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Insecticidal Efficacy of Native Diatomaceous Earth against Potato Tuber Moth, [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)], Pupae

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ABSTRACT
In this study, the insecticidal activity of native diatomaceous earth (DE) Turco004 was tested against pupae of the Potato tuber moth, [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)] in laboratory conditions. In the bioassays were used dust formulation and applied four concentrations (2.5, 5, 10 and 20 g^{-m^2}) of DE. Pupa weights and the number of adults emerging from the pupae were recorded after 24, 48, 72, 96, 120, 144 and 168 hours of diatomaceous earth exposure. It was concluded that the weights of the pupae are significantly affected depending on exposure time. In addition, pupal eclosion was prevented, and no adult emergency was observed at all concentration applied. The data obtained revealed that the native diatom soil Turco004 may have a potential in the control of *P. operculella* at all applied doses.

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Yerli Diyatom Toprağının Patates Güvesi [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)] Pupasına Karşı Böcek Öldürücü Etkisi

ÖZET
Yapılan bu çalışmada, yerli diyatom toprağı Turco004'ün Patates Güvesi [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)] pupasına karşı insektisidal etkisi laboratuvar şartlarında araştırılmıştır. Denemelerde diyatom toprağının toz halindeki formülasyonu uygulanmış ve dört farklı konsantrasyonu kullanılmıştır (2.5, 5, 10 ve 20 g^{-m^2}). Uygulamadan 24, 48, 72, 96, 120, 144 ve 168 saat sonra pupa ağırlıkları ve ergin çıkışları kaydedilmiştir. Çalışma sonunda pupa ağırlıklarının zamana bağlı olarak önemli ölçüde etkilendiği görülmüştür. Ayrıca tüm dozlarda pupa açılımı engellenmiş ve herhangi bir ergin çıkışı gözlemlenmemiştir. Elde edilen veriler yerli diyatom toprağı Turco004' ün *P. operculella*'nın kontrolünde bir potansiyele sahip olabileceğini ortaya koymuştur.

Araştırma Makalesi

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INTRODUCTION
The Potato tuber moth (PTM) (*Phthorimaea operculella* Zeller, (Lepidoptera: Gelechiidae) is an important pest that is harmful on the plants belonging to family Solanaceae including potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum*, L.), tomatoes (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and eggplant (*Solanum melongena* L.) (Das and Raman, 1994; Rondon, 2010; Kroschel and Schaub 2012, Navrozidis and Andreadis 2012). This pest is one of the most important problem to potato production in the world. Cold winters restrict the development of the pest and reduce its harm. However, global warming may change PTM population dynamics and geographic distribution in the future. This pest has become invasive and is today reported more than 90 countries (Kroschel and Sporleder, 2006; Sporleder et al. 2007). This insect is spread to all potato growing areas or regions of Turkey.

PTM causes damage in both field and storage. Female lay eggs on the underneath of the plant leaves, flower, bud, leaves, and shoots. The eggs are left near the tuber eyes, near the surface or cracks of the soil, or on the potatoes stacked in the soil. In the field, larvae feed

by mining in the leaves and shoots, and tunnelling in potato tubers. The damage due to potato moth larvae is seen principally during the storage period. *P. operculella* continues to give generation and previously infested tubers infect uninfected potatoes in the silo. Besides, damaged tubers are become more sensitive to be infected by bacteria and fungi (Golizadeh et al. 2014, Anonim 2020).

Stored crop losses in potatoes ranging from 50% in Yemen and Peru, 86% in Tunisia, Algeria, and Turkey, 90% in Kenya, and 100% in India and the Philippines have been reported (Alvarez et al. 2005). Çalışkaner et al. (1989) found that the damage of potato moth in storage period between 0.2-22% and density varies between one to 10, Bolu province in Turkey.

Synthetic chemicals are widely used for the control of potato tuber moths. However, environmental and human health problems caused by improper pesticides use, their side effects to beneficial organisms that are not targeted, the cost of insecticides used against PTM and residue problems. Researchers are trying to develop alternative methods for control of this insect due to the factors such as increasing demand for organic products, These alternative control methods include biological control, bio-pesticides, biotechnical and physical control. As an example of these studies, Shelke et al. (1985) have demonstrated that neem oil (*Azadirachta indica*, *A. juss*) has an anti-ovulation effect on PTM. Similarly, ethanol extract from *Origanum majorana* L. has a toxic effect on its larvae (Yeşilayer and Deniz 2019). Steven et al. (2008) stated that granulovirus (PoGV) and *Bacillus thuringiensis* Berliner ssp. *kurstaki* (Btk) can be used against larvae of potato tuber moth in field and storage conditions.

Diatomaceous earth (DE), which can be used within the scope of physical control methods, are fossilized silica residues of single cell microscopic algae known as diatoms. There are many studies on the toxicity of DE against insects of different pest groups (Ertürk et al. 2017). Athanassiou et al. (2004, 2005); reported that Silicosec®, PyriSec®, and Insecto® DE were given complete mortality for *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) at doses of 1000 ppm and above at 26 °C and 60% r.h. condition. Doğanay (2013) reported that the effectiveness of commercial diatom soil formulations Insecto® and native DE formulation Turco1 and Turco2 against *S. granarius* and *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) wheat, rice and corn were given 100.0% mortality at high concentrations (750 and 1000 ppm). Atay et al. (2018) stated that PTM larvae are very susceptible to Turco004 showing an effect of approximately 100% after 24 hours of the DE exposure. However, there is no study on the toxicity effect of DE on PTM pupae. This study was conducted to determine the activity of diatomaceous earth against the PTM pupae in laboratory conditions.

MATERIAL and METHODS

Phthorimaea operculella culture

The preliminary culture of *P. operculella* was supplied from available infected potatoes in the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpasa University, Tokat, Turkey. Immature stages of *P. operculella* were reared on potato tubers in a cage at 25±1°C, 65±5% r.h., and a photoperiod of 14:10 L:D. The cages were cylindrical (20 cm in diameter, and 50 cm in height) and covered by blotter paper and fine mesh gauze. The adults in the stock culture were fed with honey rubbed into the edge of the cage (Golizadeh et al. 2014).

Diatomaceous earth

Native diatomaceous earth Turco004 were acquired from a local commercial company from Ankara-Kazan and Beypazarı (Beg-tuğ Mineral). The particle sizes of the diatomaceous earth were 10-30 µm and mainly composes of SiO_2 (83.26%) and other minerals CaO (3.49%), Al_2O_3 (6.25%), Fe_2O_3 (4.91%). Scanning electron microscope (SEM) image of Turco004 is presented in Figure 1.

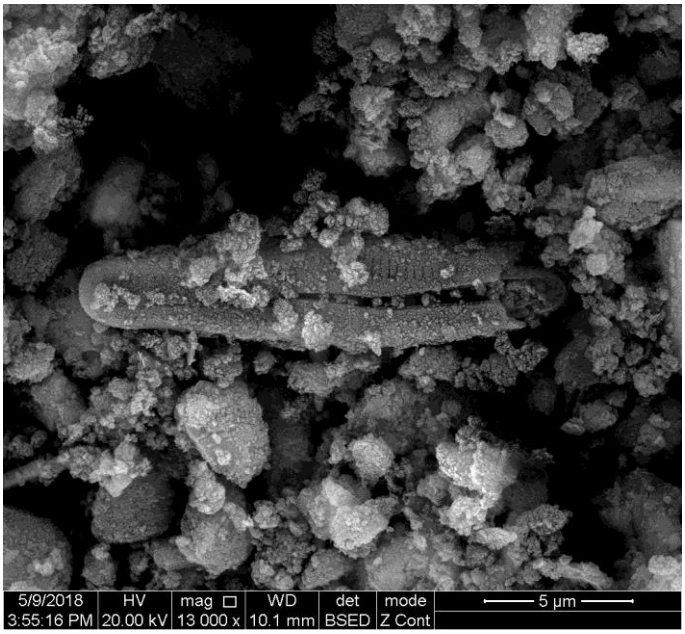

Figure 1. Scanning Electron Microscope image of Turco 004 showing general size and shape

Şekil 1. Turco 004'ün hacim ve şeklini gösteren Taramalı Elektron Mikroskop görüntüsü

Bioassay tests

The insecticidal activity of native DE Turco004 was tested against pupae of the PTM (two days old pupae). The bioassays were conducted at 25±1°C and 60±5% humidity with four concentrations of Turco004 (2.5, 5, 10 and 20 g^{-m^2}) in plastic cups with 3.5 cm diameter. For this purpose, DE was laid on the bottom of plastic cups with a camel hairbrush (No. 2) and pupae were

placed on it (5 pupae per cup). The experiment was set in randomized blog design with 4 replications and the experiment was repeated 2 times. Pupae weights and the number of adults emerging from the pupae were recorded after 24, 48, 72, 96, 120, 144 and 168 hours of DE exposure. In the experiment, the control group were not treated with the DE.

Statistical analysis

Emerge Inhibition rate of PTM pupae was calculated using the below formula (Gökçe et al. 2012) that was modified from Abdelgaleil and Al-Aswad (2005). The calculated growth rates were subjected Kruskal-Wallis test.

$$EI = \frac{CWC - TWC}{CWC}$$

Where EI: Emergence Index
CWC: Change in weight in the control (Initial weight-post treatment weight)
TWC: Change in weight in the treatment (Initial weight-post treatment weight)

The data for percent pupal emergency were subjected to arcsine transformation. Data were subjected to analysis of variance and differences among the treatments were compared by Tukey Multiple Comparison Test (α= 0.05). Statistical analysis for data on growth rate and adult emergency rate were done using MINITAB® version 18 software package (McKenzei and Goldman 2005).

RESULTS and DISCUSSION

In the study, the effects of different concentrations of DE on the development of potato tuber moth pupae were evaluated. It was found that the weights of the pupae are significantly affected by the exposure time, while their weights were not significantly affected increasing by the DE concentrations (Figure 2). While DE treatment at the 2.5 g^{-m^2} dose of the diatomaceous earth had an index value of 0.90 after at the end of 24 hours of DE exposure (H=1.02; df=3), it had an index value of -0.50 after 168 hours of DE exposure (H=0.43; df=3). In all other application concentrations (5 g^{-m^2}, 10 g^{-m^2}, 20 g^{-m^2}), these index values varied between 0.90 and -0.40 (Figure 2).

For adult emergence rates, the diatomaceous earth significantly prevented the pupal eclosion (Figure 3). No adult emergence from the pupae was observed at all DE concentrations applied. In the control group, adult emergence from the pupae was not seen after 24 hours, however, adult emergence was found after 48 hours. (F=1.75; df=2,33; P>0.05). Adult emergence started to increase after 72 hours and in this time zone, a 26.1% adult emergence rate was determined in the control group (F= 12.6; df= 2.33; P<0.05). Adult emergence rate in control group was 42.5% after 96 hours (F= 88.5; df= 2, 33; P<0.05), 52.4% after 120 hours (F= 198.1; df= 2.33; P<0.05), 55.9% after144 hours (F= 344.1; df= 2, 33; P<0.05) and 65.1% after 168 hours (F= 145.7; df= 2.33; P<0.05) (Figure 3).

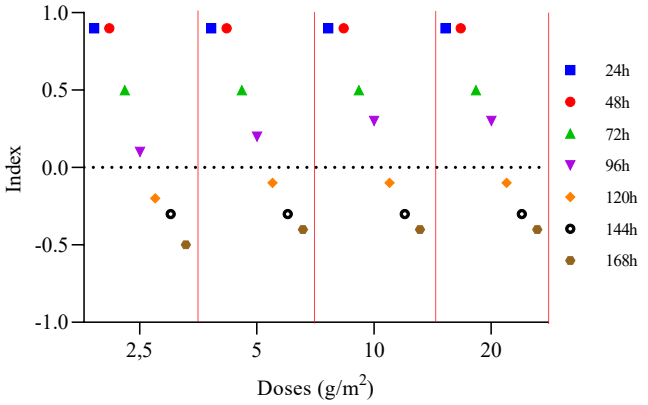

Figure 2. Weight losses caused by different doses of diatomaceous earth on potato tuber moth pupae
Şekil 2 Diyatom toprağının farklı dozlarının patates güvesi pupalarında meydana getirdiği ağırlık kayıpları

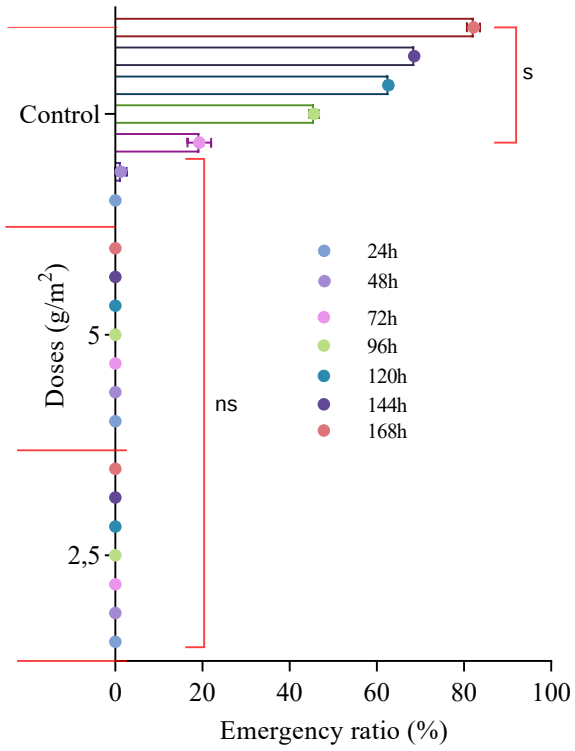

Figure 3. Effect of different doses of diatomaceous earth on emergence rates of adults of potato tuber moth
Şekil 3. Diyatom toprağının farklı dozlarının patates güvesi erginlerinin çıkış oranları üzerine etkisi

Previous studies showed that commercially available DE formulations are successfully used for controlling of stored product pests (Subramanyam et al. 1994, Ziaee 2007, Alkan et al. 2019, Bayram et al. 2019). In

addition, these studies were mostly conducted on adult of Coleopteran insect species (Athanassiou et al. 2003, 2004, 2005). Diatomaceous earth is one of the most promising methods for controlling of stored-grain insects, which can be an alternative to conventional insecticides and fumigants (Korunic et al. 1996). Collantes et al. (1986), demonstrated that synthetic pyrethroid (deltamethrin, permethrin, cyfluthrin, flucythrinate, fenvalerate) insecticides were ineffective against PTM in field conditions. El-Kady (2011) found that *P. operculella* developed resistance against fenitrothion (500g^{-1}-EC), pirimiphos-methyl (250^{-1}-EC), carbosulfan (10G 10% w/w), aldicarb (10G 10% w/w), lambda-cyhalothrin (50 g^{-1}-EC), deltamethrin (25 g^{-1}-EC) and, imidaclopride (200g^{-1}-SL) at varying rates.

Most insects make their breathing through spiracles that are part of the trachea system and open out of the body. It is known that insect's inhalation stops when these openings are closed by dust particles (Adedire et al. 2011, Ileke and Olotuah 2012). Sharaby et al. (2014) applied some essential oil compounds to the stored potato tubers using talcum powder. They concluded that these components prevent the insect to lay eggs and to enter the tubers. It is known that the diatomaceous earth causes death by damaging the insect integument (Ebeling 1971, Quarles 1992). As the diatomaceous earth mode of action does not depend on metabolic reactions, as in synthetic insecticides, more of the responses of inactive/immobil stages of insects to diatomaceous earth need to be studied. Mewis and Reichmuth (1999) applied the DE formulation Fossil Shield® against the Mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupa, revealing loss of weight in the pupa. Similarly, the data obtained from this study revealed that the DE used had a significant effect on the pupal eclosion of the potato moth and the weight of the pupae.

CONCLUSIONS

Diatomaceous earths form a physical barrier on insects, break down the epicuticular layers, cause fat absorption and therefore water loss, and also prevent the oxygen intake required for cell energy, causing death and weight loss in insects. This study reveals that diatomaceous earths significantly affected weights of the pupae and the number of adults emerging from the pupae. Despite this, exact mechanism of action of DE on the pupa yet is not known. For this reason, this study suggests that detailed and further physiological and biochemical studies are needed. The present study showed that, diatomaceous earth has a high potential as a natural preservative against pupae of *P. operculella* and could be suggested for integrated pest management programs (empty storage and tuber applications); however, further studies should be conducted under laboratory and storage conditions.

Declaration of competing interest

All authors declare that there are none interest to declare.

Author's Contributions

The contribution of the authors are equal.

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