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Insecticidal Effect of a Natural Turkish Diatomaceous Earth Formulation on Greater Wax Moth

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A B S T R A C T

In this study, the insecticidal effect of the Detech® (Turkish Diatomaceous earth) DE formulation against *Galleria mellonella* L. was determined. The study was conducted in a laboratory environment with materials taken from beehives produced at Muş Alparslan University in 2022. Diatomaceous earth (DE) was applied in two different forms (dust and slurry DE) and as positive control GüveSavar®, which is currently used against some pests in beekeeping. DE concentrations of 0, 3, 5, and 7 g/m^2 in different exposure times were tested for the control of *G. mellonella* larvae. As a result of all treatments, the highest mortality occurred at 7 g/m^2 dust DE concentration. Larvae (3rd stage) exposed to 7 g/m^2 concentration according to dust DE mortality rates reached 100% mortality after 40 hours. According to the results of the slurry DE, the larvae exposed to the slurry diatom at all concentrations achieved 100% mortality at the end of the 96 hours. When the dust and slurry DE results were examined, the direct use of dust formulations greatly accelerated the effectiveness against larvae. The study showed very promising results, suggesting that slurry DE and dust formulations could be a new alternative control method for Greater Wax Moth. In addition, for the first time, the insecticidal efficacy of DE against the honey bee pest, the greater wax moth, was determined.

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Introduction

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) are one of the most ecologically and economically valuable bee species in the world. Thanks to beekeeping, 70% of the world's valuable bee products and the pollination of plant production take part in honey bees (Klein et al., 2007). For bees to perform these valuable activities, they must be protected against diseases and pests. Varroa mites, greater wax moth, and small hive beetle, *Aethina tumida* Murray, 1867 (Coleoptera: Nitidulidae) are known as the most important pests of bees. These pests cause great damage to bees and their products (Turker et al., 1993; Core et al., 2012; Dietemann et al., 2013; Neumann et al., 2016; Gunesdogdu et al., 2021). In addition, these pests carry bee disease agents between colonies and apiaries (Charriere & Imdorf, 1999; Kwadha et al., 2017). Great wax moth causes economic losses for beehives and beekeepers (Haewoon et al., 1995; Almadani & Hiware, 2020). Greater wax moth larvae cause extreme damage to beeswax by consuming nutrients stored in the bee comb, especially in the 4th and 5th stages (Kwadha et al., 2017; Almadani & Hiware, 2020). Wax moths feed on pollen stored in the honeybee comb (Milan, 1970; Gulati & Kaushik, 2004). Beeswax can be contaminated by moths in the colony, during the honey harvest, or in storage (Rajendran & Hajira Parveen, 2005; Zhu et al., 2016; Kwadha et al., 2017; Mansour, 2020). *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) is a serious pest of weak honeybee colonies and stored combs (Shimanuki, 1980; Williams, 1997; Ritter & Akratanakul, 2006). The larva is creamy white. It feeds on beeswax during the larval stage. The insect develops in 7 larval stages (Desai et al., 2019). *Galeria mellonella* larvae were obtained from honeybee hives produced by the Animal Production and Technologies Department on the university campus. The larvae were collected from the honeycombs stored during the winter period when honey production was not produced and used in the experiments. Larvae (3rd instar) were tested under warehouse laboratory conditions. Insects and honeycombs were left in the containers as bait.

Various chemical (sulphur, aluminium phosphide, ethylene dibromide, methyl bromide, paradichlorobenzene (Napthalene), cold-hot applications, organic (vegetable oils and extracts) and biological insecticides (*Bacillus thuringiensis* Berliner, 1915) (Bacillales: Bacillaceae) control methods are applied in different ways (Burges, 1977; Ritter et al., 1992; Bisht et al., 2017; Telles et al., 2020; Beyene & Woldatsadik, 2019). These products negatively affect human health by making residues in beeswax and other bee products (Ritter & Akratanakul, 2006). Today, chemicals used to control this pest in particular cause residues in bee products. This situation negatively affects the quality and sales of bee products, which are very important for human health. Alternative control methods that do not leave chemical residues in bee products are the focus of many researchers. In particular, honey and bee products free from chemical products are important points to be considered. Therefore, the role of organic and mammalian non-toxic Diatomaceous earth in-pest control has been studied. Diatomaceous earth is one of the natural insecticides used as an alternative to chemical pesticides (Korunic, 1998).

There are different theories in the literature regarding the mechanism of action of DEs on insects. It is generally accepted that DE particles adhere to the cuticle of insects, causing death by absorption of fluids from the insect body by DE (Subramanyam & Roesli, 2000; Vayias & Vassiliki, 2009), but abrasion of the cuticle is also a complementary action through cuticular micro-wounds (Subramanyam & Roesli, 2000). Dose-dependent mortality results also show that a higher dose results in more DE particles in the insect's cuticle and faster death. Adsorbed DE particles immediately damage the protective waxy coating on the insect body, mostly through absorption and to a lesser extent, abrasion or both. It has been the common result of many studies that the main activity that causes the death of insects is the loss of water from the body of the insect through desiccation (Ebeling, 1971; Korunic et al, 1988).

Local diatomite preparation originating in Türkiye has been commercially formulated as named Detech® by Entoteam (Entoteam R&D Food Agriculture Co.). Previous studies have shown that the dust formulation of these two local diatomite preparations has high efficacy against various stored grain pests at different concentrations (Erturk et al., 2020; Saglam et al., 2020). Diatomaceous earth is non-toxic to mammals (oral LD50 value > 5000 mg/kg body weight in rats), does not leave toxic residues on crops, and is classified as GRAS (Generally Recognized as Safe) according to the US EPA because it is used as a food additive (FDA, 1995). Diatoms was formed because of the sedimentation of single-celled microscopic algae from the fossilized siliceous shells of the algae. The cell walls of diatomites are composed of amorphous silica. Diatomite dust is probably the most effective natural source that can be used as an insecticide (Korunic, 1998). Diatomite dusts are effective in the cuticle of insects, causing rapid drying of the insect and thus its death from water loss. It has been reported that Türkiye has very rich natural DE deposits and these large diatomite rock formations are in various parts of the country (Ozbey & Atamer, 1987; Mete, 1988; Sıvacı & Dere, 2006; Tas & Cetin, 2012).

In this study, the effect of dust and slurry formulations of Detech® formulation and GüveSavar® (essential oil-based) for the control of *G. mellonella* during stored honeycombs was investigated. The aim is to expand the use of natural products as alternatives to chemical pesticides against harmful insects in beekeeping. The insecticidal activity of organic DE against *Galeria mellonella* was investigated.

Materials and Methods

Turkish DE preparation (Detech®)

It has been determined that Türkiye has very rich natural DE resources and these great diatom resources are in different regions of the country. In recent years, because of scientific studies, Türkiye-origin diatom preparation Detech® has been enhanced for the physical control of pests. Detech® mainly consists of 80.6% (w/w) amorphous silicon dioxide and its median particle diameter (d (0.5)) is 14,061 µm (Bayram et al., 2019). Some properties of Detech® preparation are given in Table 1.

Commercial preparation used in beekeeping (GüveSavar®)

GüveSavar® consists of natural ingredients such as plant shells, nettle extract, walnut leaf extract, and molasses. It is reported that 250 ml of GüveSavar® protects approximately 220 Honeycombs between August and April. The concentrate is created by mixing 250 ml of GüveSavar® with 5 liters of water. The mixture is filled into clean spray bottles and sprayed homogeneously on the honeycombs and allowed to dry. The honeycombs applied with GüveSavar® are kept in suitable warehouse conditions. This commercial product is widely used in beekeeping with effective results in Türkiye, so it was used in trials as a positive control.

Table 1. Some physical and chemical properties of dust formulation of Detech® commercial local diatomite dust used in biological activity tests

DE preparation	SiO_2 (%) *	Median particle size (µm)**	pH value ± S.E.	Batch density ± S.E (g/l)	Color
Detech®	80.6	14.061	8.25±0.01	248.1±5.3	Yellowish white

*The physical and chemical of the diatomite sample was determined in the Analysis Laboratories of the General Directorate of Mineral Research and Exploration. ** Median value corresponding to 50% of the total particle volume in the volume of the cumulative particle size distributions (d(0.5)).

Table 2. Mortality (%) of greater wax moth larvae exposed to dust diatom formulations

AR	Mortality (%) ± S.E.				
	Exposure Time				
	8	24	32	40	
3	0 ± 0 Ab	46.6 ± 3.3 Aa	70 ± 10 Aa	73.3 ± 6.6 Ba	$F_{3, 8}$= 51.22 P<0.001
5	0 ± 0 Ab	50 ±15.2 Aa	80 ±10 Aa	90 ±5.7 ABa	$F_{3, 8}$= 16.97 P<0.001
7	0±0 Ac	43.3 ± 8.8 Ab	96.6±3.3 Aa	100 ±0 Aa	$F_{3, 8}$= 109.29 P<0.001
	$F_{2, 6}$= -P= -	$F_{2, 6}$= 0.12 P=0.893	$F_{2, 6}$= 2.71 P=0.145	$F_{2, 6}$= 8.93 P=0.016	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Table 3. Mortality (%) of greater wax moth larvae exposed to slurry diatom formulations

AR	Mortality (%) ± S.E.				
	Exposure Time				
	24	48	72	96	
3	0 ± 0 Ad	13.3 ± 3.3 Ac	56.6 ± 3.3 Bb	100 ± 0 Aa	$F_{3.8}$= 547.48 P<0.001
5	0 ± 0 Ad	16.6 ± 6.6 Ac	60 ± 11.5 Bb	100 ± 0 Aa	$F_{3.8}$= 81.99 P<0.001
7	0±0 Ac	23.3 ± 3.3 Ab	93.3 ± 3.3 Aa	100 ± 0 Aa	$F_{3.8}$= 166.09 P<0.001
	$F_{2, 6}$= -P= -	$F_{2, 6}$= 1.27 P=0.347	$F_{2, 6}$= 8.55 P=0.018	$F_{2, 6}$= - P= -	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Test procedures

The larvae were exposed to different dust and slurry concentrations (0, 3, 5 and 7 g/m2) for each application using a 1000 ml hand sprayer. Slurry DE formulations were prepared by mixing 0.12 g of DE/ml water. It was shaken by hand before the application so that DE and water had a homogeneous distribution. Dust and slurry diatom forms Detech® were used in biological test. The study was carried out in plastic containers (254*191*99 mm) containing bee bread (perga) for feeding the larvae. The diatomite dust form was homogeneously distributed on the plastic container surface with a small brush, the 3th instar larvae were transferred into the containers, and mortality were obtained at the exposure times (8, 24, 32, and 40 hours). Experiments were prepared in 3 replications and mortality rates were determined by leaving 10 Greater wax moth larvae in each replication. As a positive control, GüveSavar® (0, 3, 5 and 7 ml/100 ml) was applied as a spray in the form of a slurry in plastic containers and the larval mortality rates were determined at the exposure times (24, 48, 72, 96 hours). The larvae were collected from different wax frames. This study was carried out on the Muş Alparslan University campus, Faculty of Applied Sciences in 2022.

Statistical Analysis

Tables containing the obtained data, mean mortality rates, and standard errors for each application separately were created. Mortality rates were determined by recording the mortality results in the number of individuals in all treatments after certain periods. After applying the arcsin transform to individual mortality rates, they were subjected to analysis of variance using the statistical program SAS 9 (SAS Ins. 2009). One-way analysis of variance (ANOVA) was applied to the results and the differences between the averages were determined by using TUKEY test at 5% significance level.

Results

Mortality of Greater wax moth larvae was determined after 8, 24, 32, and 40 hours of exposure to dust D formulation (Table 2). According to the mortality, the larvae exposed to a concentration of 7 g/m^2 had a 100% at the end of the 40 hours. Mortality of 73.3% and 90% were obtained at an exposure time of 40 hours at concentrations of 3 and 5 g/m^2, respectively. It has been determined that dust diatom applications can be effective against larvae with increasing insecticidal efficiency results with increasing concentrations. Three different dust concentrations turned out to be ineffective on larvae after 8 hours of exposure.

Mortality of Greater wax moth larvae was determined after 24, 48, 7and 2, 96 hours of exposure to slurry DE form (Table 3). According to the mortality, the larvae exposed to three different slurry diatoms concentrations had 100% at the end the 96 hours. It has been determined that the insecticidal effect result from increasing with concentrations, slurry diatom applications could be effective against larvae. Three different concentrations of slurry diatoms appeared to be ineffective against larvae at 24 hours of exposure.

Mortality of Greater wax moth larvae were determined after exposure times of 4, 8, 12, and 16 hours to the GüveSavar® (Table 4). According to the mortality, the larvae exposed to a concentration of 7 g/m^2 had a 100% mortality rate at the end of the 12 hours. In the other concentrations (3 and 5 g/m^2), 100% mortality rates occurred after 16 hours. Insecticidal activity results increased with concentrations It was determined that moth-repellent preparation treatments conducted high-lethal activity against larvae.

Table 4. Mortality (%) of greater wax moth larvae exposed GüveSavar®

AR	Mortality (%) ± S.E.				
	Exposure Time				
	4	8	12	16	
3	6.6 ± 0 Ad	36.6 ± 3.3 Bc	70 ± 5.7 Bb	100 ± 0 Aa	$F_{3.8}$= 78.09 P<0.001
5	40 ± 5.7 Ad	76.6± 8.8 ABbc	93.3 ± 6.6 Aab	100 ± 0 Aa	$F_{3.8}$= 16.07 P<0.001
7	56 ± 8.8 Ac	90 ± 10 Aa	100 ± 0 Aa	100 ± 0 Aa	$F_{3.8}$= 10.10 P<0.004
	$F_{2,6}$= 14.23 P= 0.005	$F_{2,6}$= 8.08 P=0.020	$F_{2,6}$= 9.54 P=0.014	$F_{2,6}$= - P= -	

AR: Application rate of DE (g/m^2); *The data were subjected to one-way analysis of variance (ANOVA) and the differences between the means were determined at 5% significance level by applying TUKEY a test. Different lowercase letters in the same column and different uppercase letters in the same row indicate statistically different means.

Discussion

As a result, the potential of using the Detech® used in the study against moth pests during storage and storage of empty combs in beekeeping has emerged. In the study, it was revealed that slurry DE and dust formulations can be an important alternative as an organic insecticide to the control of Greater wax moth. During storage, when the combs are contaminated with moths, they become completely unusable. For this reason, dish combs are destroyed by burning. Therefore, the potential of DEs is promising in terms of protecting empty combs from pests during storage and reuse. In addition, diatoms are generally odorless powders, their moisture content varies between 2-6%, they are insoluble in water and do not burn, and there is no risk of flammability. DE is a highly stable organic substance that does not leave toxic chemical residues or react with other substances in its environment and is considered non-toxic to mammals (Quarles, 1992). Demirözer et al. (2022) reported that initial direct mortality to bees for DEs was kept below 25%, so DEs were classified as harmless to both honeybees and bumblebees. Also, mortality rates accelerated in parallel with increasing DE and GüveSavar concentrations. Prasantha et al. (2002), observed increased mortality linearly correlated with DE concentration. Mortality was reported to increase with increasing concentration in all treatments. Erturk et al. (2020) reported that the mortality rate of *Sitophilus oryaze* (L., 1763) (Coleoptera: Curculionidae) increased with Detech® WP dose and exposure time on both concrete and wooden surfaces. At the same time, the effectiveness of GüveSavar® swollen combs used in traditional beekeeping against the greater wax moth, which is a storage pest, was determined. Respectively, the most effective mortality percentages occurred with the GüveSavar® treatments, followed by dust DE. Almadani & Hiware (2020) found that thyme oil and homeopatic grug lodium treatment reduced the hatching of the great wax moth, Bisht et al. (2017) used neem oil, cedar oil, clove oil, pipermite oil, and karang oil in the treatment against Greater wax moth under storage conditions. Neem oil had the highest effect and Karang oil had the lowest effect. According to Ayman & Atef (2007), methyl salicylate, formic acid, clove oil, acetic acid and bacillus oil have high mortality. Fawzy et al. (2017), investigated the effects of propolis, cinnamon, clove, and peppermint ethanolic extracts on the 4th larval instar of the Greater wax moth and found that peppermint extract was the most effective and propolis extract had no effect. According to Swamy et al. (2006), mahua oil (63%), neem oil (62%), and pongamia oil (56%) cause a reduction in the Greater wax moth larvae populations. Paulraj et al. (2021) investigated the mortality of wax moth larvae using *Mentha piperita* (L., 1753) (Lamiales: Lamiaceae), eucalyptus oil and lemongrass oil. They reported mortality rates of 80.24%, 69.05%, and 50.48% respectively. Telles et al. (2020) investigated the effect of natural products of neem oil *Azadirachta indica* (A. Juss., 1830) (Sapindales: Meliaceae), eucalyptus oil (*Eucalyptus* spp.), tobacco extract *Nicotiana tabacum* L. (Solanales: Solanaceae) and malagueta pepper extract, *Capsicum frutescens* (Solanales: Solanaceae) on the control of greater wax moth. Neem oil and eucalyptus oil were reported to provide moth control at low doses and to be toxic to adult bees. They reported that tobacco and malagueta pepper extract-controlled moths and did not cause any adverse effects on bees. The untreated DE honeycombs (control group) quickly became contaminated with pests. In light of these observations, it has emerged that Diatom applications in empty honeycombs can be used as a preservative during the storage period. Ferreira et al. (2017), repellent and foraging of negramina oil *Siparuna guianensis* Aubl., 1775 (Laurales: Siparunaceae) against larvae and adults of *G. mellonella* and *Achroia grisella* F., 1794 (Lepidoptera: Pyralidae) wax moths reported that they were attracted to bees. Saglam et al. (2022) & Bayram et al. (2019) revealed that different concentrations (600 and 900 ppm) of Detech® local diatom preparation had high and moderate repellent effects against confused flour beetle *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and rice weevil *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) adults, respectively, while no or low-level repellent-effects on *Rhyzopertha dominica* F., 1792 (Coleoptera: Bostrichidae) adults.

The use of Detech® as a natural insecticide to protect combs from insect pests offers a new alternative control and protection opportunity. As a result, it was determined that Diatomaceous earth could offer an alternative physical control method in beekeeping.

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