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RESEARCH PAPER

The Use of Some Herbal Essential Oils Against *Galleria mellonella* Larvae and Testing of *Bacillus thuringiensis* Bacterium Isolated from *Galleria mellonella* Under Laboratory Conditions

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Abstract

Larvae of wax moths cause great damage in honey bee hives and especially in stored honeycombs. Biological control methods are especially important in the control of wax moth in warehouses, as they do not harm the bee, the product and the environment. This study was carried out to determine the effect of 5%, 10%, 25%, 45% and 55% of peppermint, thyme, nettle seed, and walnut herbal oils and GB1 *Bacillus* sp. (OR227363) against wax moth larvae (*Galleria mellonella*) (L1-L3) under laboratory conditions. For each group of fifteen larvae, four herbal oil and one bacterial trials were conducted and two control groups were formed. The trials were conducted in glass jars and the larvae were kept in an oven at 25°C temperature/75% relative humidity. Each jar was checked every day for two weeks and dead/viable larvae were recorded and the dead ones were removed from the jar. As a result of the dose trials, it was determined that the best dose was 2.835×10^9 cfu/mL for bacteria and 5% concentration of thyme and walnut oil for herbal oil. According to the data obtained, it is thought that GB1 *Bacillus* sp. isolate can be used as an alternative control method against wax moth larvae.

Introduction

Inadequate management of diseases and pests in honey bee rearing can lead to significant economic losses. The use of environment-friendly and bee-friendly medications against hazardous organisms in the beekeeping sector is on the rise globally. Chemical medications are solely utilized in beekeeping to combat the parasite *Varroa destructor* (Anderson & Trueman, 2000), and no other agents are treated with chemicals or antibiotics (Ertürk & Yılmaz, 2013; Aydın, 2021). Wax moth (*Galleria mellonella*) larvae, also known as honeycomb worms, wax worms. Wax moths, may cause significant damage to hives and stored combs. While the adult or pupa stage does not do any damage to the honeycombs, the larvae inflict significant economic losses by destroying honeycombs housed in dark, hot, and poorly ventilated conditions, as well as hives with weak colonies (Kwadha et al., 2017). In Türkiye, two species are recognized as giant [*Galleria mellonella* (Linnaeus, 1758)] and little wax moth [*Achroia grisella* (Fabricius, 1794)]. The two species can infest hives/combs simultaneously, and their biology is identical (Uygur & Girişgin, 2008; Girişgin, 2021).

Wax moth management in warehouses is classified into four types: technological, physical, biological, and chemical. Biological control methods that do not affect the honeycomb (indirectly, bees and humans) or the environment are gaining popularity. Today, two active compounds are commercially employed in biological control: 1. *Bacillus thuringiensis* (Berliner, 1915) and 2. *Metarhizium anisopliae* (Sorokin, 1879), (Ertürk & Yılmaz, 2013; Girişgin, 2021).

The usage of herbal essential oils against *Galleria mellonella* larvae, as well as the laboratory testing of *Bacillus thuringiensis* bacteria isolated from *Galleria mellonella*, have received attention due to their prospective uses in immune response and pathogenesis research. *Galleria mellonella*, as known as the larger wax moth, has become an important model for researching immunological responses to human infections (Pereira et al., 2018). This model enables for the study of cellular and humoral responses, such as hemocyte activity and the production of antimicrobial peptides, which are critical in understanding the immune response to many human pathogenic bacteria (Pereira et al., 2018).

Furthermore, the testing of botanical extracts and essential oils against *Galleria mellonella* larvae has shed light on their effectiveness in controlling stored goods pests such as the larger wax moth (Paulraj et al., 2021).

Furthermore, *Galleria mellonella* has been extensively studied as a model host for fungal and bacterial disease (Fuchs et al., 2010). Studies have shown a link between the virulence of human infections in *Galleria mellonella* and mammalian infection models, underlining the model's potential for pathogenesis research (Ignasiak & Maxwell, 2017; Viegas et al., 2013). Furthermore, the function of *Galleria mellonella* in determining the virulence of other pathogens, such as *Salmonella enterica* and *Pseudomonas aeruginosa*, has been studied, offering vital insights into these organisms' pathogenic processes (Bismuth et al., 2021; Sciuto et al., 2018).

In addition to pathogenesis studies, *Galleria mellonella* has been used to assess the antibacterial and antivirulence properties of essential oils, such as *Eugenia brejoensis* essential oil, against microbial pathogens (Bezerra Filho et al., 2020). This illustrates *Galleria mellonella*'s usefulness as a model for investigating the efficiency of natural substances in control microbial diseases. Furthermore, *Galleria mellonella*'s ability to assess the virulence of fungal pathogens such as *Candida species* and *Paracoccidioides* has been established, highlighting its importance in the research of fungal infections (Jacobsen, 2014; Scorzoni et al., 2015).

The laboratory settings used to investigate *Galleria mellonella* were critical in providing a controlled environment for performing infectivity trials, toxicity testing, and determining the virulence of different diseases (Ignasiak & Maxwell, 2017). These laboratory circumstances have made *Galleria mellonella* a reliable insect model host for studying the pathophysiology of a variety of human infections (Viegas et al., 2013). Furthermore, the use of *Galleria mellonella* to examine the involvement of reactive oxygen species in *Salmonella enterica* resistance demonstrates its use in researching host-pathogen interactions under controlled settings (Bismuth et al., 2021).

Using *Galleria mellonella* to research immunological responses, pathogenesis, and the efficiency of natural substances against microbial pathogens in the laboratory has offered useful insights into host-pathogen interactions. *Galleria mellonella*'s adaptability and dependability as a model host make it an invaluable tool for furthering our understanding of immune responses and pathogenic pathways, as well as assessing new antimicrobial medicines.

Considering all the information in the literature and the harm caused by *Galleria mellonella*, the purpose of this study was to examine the effects of essential oils and *Bacillus thuringiensis* bacterium derived from this pest against it. Also it is aimed to obtain an effective biological material in the control of *Galleria mellonella* larvae.

Material and Methods

Purchasing and Preparing Larvae for Experiment

Galleria mellonella larvae were obtained from Artvin Çoruh University Beekeeping Research and Application Centre and cultured in the laboratory. Larvae were also identified at Artvin Çoruh University Beekeeping Research and Application Centre. Small (L1-L3) larvae identified as *Galleria mellonella* were selected and separated for utilization. The wax were sliced into 6×6 cm pieces and put in glass jars (7 cm diameter, 13 cm height). Each wax jar had five larvae from each larval group, for a total of 15 larvae. Also control group had 15 larvae.

Preparation of Herbal Essential Oils

The peppermint, thyme, nettle seed, and walnut herbal oils utilized in the study were commercially available, and 5% solutions of each herbal oil were produced using acetone as a diluent. Finally five diluents were used (5%, 10%, 25%, 45% and 55%). Each solution was poured in a clean spray bottle and applied to the wax samples with an average of 1 mL to cover the whole surface.

Preparation of Bacterial Sample

The previously isolated bacterium GB1 *Bacillus* sp. (OR227363) were cultured in nutrition broth medium (NB) for 18 hours (72 hours for sporulation) at 30°C. After incubation, bacterial cells were centrifuged for 10 minutes at 3000 rpm (Ben-Dov et al., 1995). By adding sterile PBS, the pellet was resuspended. The cells' optical density was set to 1.89 at OD (optical density) 600 (Moar et al., 1995), and totally five concentrations were applied (0.945×10^9 cfu/mL, 1.8×10^9 cfu/mL, 2.835×10^9 cfu/mL, 3.78×10^9 cfu/mL and 5.67×10^9 cfu/mL). Bacterial culture concentration-response experiments against wax moth.

Laboratory Trials

After the bacterial sample and herbal essential oil solutions were dried, the larvae of each group were placed in jars and the jars were kept in an oven at 25°C temperature/75% relative humidity. Since the life cycle of moths can vary between 1-9 weeks, each jar was checked every day for two weeks, dead larvae were collected and removed from the jar. Twelve jars, one group of experimental and one group of control jars containing larvae of three larval stages, were used and three replications were made (total 8 experiments + 4 controls). Mortality rates were determined according to larval stages and days.

Statistical analyses

Mortality data were corrected by Abbott's formula (Abbott, 1925). Lethal concentrations (LC50) for the bacterial isolate and different concentration of herbal oils against to third stage wax moth larvae of hosts were calculated by probit analysis using MS Excel (Finney, 1952).

Results

In general, the larvae that died in the experimental groups generally died within the first one week. The average larval mortality rates were 40%, 52.6%, 47.6% and 52.6% in the mint, thyme, nettle seed and walnut groups, respectively, while it was 87.3% in the bacteria group. In the control groups, larval mortality rate was 0% in both groups (Fig 1.).

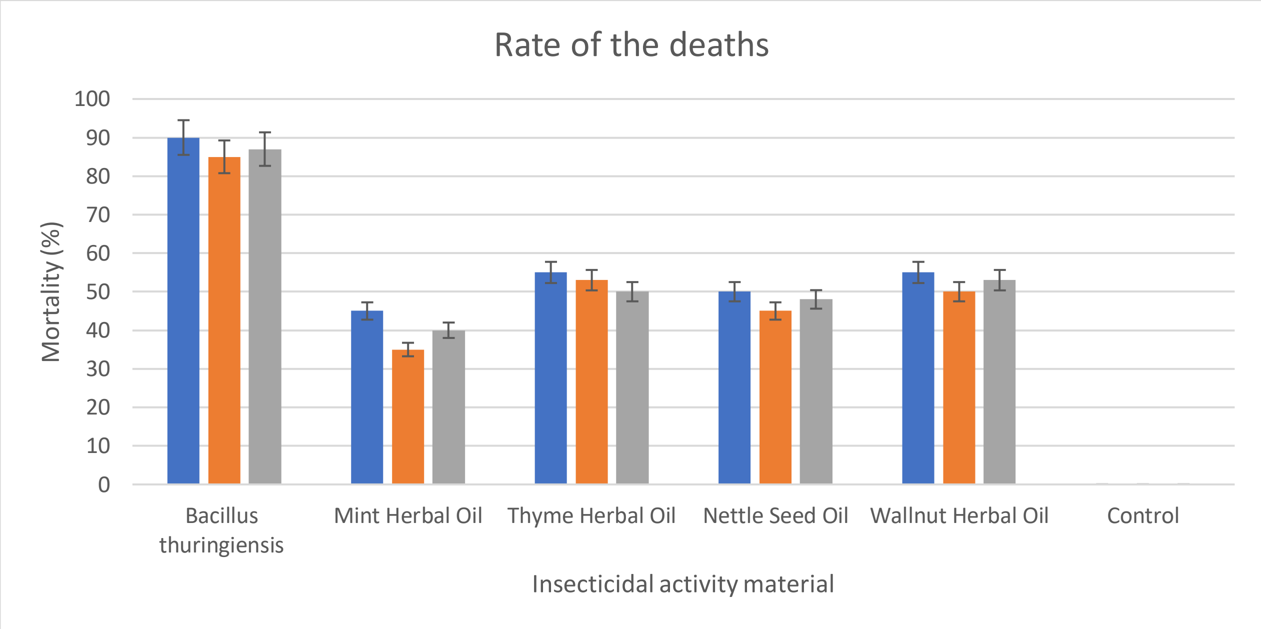

Figure 1. Insecticidal activity of *Bacillus thuringiensis* (OR227363) and commercially herbal oils. (The colors in the graph show the repetitions in the application. The blue color shows the ratios in the first iteration, the orange color the ratios in the 2nd iterations, and the gray color the ratios in the 3rd iterations.)

The mortalities of all doses of insects infected with herbal oils are shown in Figure 2. The highest mortality rates were 55, 58, 60 and 65% for mint, thyme, nettle seed and walnut oil treatments at 5% concentration, respectively. Also mortality of all doses of insects infected with *B. thuringiensis* is shown in Figure 3. The highest mortality with bacterium treatment was 90% for *G. mellonella* at the 2.835x10^9 cfu/mL.

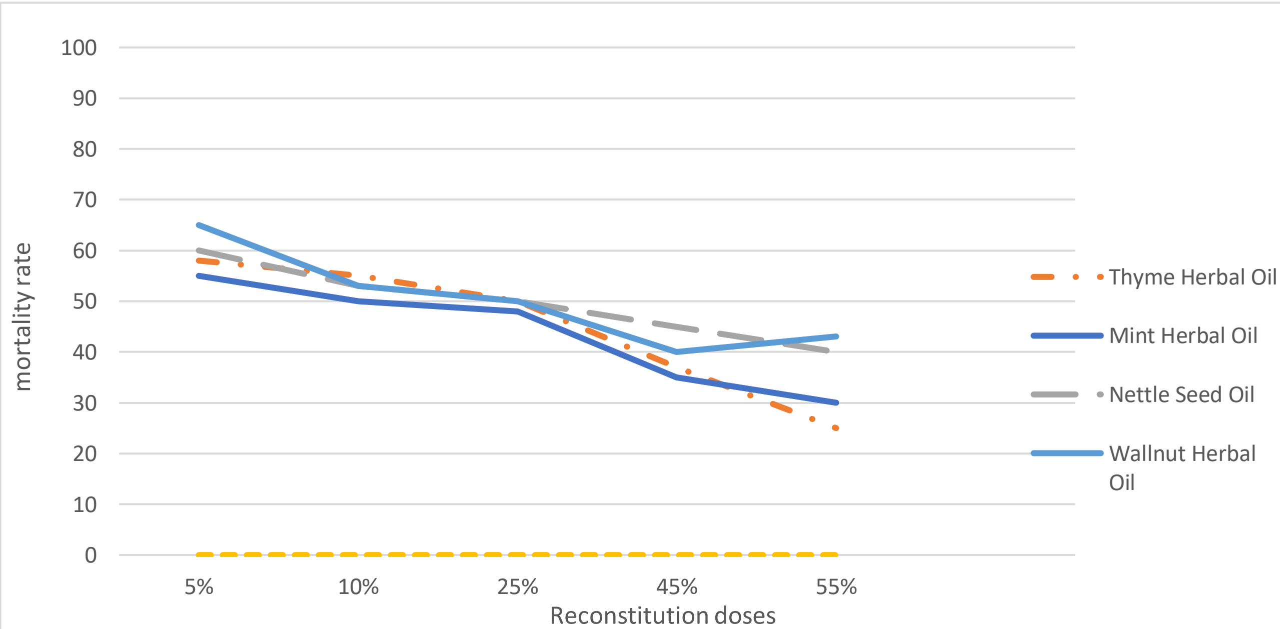

Figure 2. Mortality rate of insect larvae resulting from herbal oils reconstitution doses. (The colors in the graph show the herbal oils. The light blue color shows the mint herbal oil, the dark blue color shows the wallnut herbal oil, orange color shows the thyme herbal oil, grey color shows the nettle seed oil and the yellow color shows the control)

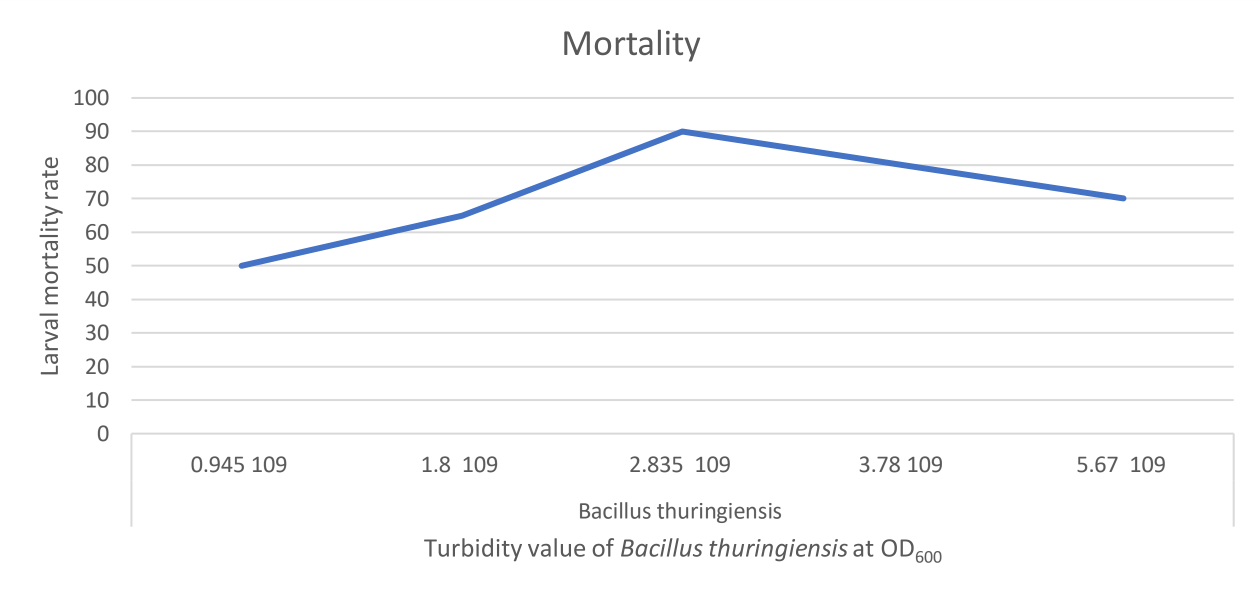

Figure 3. Mortality of insect larvae resulting from *Bacillus thuringiensis.* X axis shows concentrations of *Bacillus thuringiensis* (0.945×10^9 cfu/mL, 1.8×10^9 cfu/mL, 2.835×10^9 cfu/mL, 3.78×10^9 cfu/mL and 5.67×10^9 cfu/mL). Y axis shows the rates of mortality.

The LC50 values calculated in the experiments are presented in Table 1. The LC50 calculated by probit analysis were lower at 1011.7 % concentration for nettle seed oil (F= 1.104, df= 1), 1014.6% concentration for mint herbal oil (F=1.646, df=1) and the highest mortality 3854.5% concentrations for thyme herbal oil and wallnut herbal oil (F=2.656, df=1) for *G. mellonella* and 4164.8 OD for *G. mellonella* for bacterial isolate. Based on mortality rates and statistical analysis, the benefits of thyme herbal oil and wallnut herbal oil appear to be equivalent. A more in-depth investigation should be conducted to see whether there is a difference between them.

Table 1. Median lethal concentration (LC50) of bacterial isolate and herbal oils.

Isolates	LC 50 %concentration (OD for bacterial isolate)	Df (degree of freedom)	X^2 (x squared)	SS (Sum of square)	F (Varyans analysis F test)	Slope±SE
Bacillus thuringiensis	4164.8	1	0.97	1.399	6.016	0.415±0.303
Mint Herbal Oil	1014.6	1	0.53	0.467	1.646	0.312±0.547
Thyme Herbal Oil	3854.5	1	0.85	0.583	2.656	0.413±0.473
Nettle Seed Oil	1011.7	1	0.36	0.323	1.104	0.213±0.532
Wallnut Herbal Oil	3854.5	1	0.85	0.583	2.656	0.413±0.473

Discussions

The use of products obtained from biological and natural products in honey bee diseases has gained importance in recent years in terms of human/bee health and food/environmental safety. In this direction, natural protection methods against wax moths, one of the bee pests, are being tried to be found. Until now, methods such as spraying a solution containing bacteria (Boşgelmez et al., 1983), carbon dioxide gas application (Akyol et al., 2009), cold application (Akyol & Korkmaz, 2008) have been carried out and successful results have been obtained.

In this study, the results showed that *Bacillus thuringiensis* show high mortality rate (Fig 1 and Fig 3). The highest mortality with bacterium treatment was 90% for *G. mellonella* at the 2.835x10^9 cfu/mL. The utilization of *Bacillus* bacteria on *Galleria mellonella* pests has been extensively studied in various research articles. *Galleria mellonella* larvae have been used as a model to evaluate the virulence of different bacterial strains, including *Bacillus thuringiensis* and *Bacillus cereus*, and a correlation with the virulence of these microbes in mice has been established (Kavanagh & Reeves, 2004). Additionally, the opportunistic properties of acrystalliferous *B. thuringiensis* and *B. cereus* strains were investigated in *G. mellonella*, demonstrating the potential of these bacteria in both insect and mammalian hosts (Salamitou et al., 2000). Furthermore, *G. mellonella* has been recognized as a suitable model for biochemical research, making it an ideal candidate for studying the immunity of insects and host-pathogen interactions (Wojda, 2016). Studies have also focused on the effect of *Bacillus thuringiensis* on the biological aspects of *G. mellonella*, indicating the potential of this bacterium in pest control (Al-Mashhadani & Al-Joboory, 2022). Moreover, the isolation, characterization, and identification of entomopathogenic bacterial strains of the genus *Bacillus* from *G. mellonella* larvae have been conducted, with a preliminary study of the use of these entomopathogenic bacteria on the larvae under controlled conditions (Farida et al., 2017). These findings highlight the potential of *Bacillus* bacteria in controlling *G. mellonella* pests. Furthermore, the involvement of *Bacillus thuringiensis* in the infectious cycle of *G. mellonella* has been investigated, demonstrating the ability of these bacteria to control their insect host, survive in its cadaver, and form spores by sequentially activating virulence, necrotrophism, and sporulation genes (Rejeb et al., 2017). Additionally, the identification of new *Alcaligenes faecalis* strains and their toxicity and pathogenicity to insects, including *G. mellonella* larvae, further emphasizes the potential of various bacterial species in controlling insect pests (Quiroz-Castañeda et al., 2015). In conclusion, the application of *Bacillus* bacteria, particularly *Bacillus thuringiensis* and *Bacillus cereus*, has shown promising potential in controlling *Galleria mellonella* pests. These bacteria have been demonstrated to exhibit pathogenicity towards *G. mellonella* larvae, making them viable candidates for biocontrol agents. The studies conducted on the interaction between *Bacillus* bacteria and *G. mellonella* provide valuable insights into the potential use of these bacteria for pest management.

G. mellonella larvae have been used to assess the effect of bio-pesticides and plant extracts on larval mortality, indicating their potential for studying the insecticidal activity of herbal oils (Balpande & Yadav, 2021; Omer et al., 2023). Various experiments were carried out with essential oils of different plants and different results were obtained. Mahmoud and Abdel-Rahman (Mahmoud & Abdel-Rahman, 2021) tested clove, garlic and rosemary oils at 1.5% and 3% ratios on 4th instar of wax moth larvae and found that the average efficacy of the oils against larvae after one week was 68.3%, 51.6% and 38.6%, respectively. Said et al. (2019) tested five different essential oils at four different ratios on 3rd instar of wax moth larvae and made measurements at 24 and 48 hours after the experiments. Contrary to the previous researchers, the highest effect of 100% was found in 20% rosemary essential oil. In the other essential oils, lavender, eucalyptus, clove and peppermint, the effect increased as the oil ratio increased (72-92%). The average larval mortality rates were 40%, 52.6%, 47.6% and 52.6% in the mint, thyme, nettle seed and walnut groups, respectively. In the control groups, larval mortality rate was 0% in both groups (Fig 1.).

In this study, the mortalities of all doses of insects infected with herbal oils are shown in Figure 2. The highest mortality rates were 55, 58, 60 and 65% for mint, thyme, nettle seed and walnut oil treatments at 5% concentration, respectively.

The effect of bacteria and herbal oil used in the current study on bees has not been determined. These trials will be carried out as part of the following research. According to the literature, Telles et al. (2020) used neem and eucalyptus oils, as well as tobacco and malagueta pepper extracts, to treat both larvae and adult bees. They discovered that all treatments were effective against moth larvae, but neem and eucalyptus oils were hazardous to adult bees. According to a research done by Girişgin et al. (2022) on the application of various herbal oils and fungal samples, the fungal sample was found to be more successful and had a great potential for usage in storage facilities. It was determined not by directly administering the items to the bees, but by providing the bees with product-applied honeycombs and noting their preferences for climbing / knitting honeycombs (Girişgin et al., 2022). The bacterial sample and herbal oil employed in this study produced good results in the management of wax moths (Lepidoptera: Pyralidae), one of the most common pests of honey bees and honeycombs in storage. Further research into product standardization and use on honeycombs is expected to yield a viable and beneficial strategy for controlling honeycomb moth with natural products.

Overall, the observed larval mortality rates in the study reflect the potential of plant extracts and bacterial interventions in controlling insect populations. These findings contribute to the growing body of research on natural and sustainable methods for pest management, highlighting the importance of exploring alternative strategies to reduce reliance on synthetic pesticides and mitigate environmental impacts.

Ethical Statement

Since the study concerns invertebrates does not require any ethics committee authorisation. (Article 4, Paragraph 1-d of the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees published in the Official Gazette dated 15/2/2014 and numbered 28914 based on Article 14 of the Higher Education Law No. 2547.)

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Conflict of Interest

The author/s declare that they have no potential conflict of interest in relation to the study in this paper.

Author Contributions

The author/s contributed equally.

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References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18 (2), 265-267.

Akyol, E., & Korkmaz, A. (2008). The Effect of –5°C Cold Application to Protect Honeycombs against Greater Wax-Moth *Galleria mellonella* Damage. *Uludağ Bee Journal*, 8, 26-9.

Akyol, E., Yeninar, H., Şahinler, N., & Ceylan, D. A. (2009). The Using of Carbon dioxide (CO_2) on Controlling of The Greater Wax Moth's *Galleria mellonella* L. (Lepidoptera: Pyralidae) Damages. *Uludağ Bee Journal*, 9, 26-31.

Al-Mashhadani, M., & Al-Joboory, R. (2022). Effect of *Bacillus thuringiensis* on the biological aspects of the great waxworm *Galleria mellonella*. *International Journal of Health Sciences*, 14886-14893.

Anderson, D. L., & Trueman, J. W. H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental & Applied Acarology*, 24, 165-189.

Aydın, L. (2021). Varroosis. In: A. Doğanay, & L. Aydın (Eds.), *Bal Arısı Yetiştiriciliği, Ürünleri, Sağlığı* (pp 250-264). Dora Basımevi.

Balpande, S., & Yadav, A. (2021). Effect of bio-pesticides on mortality of greater wax moth *Galleria mellonella* L. *Current Journal of Applied Science and Technology*, 39-47.

Ben-Dov, E., Boussiba, S., & Zaritsky, A. (1995). Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from *Bacillus thuringiensis* subsp. *israelensis*. *Journal of Bacteriology*, 177(10), 2851-2857.

Bezerra Filho, C. M., da Silva, L. C. N., da Silva, M. V., Løbner-Olesen, A., Struve, C., Krogfelt, K. A., & Vilela Oliva, M. L. (2020). Antimicrobial and antivirulence action of Eugenia brejoensis essential oil in vitro and in vivo ınvertebrate models. *Frontiers in Microbiology,* 11, 506073.

Bismuth, H., Brasseur, G., Ezraty, B., & Aussel, L. (2021). Bacterial genetic approach to the study of reactive oxygen species production in *Galleria mellonella* during *Salmonella* infection. *Frontiers in Cellular and Infection Microbiology*, 11.

Boşgelmez, A., Cakmakçi, L., Gürkan, B., Gürkan, F., & Cetinkaya, G. (1983). The effects of *Bacillus thuringiensis* on the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Galleriidae). *Mikrobiyoloji Bülteni*, 17, 233-42.

Ertürk, Y. E., & Yılmaz, O. (2013). Organic beekeeping in Turkey. *COMU Journal of Agriculture Faculty*, 1, 35-42.

Farida, B., Oulebsir-Mohandkaci, H., Belaid, M., Irnatene, H., & Mammeri, S. (2017). Isolation of entomopathogenic bacteria from larvae of a Lepidopteran specie; *Galleria mellonella* and study of their insecticidal effect. *The Journal Agriculture and Forestry*, 63(4).

Finney, D. J. (1952). *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve.* Cambridge University Press.

Fuchs, B., O'Brien, E., Khoury, J., & Mylonakis, E. (2010). Methods for using *Galleria mellonella* as a model host to study fungal pathogenesis. *Virulence*, 1(6), 475-482.

Girişgin, A. O. (2021). Petek güveleri (Büyük petek güvesi). In: A. Doğanay, & L. Aydın (Eds.), Bal Arısı Yetiştiriciliği, Ürünleri, Sağlığı (pp 267-273). Dora Yayıncılık.

Girişgin, A. O., Çimenlikaya, N., Aydın, L., & Zengin, S. A. (2022). Experimentation of Essential Oils and Entomopathogenic Fungi Against Wax Moth Larvae in Laboratory Conditions. *Turkiye Parazitol Dergisi*, 46(4), 322-326.

Ignasiak, K., & Maxwell, A. (2017). *Galleria mellonella* (Greater wax moth) larvae as a model for antibiotic susceptibility testing and acute toxicity trials. *BMC Research Notes*, 10(1).

Jacobsen, I. (2014). *Galleria mellonella* as a model host to study virulence of *Candida*. *Virulence*, 5(2), 237-239.

Kavanagh, K., & Reeves, E. (2004). Exploiting the potential of insects for in vivo pathogenicity testing of microbial pathogens. *Fems Microbiology Reviews*, 28(1), 101-112.

Kwadha, C. A., Ongamo, G. O., Ndegwa, P. N., Raina, S. K., & Fombong, A.T. (2017). The biology and control of the greater wax moth, *Galleria mellonella*. *Insects,* 8, 61.

Mahmoud, M. A., & Abdel-Rahman, Y. A. (2021). Influence of some honey bee products and plant oils on the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Egyptian Academic Journal Biological Science (A. Entomology),* 14, 9-20.

Moar, W.J., Pusztzai-Carey, M., & Mack, T.P. (1995). Toxity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* againts lesser cornstalk borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, 88, 606-609.

Omer, S., Faraj, N., & Faraj, N. (2023). Insecticidal activity of some plant extract against greater wax moth larvae (*Galleria mellonella* L.). *Agricultural Science*, 6(2), 78-88.

Paulraj, S., Govindasamy, U., & Pernamallur, S. (2021). Büyük mum güvesi, *Galleria mellonella Linnaeus*'un (Lepidoptera: Pyralidae) depolanmış durumda yönetimi için botanik özütlerin değerlendirilmesi. *Uludağ Arıcılık Dergisi*, 21(2), 227-236.

Pereira, T., Barros, P., Fugisaki, L., Rossoni, R., Ribeiro, F., Menezes, R., & Scorzoni, L. (2018). Recent advances in the use of *Galleria mellonella* model to study immune responses against human pathogens. *Journal of Fungi*, 4(4), 128.

Quiroz-Castañeda, R., Mendoza-Mejía, A., Obregón-Barboza, V., Martínez-Ocampo, F., Hernández-Mendoza, A., Martínez-Garduño, F., & Dantán-González, E. (2015). Identification of a new *Alcaligenes faecalis* strain MOR02 and assessment of its toxicity and pathogenicity to insects. *Biomed Research International*, 1-10.

Rejeb, S., Lereclus, D., & Slamti, L. (2017). Analysis of *abrb* expression during the infectious cycle of *Bacillus thuringiensis* reveals population heterogeneity. *Frontiers in Microbiology*, 8.

Said, S. M., Hammam, M.A., & Abd-el Kader, S. K. (2019). Insecticidal activity against the greater wax moth (*Galleria mellonella* L.) and chemical composition of five plant essential oils. *Menoufia Journal of Plant Protection*, 4, 145-61.

Salamitou, S., Ramisse, F., Brehélin, M., Bourguet, D., Gilois, N., Gominet, M., & Lereclus, D. (2000). The plcr regulon is involved in the opportunistic properties of *Bacillus thuringiensis* and *Bacillus cereus* in mice and insects. *Microbiology*, 146(11), 2825-2832.

Sciuto, A., Martorana, A., Fernández-Piñar, R., Mancone, C., Polissi, A., & Imperi, F. (2018). *Pseudomonas aeruginosa* lpte is crucial for lptd assembly, cell envelope integrity, antibiotic resistance and virulence. *Virulence*, 9(1), 1718-1733.

Scorzoni, L., Silva, A., Singulani, J., Leite, F., Oliveira, H., Silva, R., & Mendes-Giannini, M. (2015). Comparison of virulence between *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii* using *Galleria mellonella* as a host model. Virulence, 6(8), 766-776.

Telles, D. M., Martineli, G. M., Scaloppi, M. F., Ferreira Da Luz, M. P., & Kadri, S. M. (2020). Natural products can efficiently control the greater wax moth (Lepidoptera: Pyralidae), but are harmless to honey bees. *Sociobiology*, 67,89-93.

Uygur, Ş. Ö., & Girişgin, A. O. (2008). Diseases and pests of honeybee. *Uludağ Bee Journal*, 8, 130-42.

Viegas, S., Mil-Homens, D., Fialho, A., & Arraiano, C. (2013). The virulence of *Salmonella enterica* serovar *typhimurium* in the insect model *Galleria mellonella* is impaired by mutations in Rnase e and Rnase iii. *Applied and Environmental Microbiology*, 79(19), 6124-6133.

Wojda, I. (2016). Immunity of the greater wax moth *Galleria mellonella*. *Insect Science*, 24(3), 342-357.