

Original article (Orijinal araştırma)

Insecticidal effect of diatomaceous earth formulation against Pear lace bug, *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae)

Armut kaplanı, *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae) üzerinde diatom toprağı formülasyonunun etkisi

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Abstract

The pear lace bug, *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae), is an economically important pest of pome fruit trees. This study was conducted under controlled laboratory conditions to determine the insecticidal efficacy of Detech® WP 95 against the nymphal instars and adults of *S. pyri*. Experiment was carried out on apple, *Malus domestica* Borkh. (Rosales: Rosaceae) leaves using aqueous DE suspensions at three concentrations (2.5, 5, and 10 g/m²), and mortality was recorded daily from the first to the fifth day after treatment. 100% mortality was achieved within 24h in all treatments with 1st nymphal instars, indicating a high susceptibility of early instar nymphs to DE exposure. In the 5th nymphal instar, at a rate of 2.5 g/m², mortality increased from 70 to 100% on the third and fifth day, respectively. At 5 g/m², mortality was 80% on the second day and 100% on the third day of the treatment. Overall, mortality was positively correlated with both exposure time and DE concentration. Lethal time (LT₅₀) value for adults at the lowest concentration was 1.29 days, which was lower than that of fifth-instar nymphs (2.56 days). In conclusion, DE shows significant toxicity against *S. pyri* and highlights its potential as a natural insecticidal agent in integrated pest management programs.

Keywords: Detech, IPM, natural insecticide, nymphal instars, toxicity

Öz

Armut kaplanı, *Stephanitis pyri* (Fabricius, 1775) (Hemiptera: Tingidae), yumuşak çekirdekli meyve ağaçlarının ekonomik açıdan önemli bir zararlısıdır. Bu araştırma Detech® WP 95 preparatının *S. pyri*'nin 1. ve 5. dönem nimf ile ergin dönemlerine karşı öldürücü etkisini belirlemek amacıyla, kontrollü laboratuvar koşullarında 2024 yılında Tekirdağ'da yürütülmüştür. Denemeler elma, *Malus domestica* Borkh. (Rosales: Rosaceae) yaprakları üzerinde üç konsantrasyon düzeyinde (2.5, 5 ve 10. g/m²) sulu DE süspansiyonları ve kontrol grubu kullanılarak gerçekleştirilmiştir. Ölüm oranları uygulamadan 1-5 gün sonra değerlendirilmiştir. Birinci dönem nimfler üzerinde tüm konsantrasyonlarda yüksek etkinlik göstermiş ve 24 saat içinde %100 ölüm meydana gelmiştir. Bu durum, erken dönem nimflerin DE'ye karşı oldukça duyarlı olduğunu göstermiştir. Beşinci dönem nimflerde ise 2.5 g/m² konsantrasyonlarda ölüm oranı 3. günde %70'ten 5. günde %100'e yükselmiştir, 5 g/m² konsantrasyonlarda ise 2. günde %80 ve 3. günde %100 ölüm oranına ulaşılmıştır. Genel olarak, ölüm oranının hem maruz kalma süresi hem de DE konsantrasyonu ile pozitif korelasyon gösterdiği belirlenmiştir. Öldürücü süre (LT), en düşük konsantrasyonda erginlerin LT₅₀ değeri 1.29 gün olup beşinci dönem nimflerden (2.56 gün) daha düşük bulunmuştur. Bu sonuçlar, DE'nin *S. pyri* üzerinde önemli toksik etkiler gösterdiğini ve entegre zararlı yönetimi kapsamında doğal bir böcek öldürücü olarak kullanılabilme potansiyelini ortaya koymuştur.

Anahtar sözcükler: Detech, IPM, doğal böcek ilacı, nimf evreleri, toksisite

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Introduction

Apple, *Malus domestica* Borkh. (Rosales: Rosaceae) is one of the most widely cultivated fruit crops globally, accounting for approximately 45% of the world's land area allocated to fruit tree cultivation (Khan et al., 2018). During the 2024-2025 production season, Türkiye ranked as the fourth-largest apple producer worldwide, after China, the European Union, and the United States, contributing approximately 5% of global output, with an estimated annual production of 4.15 million metric tons (USDA, 2025). In Türkiye, apple cultivation is concentrated in five major provinces: Isparta, Karaman, Antalya, Niğde, and Denizli, which together account for 53.29% of national production (Oğuz et al., 2009). These regions continue to benefit from favorable geographical and ecological conditions, reinforcing the strategic importance of apple cultivation (Aslan & Karaca, 2005). However, apple production faces multiple constraints, including limitations in farm size, technological capacity, infrastructure, pest and disease pressure, and market-related challenges (Yilmaz et al., 2015; Giray et al., 2019). In Türkiye, more than 50 insect pests and 10 plant diseases have been recorded in apple orchards, causing significant economic losses, with insect pests alone responsible for an estimated 20% reduction in yield (Atlamaz et al., 2007; Khan et al., 2018).

Among the various pest species affecting fruit orchards, the pear lace bug, *Stephanitis pyri* (Fabricius, 1775) (Tingidae: Hemiptera) is recognized as one of the most polyphagous and economically damaging species, infesting a wide range of pome and stone fruit trees, particularly within the family Rosaceae, across the Palearctic region (Kment & Jindra, 2005; Golub & Soboleva, 2018). Both nymphs and adults feed on the abaxial surface of leaves by piercing parenchyma cells and extracting sap, causing chlorosis, stippling, and the accumulation of black excreta spots on the leaf surface (Maral, 2021). High-density infestations result in premature defoliation, reduced fruit size, fruit drop, and overall tree decline, leading to substantial yield and quality losses (Vergnani & Caruso, 2008). In Türkiye, apple has been reported as the most preferred host, followed by pear, *Pyrus communis* L. (Rosales: Rosaceae) and quince, *Cydonia oblonga* Mill. (Rosales: Rosaceae) (Anonymous, 2025).

Chemical insecticides, particularly organophosphates such as malathion and dimethoate, remain the primary method for controlling *S. pyri* in apple orchards due to their ease of application and rapid knockdown effect (Vergnani & Caruso, 2008; Anonymous, 2025). It has been reported that more than 25 pesticide applications with 80 different active ingredients were applied on an apple orchard in one season for insect pest management (Zaller et al., 2023). Moreover, excessive reliance on synthetic chemicals presents serious drawbacks, including environmental contamination, toxicity to non-target organisms, the development of pesticide resistance, and adverse effects on human health (Peiris-John & Wickremasinghe, 2008; Srivastava & Kesavachandran, 2019). In response to these challenges, there is growing global interest in adopting more sustainable, ecologically sound pest management approaches, notably Integrated Pest Management (IPM) (Pimentel & Peshin, 2014).

Inert dusts, such as kaolin and diatomaceous earth (DE), have attracted increasing interest as alternative pest management tools due to their physical mode of action, low environmental toxicity, and compatibility with IPM programs. Kaolin, a naturally occurring aluminosilicate clay, and DE is a silica-rich material derived from fossilized remains of unicellular algae known as diatoms; both have demonstrated insecticidal or repellent and oviposition deterrent effects across a variety of agricultural systems (Korunic, 1998; Subramanyam & Roesli, 2000; Glenn, 2012). The abrasive and adsorptive properties of DE allow it to adhere to the insect cuticle, disrupt the protective lipid layer, cause desiccation, and, in some cases, damage internal tissues or obstruct spiracles, ultimately leading to death or repellence (Jackson & Webley, 1994; Korunić, 2013). Previous studies have demonstrated the effectiveness of DE on various stored-grain insects, including *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius, 1775) (Coleoptera: Bostrichidae), and *Tribolium confusum* du Val, 1863 (Coleoptera: Tenebrionidae) (Sağlam et al., 2022a; Mortazavi et al., 2025), *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) (Sağlam et al., 2022b; Abdelgaleil et al., 2025), *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae) (Sağlam et al., 2022c; Novljan et al., 2025), and *Sitophilus oryzae*

(L., 1763) (Coleoptera: Curculionidae) (Ertürk et al., 2020). In addition, the efficacy of DE has been evaluated against *Plodia interpunctella* (Hübner 1813) (Lepidoptera: Pyralidae) (Predojević et al., 2025), thrips species *Frankliniella occidentalis* (Pergande, 1895), *Frankliniella fusca* (Hinds, 1902) (Thysanoptera: Thripidae) (Mitchell et al., 2018; Ge et al., 2020), *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) (Penn et al., 2025), and *Musca domestica* L., 1758 (Diptera: Muscidae) (Rahman et al., 2016).

Diatomaceous earth (DE) can be applied directly to stored grains without the need for specialized equipment (Athanasios et al., 2005) or formulated with additives or attractants to enhance insecticidal efficacy (Islam et al., 2010). In orchard systems, DE may also be utilized as a foliar spray or soil amendment to suppress pest populations or reduce oviposition rates. Ingestion of DE may damage insect mouthparts and lead to additional physiological disruptions (Penn et al., 2025). In horticultural crops, kaolin has been shown to reduce pest pressure and mitigate abiotic stress (Glenn, 2012). For example, Lalancette et al. (2005) found that both hydrophobic and hydrophilic formulations of kaolin significantly suppressed key peach pests such as the *Popillia japonica* Newman, 1841 (Coleoptera: Scarabaeidae), *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae), and *Conotrachelus nenuphar* (Herbst, 1797) (Coleoptera: Curculionidae) and concurrently reduced brown rot incidence (caused by *Monilinia* species), as well as symptoms of peach scab (caused by *Cladosporium* species) and rusty spot (caused by *Tranzschelia* species). According to Maral (2021), in combination with spinosad and botanical insecticides such as azadirachtin, kaolin was highly effective against *S. pyri*. Additionally, Joseph (2020) demonstrated that certain insecticides have repellent effects on *Stephanitis pyrioides* (Scott, 1874) (Hemiptera: Tingidae). The aim of this study was to evaluate, for the first time, the insecticidal activity of a diatomaceous earth-based formulation (Detech® WP 95) against the nymphal instars and adult stages of *S. pyri* on apple leaves under laboratory conditions. By establishing DE concentration-response relationships and stage-specific susceptibility patterns, this research seeks to contribute to the development of safer, non-chemical control options suitable for integration into IPM programs targeting the pear lace bug in apple orchards.

Materials and Methods

Collection and rearing of *Stephanitis pyri*

Overwintered adults of *S. pyri* were collected in July 2024 from *Malus sylvestris* (L.) Mill. (Rosales: Rosaceae) (wild ornamental apple) trees located in fruit orchards in Süleymanpaşa, Tekirdağ, Turkey (40°58'47" N, 27°33'08" E), and transferred to the laboratory of the Department of Plant Protection at Tekirdağ Namık Kemal University for further experiments. Ten pairs of adults were each placed in 9 cm diameter Petri dishes containing fresh apple leaves to establish a homogeneous population. To prevent leaf desiccation, a piece of blotting paper moistened with distilled water was placed at the bottom of each Petri dish, and the leaf petioles were wrapped in moistened cotton. Each Petri dish was covered with a lid to prevent adult escape. Apple leaves were replaced daily throughout the rearing period. After oviposition, leaves containing eggs were transferred to new Petri dishes, where egg hatching was monitored daily. The hatched nymphs were gently transferred onto fresh apple leaves using a fine camel hair brush and reared until adult emergence, following the revised method described by Aysal & Kıvanç (2008). Nymphs and newly emerged adults obtained from this colony were subsequently used in the experiments. All experiments were conducted under controlled laboratory conditions: in a thermostatic chamber at 26±1°C, 60±5% relative humidity, and a 16:8 h (light: dark) photoperiod.

Diatomaceous earth formulation

The diatomaceous earth (DE) formulation used in the study was Detech® WP 95 (Entoteam R&D Food Agriculture Industry and Trade Ltd. Co., Türkiye), developed as a grain protectant for the control of stored-grain pests and for surface application in empty storage facilities. Detech® WP 95 contains 95% amorphous DE and 5% surfactant. Its chemical composition consists of 80.6% SiO₂, 4.75% CaO, 4.7% Al₂O₃, 1.5% Fe₂O₃, 0.85% MgO, 0.5% K₂O, 0.4% Na₂O, and <0.01% TiO₂, with an average median particle diameter (d₅₀) of 14.06 µm. The formulation was supplied directly from Entoteam R&D Company (Türkiye) and applied in bioassays as a water-based wettable powder (WP) formulation.

Experimental design and procedure

Newly emerged adults and individuals representing each of the five nymphal instars, obtained from the laboratory colony, were used in the bioassays. Nymphal instars used in the experiments were observed daily during rearing period and differentiated according to their age (expressed in days) based on previously reported developmental data for *S. pyri* (Montazersaheb et al., 2024). Bioassays were conducted in sterile 5 cm diameter Petri dishes. Leaves were collected from a chemically free apple orchard field, cleaned by rinsing with distilled water before being placed on a Petri dish and dried with clean filter paper, and left it for 10 minutes. Each Petri dish contained a circular 5 cm in length apple leaf disk placed on moistened blotting paper to prevent desiccation. DE formulation (Detech® WP 95) was applied as an aqueous spray at concentrations of 2.5, 5.0, and 10.0 g/m² using an HSENG Airbrush AS18 model sprayer (Ningbo Co., China). The required amount of DE for each concentration was calculated based on the internal surface area of the Petri dish. The calculated amount of DE for each dose was suspended in the distilled water, and suspension was mixed thoroughly. For each Petri dish, 1.0 mL of the DE suspension was evenly applied from a standardized distance of 10 cm to ensure uniform coverage. Treated leaf disks were air-dried for 15 minutes at room temperature before being placed in the Petri dishes. In the control replicates, leaves were only treated with distilled water. Four treatments, including three diatomaceous earth concentrations and an untreated control, were evaluated, with five independent replicates per treatment (total n = 20). Each replicate contained ten nymphs (of a certain instar) or five adults, which were individually placed into Petri dishes to avoid inter-stage interactions. Leaf disks were replaced daily with freshly treated ones at the same concentration, and moistened blotting paper was refreshed to maintain humidity levels. The replicates were kept under the same conditions as those for rearing the *S. pyri* experimental population. Mortality was assessed daily for five consecutive days. An individual was considered dead if it exhibited no movement upon gentle stimulation with a camel hairbrush (Figure 1). All experimental units and Petri dishes were disinfected with 70% ethanol between treatments to prevent cross-contamination.

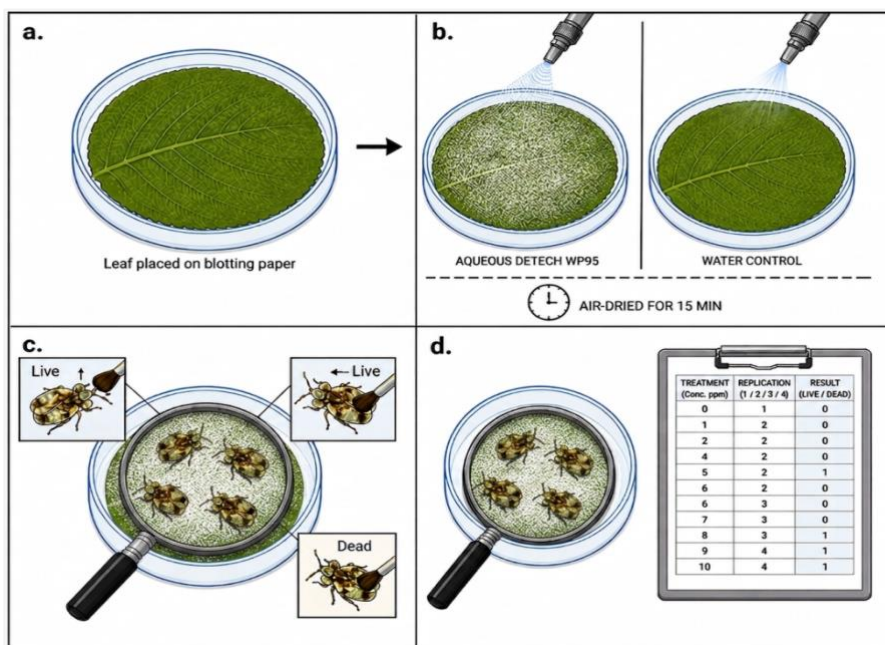


Figure 1. Overview of the experimental workflow applied uniformly to each treatment type, Detech® WP 95 tested against *Stephanitis pyri*. The figure illustrates the bioassay procedure, a) fresh leaf placed on moistened blotting paper in a Petri dish, b) spray treatment, c) mortality assessments after releasing pear lace bug, and d) data evaluation.

Statistical analysis

Mortality data (%) obtained from each treatment and observation day were first tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test (Royston, 1992). As percentage mortality data are proportional in nature, they were subjected to arcsine square root transformation prior to analysis to meet the assumptions of parametric statistical tests. However, since the transformed data did not meet the normality assumption, non-parametric statistical methods were employed: the Mann-Whitney U test was used for pairwise comparisons, and the Kruskal-Wallis test for comparisons among more than two groups (Rozaini & Khalid, 2024). All statistical analyses, including descriptive statistics, were performed using Minitab® software (Version 22; Minitab LLC, State College, PA, USA). Where appropriate, treatment means were separated using the least significant difference (LSD) test at a significance level of $p \leq 0.05$.

To assess the time-dependent toxicity of diatomaceous earth (DE) against *S. pyri*, mortality data obtained from different DE concentrations (2.5, 5.0, and 10.0 g/m²) and the untreated control were subjected to Probit regression analysis (Finney, 1971). This analysis was conducted to estimate the lethal time₅₀ (LT₅₀) and lethal time₉₀ (LT₉₀), the time required to kill 50% and 90% of the exposed individuals, respectively, for each concentration. Probit analysis assumes a sigmoid time-response relationship and allows estimation of LT₅₀ and LT₉₀ values along with 95% confidence intervals (CIs), slope values, and chi-square (χ^2) goodness-of-fit statistics. All Probit analyses were performed using PoloPlus software (Leora, 2002), which automatically calculates the lethal time estimates and associated parameters based on cumulative mortality data. Only datasets with mortality rates between 10% to 90% were included in the analysis, as required for accurate regression modeling. Treatments showing insufficient mortality (<10%) or complete (100%) mortality were excluded from LT₅₀ estimation due to violation of model assumptions. LT₅₀ and LT₉₀ values were compared across concentrations by examining the overlap of 95% confidence intervals, where non-overlapping intervals were considered significantly different (Robertson et al., 2017).

Results

Concentration response and lethal effects of diatomaceous earth on adult of *Stephanitis pyri*

Median mortality values (%) and interquartile ranges (Q1-Q3) of *S. pyri* adults after 5 days of exposure to different concentrations of a diatomaceous earth (DE) formulation applied to apple leaves under laboratory conditions are presented in Table 1.

Table 1. Median mortality values (%) and interquartile ranges (Q1-Q3) of *Stephanitis pyri* adults after 5 days of exposure to different concentrations of a diatomaceous earth (DE) formulation applied to apple leaves under laboratory conditions (n=5)

DE concentration (g/m ²)	Median mortality (%) Interquartile ranges (Q1-Q3)					Statistical value
	Day 1	Day 2	Day 3	Day 4	Day 5	
2.5	20.00 Bc* (20.00-60.00)	60.00 Bb (60.00-100.00)	100.00 Aa (60.00-100.00)	100.00 Aa (80.00-100.00)	100.00 Aa (100.00-100.00)	H=10.89, df=4 $p \leq 0.028$
5	40.00 Bb (40.00-80.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=18.18, df=4 $p \leq 0.001$
10	100.00 Ba (40.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=8.35, df=4 $p = 0.080$
Control	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-20.00)	H=8.35, df=4 $p = 0.080$
Statistical value	H=5.20, df=2 $p = 0.074$	H=7.00, df=2 $p \leq 0.030$	H=4.31, df=2 $p = 0.116$	H=2.00, df=2 $p = 0.368$	H=-- $p = --$	

*A non-parametric Mann-Whitney U test was applied to the mortality data. Different uppercase letters within columns and different lowercase letters within rows indicate statistically significant differences at the 5% significance level ($p \leq 0.05$). Q1 refers to the first quartile (25th percentile) of the mortality distribution. Q3 refers to the third quartile (75th percentile) of the mortality distribution.

The Anderson-Darling normality test indicated that mortality data across all concentrations and the control were not normally distributed (AD=3.260-8.490; $p < 0.005$), necessitating the use of non-parametric analyses. The Kruskal-Wallis test revealed statistically significant differences in adult mortality among DE concentrations (H=73.57, $p < 0.001$) and across exposure periods (H=8.72, $p < 0.006$). Mann-Whitney U tests confirmed significant differences in mortality between all pairwise concentration comparisons (2.5 vs. 5, 2.5 vs. 10, 5 vs. 10 g/m²; $p < 0.05$). At 2.5 g/m², adult mortality increased steadily from 20% on day 1 to 100% by day 5 ($p < 0.028$), whereas both 5 and 10 g/m² achieved complete mortality by day 2. Significant differences across periods were detected only at 10 g/m² ($p = 0.080$), suggesting a more rapid onset of lethality at higher concentrations. Mortality at all DE concentrations was significantly higher than in the control group ($p < 0.001$), where no mortality was observed throughout the experimental period. These results confirm that DE induces rapid and complete mortality in *S. pyri* adults in a concentration and time-dependent manner, with higher concentrations leading to faster lethality.

Concentration response and lethal effects of diatomaceous earth on nymphal instars of *Stephanitis pyri*

Median mortality values (%) and interquartile ranges (Q1-Q3) of first-instar nymphs of *S. pyri* after 5 days of exposure to different concentrations of a diatomaceous earth (DE) formulation applied to apple leaves under laboratory conditions are presented in Table 2. For the first-instar nymphs, the Kruskal-Wallis test revealed a significant difference in mortality between all DE-treated concentrations and the control (H=96.39, $p < 0.001$) (Table 2). No significant differences in mortality were detected across exposure periods within any DE concentration (H=0.47, $p = 0.976$). Mann-Whitney U tests confirmed non-significant differences among the three DE concentrations (2.5 vs. 5, 2.5 vs. 10, 5 vs. 10 g/m²). All DE treatments resulted in 100% mortality of first instar nymphs within 24 h, which was maintained throughout the five-day observation period, whereas mortality in the control group increased gradually from 20% to 50% by day 5 ($p < 0.001$). These findings demonstrate that first-instar nymphs of *S. pyri* are highly susceptible to DE, even at the lowest tested concentration, and that no concentration-dependent variation was observed due to uniform and immediate lethality.

Table 2. Median mortality values (%) and interquartile ranges (Q1-Q3) of first-instar nymphs of *Stephanitis pyri* after 5 days of exposure to different concentrations of a diatomaceous earth (DE) formulation applied to apple leaves under laboratory conditions (n=5)

DE concentration (g/m ²)	Median mortality (%) Interquartile ranges (Q1-Q3)					Statistical value
	Day 1	Day 2	Day 3	Day 4	Day 5	
2.5	100.00 Aa* (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=-- $p = -$
5	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=-- $p = -$
10	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=-- $p = -$
Control	0.00 (0.00-10.00)	20.00 (05.00-25.00)	20.00 (10.00-40.00)	40.00 (30.00-65.00)	50.00 (40.00-70.00)	H=-- $p = -$
Statistical value	H=-- $p = -$	H=-- $p = -$	H=-- $p = -$	H=-- $p = -$	H=-- $p = -$	

*A non-parametric Mann-Whitney U test was applied to the mortality data. Different uppercase letters within columns and different lowercase letters within rows indicate statistically significant differences at the 5% significance level ($p \leq 0.05$). Q1 refers to the first quartile (25th percentile) of the mortality distribution. Q3 refers to the third quartile (75th percentile) of the mortality distribution.

Median values and interquartile ranges (Q1-Q3) of the mortality (%) of *S. pyri* fifth-instar nymphs after five days of exposure to different concentrations of the DE formulation applied to apple leaves under laboratory conditions are presented in Table 3. For the fifth-instar nymphs, the Anderson-Darling tests indicated that mortality data for all DE concentrations significantly deviated from normality (AD=1.878-4.871; $p < 0.005$).

Kruskal-Wallis results showed that both DE concentration and exposure time had a significant effect on mortality ($H=49.35$, $p\leq 0.001$; $H=33.30$, $p\leq 0.001$) (Table 3). At 2.5 g/m², mortality increased from 70% by day 3 to 100% by day 5. The 5 g/m² concentration caused 80% mortality by day 2 and complete mortality by day 3. The 10 g/m² concentration reached 20% mortality on day 1, with 100% mortality achieved by day 2. Control group mortality remained low (0-10%) throughout the observation period. These findings demonstrate that DE is highly effective against late-instar nymphs of *S. pyri*, with higher concentrations accelerating the onset of mortality, and even the lowest concentration resulting in incomplete mortality within five days.

Table 3. Median mortality values (%) and interquartile ranges (Q1-Q3) of fifth-instar nymphs of *Stephanitis pyri* after 5 days of exposure to different concentrations of a diatomaceous earth (DE) formulation applied to apple leaves under laboratory conditions (n=5)

DE concentration (g/m ²)	Median mortality (%) Interquartile ranges (Q1-Q3)					Statistical value
	Day 1	Day 2	Day 3	Day 4	Day 5	
2.5	0.00 Bc* (0.00-05.00)	20.00 Bb (10.00-50.00)	70.00 Ba (55.00-85.00)	90.00 Ba (90.00-100.00)	100.00 Aa (100.00-100.00)	H=20.67, df=4 $p\leq 0.000$
5	0.00 Bc (0.00-20.00)	80.00 Ab (70.00-80.00)	100.00 Aa (90.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=22.12, df=4 $p\leq 0.000$
10	20.00 Ab (10.00-70.00)	100.00 Aa (75.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	100.00 Aa (100.00-100.00)	H=19.67, df=4 $p\leq 0.001$
Control	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-05.00)	10.00 (0.00-10.00)	10.00 (0.00-20.00)	H=9.02, df=4 $p=0.060$
Statistical value	H=7.28, df=2 $p\leq 0.026$	H=7.69, df=2 $p\leq 0.021$	H=8.32, df=2 $p\leq 0.016$	H=7.00, df=2 $p\leq 0.030$	H=-- $p=--$	

*A non-parametric Mann-Whitney U test was applied to the mortality data. Different uppercase letters within columns and different lowercase letters within rows indicate statistically significant differences at the 5% significance level ($p\leq 0.05$). Q1 refers to the first quartile (25th percentile) of the mortality distribution. Q3 refers to the third quartile (75th percentile) of the mortality distribution.

Time mortality relationship and probit parameters of *Stephanitis pyri* exposed to diatomaceous earth formulation

Probit analysis parameters and lethal time estimates (days) of *S. pyri* adult and fifth-instar nymphs exposed to different concentrations of the DE formulation in Table 4. The probit analysis revealed clear differences in the susceptibility of *S. pyri* adults and fifth-instar nymphs to DE treatments across tested concentrations. At the lowest concentration (2.5 g/m²), adults exhibited a shorter LT₅₀ value (1.293 days) compared to fifth-instar nymphs (2.555 days), indicating a higher susceptibility of adults to DE exposure. This difference is further supported by non-overlapping 95% confidence intervals, suggesting a statistically meaningful variation in susceptibility between developmental stages. In fifth-instar nymphs, a clear concentration-dependent decrease in lethal time values was observed, demonstrating that mortality accelerated with increasing DE concentration. The LT₅₀ values decreased from 2.555 days at 2.5 g m⁻² to 1.702 days at 5 g/m² and further to 1.205 days at 10 g/m². Similarly, LT₉₀ values decreased from 3.620 to 2.407 and 1.955 days, respectively. These results indicate a strong and consistent concentration-response relationship. However, the reduction in lethal times was most pronounced between 2.5 and 5 g/m², while further increase to 10 g/m² yielded only marginal improvement, suggesting diminishing returns at the highest concentration. From a practical standpoint, this suggests that 5 g/m² may represent an operationally efficient concentration for *S. pyri* control, balancing insecticidal efficacy with economic considerations.

The slope values of the probit regression lines, ranging from 2.07 to 3.21, provide insights into the distribution of mortality and population heterogeneity. Steeper slopes, observed at higher concentrations and in adult individuals, indicate a more uniform mortality response, whereas shallower slopes, particularly in nymphs at 2.5 g/m², reflect greater individual variability in susceptibility. The LT₉₀/LT₅₀ ratios further support this interpretation: nymphal stages displayed more synchronous mortality patterns (ratios ~1.4-1.6) compared to adults (ratio ~2.5), indicating a narrower spread of mortality times among nymphs.

Goodness-of-fit statistics (χ^2/df) ranged from 1.19 to 1.67, suggesting an acceptable fit of the probit model, although some overdispersion was observed, particularly in adult datasets. This may be attributed to variability in individual contact with DE particles. Applying a heterogeneity correction factor (\hat{c}) to standard errors could improve the reliability of parameter estimates by providing more conservative confidence intervals. Future studies are recommended to increase replication or refine the time-interval sampling regime, particularly for adult individuals, to improve the precision of lethal time estimations.

Table 4. Probit analysis parameters and lethal time estimates (days) of *Stephanitis pyri* adults and fifth-instar nymphs exposed to different concentrations of a diatomaceous earth (DE) formulation

Stage	DE Treatment (g/m ²)	LT ₅₀ (95% CIs) ^a days	LT ₉₀ (95% CIs) ^a days	Slope±S. E ^b	N ^c	df ^d	χ^2 ^e
Adult	2.5	1.293 (0.719-1.724)	3.242 (2.432-5.832)	3.21±0.60	125	23	38.3
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
5 th Nymph	2.5	2.555 (2.29-2.80)	3.620 (3.29-4.14)	2.07±0.25	250	23	32.03
	5	1.702 (1.48-1.89)	2.407 (2.17-2.81)	3.11±0.46	250	23	29.24
	10	1.205 (0.97-1.40)	1.955 (1.70-2.44)	2.92±0.50	250	23	27.38

^a95% CIs: 95% Confidence Intervals, ^bS.E: Standard Error, ^cN: Total number of individuals tested, ^ddf: Degree of freedom, ^e χ^2 : Chi-square, ^f: Since due to 100% or near-complete mortality of *S. pyri* adults at 5 and 10 g/m², LT₅₀ and LT₉₀ estimates could not be calculated.

Discussion

The pear lace bug, *S. pyri*, is a highly polyphagous and economically significant pest that attacks a broad range of fruit trees, particularly pome fruits such as apple and pear. Feeding by both nymphs and adults causes characteristic chlorotic stippling and leaf necrosis, which can lead to premature leaf drop, reduced photosynthetic capacity, and considerable yield losses (Göksu, 1964; Kivan & Aysal, 2011). Despite being ranked as the world's fourth-largest apple producer, fruit export volumes remain below potential, in part because of pest management deficiencies that can lead to yield losses of up to 65% (Oerke et al., 2012). Consequently, there is an urgent need for effective, environmentally sustainable, and stage-specific control tools for *S. pyri* that can be integrated into existing Integrated Pest Management (IPM) programs (Flint & Van den Bosch, 2012). The present study provides insights into the efficacy of Detech® WP 95 against *S. pyri* under laboratory conditions.

This study provides the first laboratory-based evaluation of diatomaceous earth (DE) as a potential control agent against both nymphal and adult stages of *S. pyri*. The results clearly demonstrate that DE exerts potent insecticidal activity across all tested concentrations (2.5, 5, and 10 g/m²), with mortality increasing as concentration and exposure time increased. Even the lowest concentration (2.5 g/m²) resulted in complete mortality of first-instar nymphs and adults within three days, while fifth-instar nymphs required approximately five days to reach 100% mortality. The observed stage-specific susceptibility is likely due to morphological and physiological differences among developmental stages. Early instars generally have thinner and less sclerotized cuticles, providing limited protection against the abrasive and desiccating effects of DE. Similar age-related differences in susceptibility to inert dusts have been reported for *Tenebrio molitor* L., 1758 (Gourgouta et al., 2022), *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), and other species (Vayias & Athanassiou, 2004; Athanassiou et al., 2006).

Probit regression analysis clarified the quantitative relationship between exposure time and mortality. Lethal time values (LT₅₀ and LT₉₀) decreased consistently with increasing DE concentration, reflecting a clear concentration-dependent acceleration of mortality. For fifth-instar nymphs, LT₅₀ declined from 2.555 days at 2.5 g/m² to 1.205 days at 10 g/m², while LT₉₀ values dropped from 3.620 to 1.955 days. These reductions indicate that higher concentrations significantly enhance the rate of desiccation-induced death.

The corresponding slope values (2.07-3.11) became steeper as concentration increased, suggesting more synchronized mortality and reduced inter-individual variability.

Adults exhibited a lower LT_{50} (1.293 days) than fifth-instar nymphs at the lowest concentration, demonstrating greater initial susceptibility; however, the adult data also showed greater variability ($\chi^2/df=1.67$), likely due to a smaller sample size and behavioral differences in contact with treated surfaces. Despite this, the goodness-of-fit values remained acceptable ($\chi^2/df=1.19-1.67$), confirming the adequacy of the probit model. Notably, LT_{50} and LT_{90} values could not be estimated for adults could not be estimated at 5 and 10 g m⁻² due to near-instantaneous mortality, implying an overwhelming toxic desiccant effect at these concentrations., implying an overwhelming toxic desiccant effect at higher doses. This pattern agrees with earlier findings for stored-product beetles exposed to DE, where excessive mortality in early observation periods prevented full regression fitting (Finney, 1971; Robertson et al., 2017). Future assays should include earlier time-point observations to improve estimation accuracy under rapid-mortality conditions. The Mann-Whitney U test further confirmed significant interactions between concentration and exposure time. For example, at 5 g/m², adult mortality reached 40% after 24 h and 100% after 48 h, reflecting a steep mortality curve typical of DE contact action. Similar time-dependent mortality increases have been recorded in stored-product pests, where extended exposure enhances cuticular abrasion and water loss (Subramanyam & Hagstrum, 2012; Aisvarya et al., 2021). Collectively, these findings confirm that DE functions as a cumulative, contact-based insecticide with strong time-concentration synergy.

The mechanism of DE particles composed of amorphous silica with sharp, porous structures abrades the waxy cuticle and absorbs surface lipids, leading to dehydration and eventual death (Korunic, 1998, 2013). As mortality driven by contact, DE affects all motile stages that come into contact with treated surfaces. The faster mortality at higher concentrations likely results from greater particle density and contact frequency, which accelerate lipid loss and dehydration. The greater susceptibility of adults at lower concentrations could be related to thinner cuticular layers or prolonged surface contact behaviors (Ebeling, 1971; Aldryhim, 1990).

Compared to other mineral-based materials such as kaolin and zeolite, DE demonstrated a distinctly stronger lethal mode of action. Kaolin, a hydrated aluminum silicate clay, is widely used as an inert barrier or particle film in horticulture but functions primarily through non-lethal mechanisms. Its fine white coating masks visual and chemical cues, creating a deterrent effect that reduces pest landing, feeding, and oviposition (Daniel et al., 2005; Glenn & Puterka, 2010). While kaolin applications have successfully reduced *Cacopsylla pyri* (L., 1758) (Hemiptera: Psyllidae) populations in pear orchards (Daniel et al., 2005) and *Monosteira uncostata* (Mulsant & Rey, 1852) (Hemiptera: Tingidae) infestations in almonds (Marcotegui et al., 2015), the effect is mainly behavioral, stemming from physical obstruction or light reflection rather than acute toxicity.

Maral (2021) further reported that kaolin and azadirachtin significantly reduced feeding and oviposition in *S. pyri* but induced only limited direct mortality. Likewise, Glenn et al. (1999) demonstrated that kaolin coatings act as a reflective film that alters microclimate and host recognition but rarely causes death. In contrast, the present study shows that DE acts primarily through direct lethality. Its abrasive, highly sorptive particles damage the integument and adsorb cuticular lipids, disrupting water balance and leading to irreversible desiccation (Korunić, 2013). The physical destruction of the cuticle ensures that mortality is inevitable once adequate contact is established, regardless of feeding behavior. From an IPM standpoint, this distinction is crucial. While kaolin and botanical products may protect crops by deterring pest activity, DE eliminates pests outright, thereby reducing population density and potentially decreasing the frequency of applications. The integration of DE with kaolin films could be particularly promising: kaolin may prevent colonization, while DE can suppress residual individuals that bypass the barrier. Such complementary use could enhance efficacy while maintaining environmental safety.

Although DE shows strong laboratory efficacy, its performance in the field can be influenced by several abiotic factors. Relative humidity is particularly critical, as DE's desiccant capacity diminishes under moist conditions due to saturation of its pores, which impairs lipid adsorption (Aldryhim, 1990). Similarly, high temperature can increase insect activity and thus enhance contact with DE particles, while low temperature may reduce efficacy by limiting movement. The particle size distribution and formulation type also affect DE performance; smaller, more porous particles generally exhibit higher insecticidal activity due to increased surface area (Korunic, 1998; Henteş & Işıkber, 2024; Bozkurt, 2025). Field trials should therefore consider climatic and physical factors to determine the optimal formulation and application timing. Kaolin faces its own environmental constraints: rainfall and wind can erode coatings, reducing their persistence, while excessive film buildup can alter leaf physiology and photosynthesis (Glenn et al., 1999). Consequently, both materials require formulation refinement, such as improved adhesion agents or micronization, to enhance durability and efficacy under variable orchard conditions.

As a mechanical insecticide, DE offers several advantages for IPM: (i) it acts through a non-chemical, physical mechanism, (ii) it is non-toxic to non-target organisms and mammals, and (iii) it has a low risk of resistance development, since its action is non-metabolic (Korunic, 1998; Wakil et al., 2025). Moreover, DE is compatible with organic production systems and can be combined with other low-risk control methods such as entomopathogenic fungi or kaolin coatings to develop integrated multi-component strategies.

The results also highlight the importance of stage-specific targeting. Because early instars were the most susceptible and adults respond quickly even at low concentrations, targeted DE applications during peak nymphal development could maximize efficacy, while minimizing treatment frequency, a core principle of sustainable pest control.

Diatomaceous earth has been widely used against stored-product pests such as *S. oryzae*, *T. castaneum*, and *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae) (Baliota & Athanassiou, 2020; Korunić et al., 2020; Wahba et al., 2025; Wakil et al., 2025). Recent studies also report its efficacy against field pests, including *M. domestica* (Islam & Rahman, 2016), *D. saccharalis* (Penn et al., 2025), and *P. interpunctella* (Predojević et al., 2025). The current study contributes to this body of research by providing new evidence on the effectiveness of DE against *S. pyri* under laboratory conditions. Future research should evaluate field performance, residual activity, and non-target effects, as well as explore formulation advancements (e.g., microencapsulation or oil-based carriers) to improve leaf adherence and rainfastness.

Overall, the present study demonstrates that diatomaceous earth is a highly effective control agent for *S. pyri*. Mortality was clearly concentration- and time-dependent, as confirmed by probit regression parameters. Adults exhibited greater initial susceptibility, while mortality of later nymphal stages accelerated markedly at higher concentrations. Compared with kaolin, DE provides a direct, irreversible killing mechanism rather than a mere deterrent effect, making it an ideal candidate for inclusion in IPM programs. The incorporation of DE into orchard management could reduce reliance on chemical insecticides, lower environmental impact, and support sustainable production. However, successful field implementation will require optimizing formulation, application timing, and integration with complementary tools to ensure consistent performance under variable environmental conditions.

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