	8.42 b
Comparison of natural shedding	without treatment	4.33 c	4.54 c	3.92 c	4.04 d	5.83 c
Comparison pesticides	2 bars	34.25 a	43.00 a	31.46 a	27.04 a	42.25 a
L.s.d	0.05	1.44	1.25	1.38	0,78	1.44

[1] The averages with different letters within the same column have significant differences in comparison with L.S.D. at the level of significance 0.05

Table 1. compares the effectiveness of different concentrations of the bacterial suspension B. thuringiensis in the average mortality of V. destructor with the Epistan pesticide and natural fall in the field.

Study the effectiveness of essential oils soaked with cotton at a concentration of 100% (clove, anise) in the average mortality of V. destructor in the field.

The results in Table (2) showed that the treatment of bee hives by the method of cotton soaked in essential oil (for cloves, anise) had a significant effect on the average mortality of Varroa mites. It was shown that clove essential oil was significantly superior to the essential oil of anise with two concentrations (1, 2) ml/cell in the periods (24,48) hours after treatment. Whereas the average mortality of Varroa at a concentration of (1) ml/cell of clove oil (was 19.12, 23.17), while at a concentration of (2) ml/cell, it reached (27.17, 32.12) after (24, 48) hours, respectively. At the same time, the average mortality of Varroa mites was at a concentration of (1) ml/cell of anise oil (17.4, 21.00), while at a concentration of (2) ml/cell, it reached (24.96, 28.83) after (24, 48) hours, respectively.

Treatment (T)	Concentration CFU/ml	Reading rate after 24 hours	Reading rate after 48 hours	Reading rate after 72 hours	Reading rate after 96 hours	Reading rate after 144 hours
Control only cotton	0	3.92 g	3.92 g	3.54 d	3.92 f	5.96 e
Oil carnation	0.5	9.62 f	13.96 f	6.12 c	4.33 f	6.00 e
anise oil		9.83 f	13.25 f	5.37 c	3.75 f	5.79 e
Oil carnation	1	19.12 d	23.17 e	23.71 b	13.54 d	7.75 d
anise oil		17.04 e	21.oo d	23.46 b	11.38 e	7.79 d
Oil carnation	2	27.17 b	32.12 b	32.62 a	20.79 b	10.33 c
anise oil		24.96 c	28.83 c	31.58a	16.62 c	11.67 b
Comparison of natural shedding	without treatment	4.33 g	4.54 g	3.92 d	4.04 f	5.83 e

Comparison pesticides	2 Bars	34.25 a	34.00 a	31.46 a	27.04 a	42.25 a
L.s.d	0.05	1.08	1.09	1.18	1.42	1.31

[1] The averages with different letters within the same column have significant differences in comparison with L.S.D. at the level of significance 0.05

Table 2. shows the effectiveness of essential oils soaked with cotton at a concentration of 100% (cloves, anise) in the average mortality of V. destructor and compared with the Epistan pesticide and natural fall in the field.

Discussion

The results of Table (1) in this field study did not agree with the laboratory results obtained by [23], which states that three isolates belonging to the type of bacteria (Bt) are (EA 11.3, EA3, EA 26.1), which killed the percentage of (96.7%, 96.3%, 93.3%) respectively after 48 hours of treatment. The reason for the inefficiency of bacteria B. thuringiensis in the average field mortality of Varroa mites may be attributed to the difference in conditions between the laboratory and field or to the nature of the Varroa mite food on fatty bodies or the hemolymph of honey bees that is not contaminated with toxic protein crystals produced by Bt bacteria, in addition to That is, honey bee workers spread the propolis substance throughout the hive from the inside, as well as spread it by clicking on the bodies of adult honeybees and larvae, which is an anti-bacterial and other microorganism. The results in Table (1) agree with what was mentioned [19] that no field studies allow bacteria B. thuringiensis to be considered a critical method against Varroa mites. Epistan pesticide was used to compare it with the rest of the study results, where the pesticide treatments were significantly superior in all periods on the concentrations of the bacterial suspension. The reason for this may be due to the fact that the Epistle tapes contain the active substance Fluvalinate at a rate of 10%, which is a pyrethroid insecticide that kills the dream and works through contact with the digestive system. In Table (2), The mechanism of action of Eugenol is considered a narcotic substance that causes permanent or temporary nervous paralysis, which leads to its falling from the bodies of bees and collecting at the bottom of the hive equipped with a hive containing wire mesh and petroleum jelly, or the time exposure to the smell of cloves may cause respiratory toxins to dream and cause them to fall off. As for the anise oil, it may be attributed to the fact that the reason for its effect in the destruction of varroa mites is the presence of the active compounds present in it, especially the compound anthol, which constitutes 80% of the other effective compounds, which is a volatile substance and prevents feeding to insects and mites. As confirmed by [20]. This study agrees with what was mentioned [21], which indicated that some essential oils, including clove oil, had the same properties as acaricides. This study also agreed with [17], who showed in his laboratory study using essential oils (menthol, clove, origanum), where cloves gave a 96% percentage in the killing of Varroa mites. This study agrees with what was reached by [22] in his field study, which stated that using anise essential oil soaked with cotton at a concentration of 100% gave a death rate of 41.6%.

Conclusion

We conclude from the study that using Bacillus thuringin suspension concentrations had little effect in controlling Varroa mites and was harmful to adult bees and larvae.

We conclude from the study that using essential oils (anise, clove)With different concentrations soaked in a piece of cotton greatly impacted the fight against Varroa mites, as clove oil outperformed aniseed oil on the first and second day. Their effect was equal with pesticide on the third day after the labs in controlling Varroa mites. As for the effect of oils on adult honeybees, it was very little.

References

1. Humagain, S. Life cycle of honey bee and uses of honey. September 30, 2018. (2019). Identification and measurement of veterinary drug residues in beehive 2019.48(4): 263–269.
2. Li, J.; Qin, H.; Wu, J.; Sadd, B. M.; Wang, X.; Evans, J. D.; Chen, Y. The prevalence of parasites and pathogens in Asian honeybees Apis cerana in China. Plos one, 2012, 7.11: e47955.
3. Rossi, M.; Marrazzo, P. The potential of honeybee products for biomaterial applications. Biomimetics, 2021, 6.1: 6.
4. Anderson, D. L.; Trueman, J. W. H. Varroa jacobsoni (Acari: Varroidae) is more than one species. Experimental & applied acarology, 2000, 24.3: 165-189.
5. ielmanowicz, M. G.; Inberg, A.; Lerner, I. M.; Golani, Y.; Brown, N.; Turner, C. L.; Hayes, G. J. R.; Ballam, J. M.. 2015. A prospective large-scale field study generates a predictive model identifying major contributors to colony losses. PLoS Pathogens, 2015, 11.4: e1004816.
6. Eliash, N.; Mikheyev, A. Varroa mite evolution: a neglected aspect of worldwide bee collapses?. Current Opinion in Insect Science, 2020, 39: 21-26.
7. Jamal, M; Aziz, M.A.; Naeem, M.; Iqbal, Z.;Khalid, A; Siddique, F.; Khan, K.A.; Ghramh, H.A. Detection of flumethrin acaricide residues from honey and beeswax using high-performance liquid chromatography (HPLC) technique. Journal of King Saud University-Science, 2020, 32.3: 2229-2235.
8. Lozano, A., Hernando, M. D., Uclés, S., Hakme, E., and Fern.ndez-Alba, A. R.Nat. Prod .2011.. 6: 206–210.
9. Adang, M. J.; Crickmore, N.; Jurat-Fuentes, J. L.. Diversity of Bacillus thuringiensis crystal toxins and mechanism of action. In: Advances in insect physiology. Academic Press, 2014. p. 39-87.
10. Delaplane, K. S. ; Berry J. A.; Skinner J. A.; Parkman J. P.; Hood W. M. Integrated pest management against Varroa destructor reduces colony mite levels and delays treatment threshold. Journal of Apicultural Research, 2005, 44.4: 157-162
11. Flores, J. M.; Gil, S.; Padilla, F. Reliability of the main field diagnostic methods of Varroa in honey bee colon. Archivos de zootecnia, 2015, 64.246: 161-165
12. Allen M. The incidence and world distribution of honey bee viruses, Bee World , 2004 . 77:141– 162.
13. Stahly, D.P.; Andrews R.E.; yousten, A.A.The genus Bacillus-insect pathogens . In:Balows A.truper HG,D workin M,Harderw. Qschleifer K.H ed . The prokaryotes , 2nd ed .New York, Basel , Springer – ver lag 1991, vol II, P.P..1697 – 1735.
14. Clark, F.E. Agar-plats method for total microbial C.F.Black, methods of soil analysis part 2 publisher madeson ,Wisconsin .1965. U.S.A. 1572p.
15. Thiery, I.; Frachon, E. Identification, isolation, culture and preservation of entomopathogenic bacteria. In: Manual of techniques in insect pathology. Academic Press, 1997. p. 55-IX
16. Tongnuanchan, P.; Benjakul, S. Essential oils: extraction, bioactivities, and their uses for food preservation. Journal of Food Science, 2014, 79.7: R1231-R1249
17. Noaman A I, Khalaf R M, Emad GH, Al-Abbasy, Mohammed Th. T. Effect of flaxseed oil dosing on fertility, growth characteristics and some physical, biochemical, and hormonal blood parameters during the early pregnancy of Awassi ewes. Revis Bionatura. 2022;7(4) 5. http://dx.doi.org/10.21931/RB/2022.07.04.5.
18. Steel, R. G. D.; Torrie, J. H. Principles and procedures of statistics, a biometrical approach. McGraw-Hill Kogakusha, Ltd., 1980.
19. Jouzani, G.S.; Valijanian, E.; Sharafi, R. Bacillus thuringiensis: a successful insecticide with new environmental features and tidings. Applied microbiology and biotechnology, 2017, 101.7: 2691-2711.
20. Suwaid, A. H. .; Rashid, M. A. .; Taha, M. M. . GENETIC ANALYSIS FOR COMBINING ABILITY AND ESTIMATION OF SOME Genetic Parameters Of Yield And Its Components In Maize Using Half Diallel Cross. JLSAR 2020, 1, 60-64.
21. Goswami, V.; Khan, M. S. Management of varroa mite, Varroa destructor by essential oil and formic acid in Apis mellifera Linn. colonies. J. Nat. Prod, 2013, 6: 206-210
22. Abd El-Wahab, T. E.; Ebadah, I. M. A.; Zidan, E. W. Control of Varroa mite by essential oils and formic acid with their effects on grooming behavior of honey bee colonies. J. Basic. Appl. Sci. Res, 2012, 2.8: 7674-7680

23. Alquisira-Ramírez, E. V.; Paredes-Gonzalez, J. R.; Hernández-Velázquez, V. M.; Ramírez-Trujillo, J. A.; Peña-Chora, G. In vitro susceptibility of Varroa destructor and Apis mellifera to native strains of Bacillus thuringiensis. Apidologie, 2014, 45.6: 707-718

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