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Oviposition Deterrent Effect of a High Quality Natural Zeolite for the Olive Fruit Fly *Bactrocera oleae*, Under Different Conditions of Temperature and Relative Humidity

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Simple Summary: In pest control, the number of available chemical pesticides has been dramatically reduced the last two decades due to their possible negative effects on human health and environment. Therefore, there arises an urgent need for discovery of alternative to chemical pesticides products for pest control, as among others the natural zeolites (zeolitic rocks). The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), is one of the most harmful pests of olives in Mediterranean countries, and worldwide. In this study, we evaluated the oviposition deterrent effect of a natural zeolite on the olive fruit fly, under different temperature and relative humidity conditions. The tested natural zeolite was highly effective in deterring egg laying of *B. oleae* females, under all tested conditions. These findings are poised to advance our understanding of sustainable and eco-friendly strategies for pest control in olive orchards, presenting opportunities for the development of targeted interventions that align with both environmental conservation and effective agricultural practices.

Abstract: In recent years the number of available chemical pesticides has been significantly reduced and there have been an urgent need for discovery of alternative to chemical pesticides products, as among others the natural zeolites (zeolitic rocks). We determined the mineralogical and chemical composition of a specific and continuous layers of zeolitic rock sample (ZeotP) from Petrota, Evros, Greece and evaluated its oviposition deterrent effect for the olive fruit fly *Bactrocera oleae* (Diptera: Terphritidae). The tested natural zeolite contained 70 wt. % Clinoptilolite, 18 wt. % Amorphous material, 7 wt. % Feldspars, 4 wt. % Cristobalite and 1 wt. % Quartz. We tested the oviposition deterrent effect of ZeotP mixed or not with an emulsifier adjuvant NU-FILM-P® in water and applied on the surface of olive fruits. The ZeotP oviposition deterrent effect for the olive fly was very high under a series of tested temperatures (17°C, 20°C, 25°C and 30°C) and RHs (23%, 33%, 55%, 75%, 94%). In addition, the ZeotP residual deterrent effect after equable water spraying was high and similar to the respective effect of a pyrethroid insecticide (Decis®). Our results may contribute to an effective control of the olive fruit fly using an alternative to chemical pesticides natural zeolite (zeolitic rocks) product.

Keywords: olive fly; control; oviposition deterrent effect; natural zeolite (zeolitic rock); clinoptilolite; temperature; relative humidity

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1. Introduction

The continuous efforts for a greener and more sustainable world, in order to protect the environment from industrial and agricultural uses, lead among others to the use of alternative to chemicals methods, efficient and friendly to the environment and public health. In agriculture, and specifically in pest control, the available chemical products (insecticides, herbicides, fungicides) have been significantly reduced in the last decades due to their multiple negative effects, such as soil and water contamination, toxicity to animal and humans and inefficient pest control due to the development of resistant populations [1,2]. Therefore, the development and use of alternative to chemical pesticides products such as the inert-sorbent materials (mineral- and dust-based products) could be a promising solution. In recent years, many studies have confirmed the effectiveness of inert dust such as kaolin, for the control of crop and stored products pests [2–4]. There are limited studies, however, concerning the insecticidal effect of zeolites and their use for pest control.

Zeolites are in abundance among microporous materials worldwide and characterized as hydrous, microporous crystalline aluminosilicate minerals of alkali and alkaline earth elements. They consist of a unique three-dimensional framework structure, with bonding silica (SiO_4) and alumina (AlO_4) tetrahedral units, connected together with the sharing of all the oxygen atoms. They can be also defined as crystalline inorganic polymers, with their frameworks forming pores and channels containing significant contents of water molecules, and exchangeable cations (calcium, potassium, sodium etc.) [1–5].

Approximately 67 types of zeolites occur naturally, and more than 200 types are synthetically prepared in the laboratory. Natural zeolites are hydrothermal and of mainly volcanic origin. They can occur both in crystallized forms found in igneous and metamorphic rocks, as well as in grains of smaller diameters accumulated in sedimentary rocks [6,7]. The main rock-type, which contains great amounts of zeolite minerals, is characterized as zeolitic rock. In nature, apart from the fibrous zeolites (erionite, mordenite, roggianite, mazzite, etc.), which are hazardous for animal and human health, other non-toxic types exist such as chabazite, phillipsite, clinoptilolite, heulandite, analcime. Among them, HEU-type zeolites (clinoptilolite – heulandite) are of great research and economic interest. They are characterized by the presence of tabular crystals (typically 1-100 μm in size), and the presence of micro/nano-pores, that create infinite three-dimensional frameworks of silica, alumina and oxygen atoms in a structure of 10- and 8-membered rings of dimensions (7.5×3.1 Å (V=23.25 $Å^3$), 4.6×3.6 Å (V=16.56 $Å^3$), and 4.7×2.8 Å (V=13.16 ^{Å3}) [7,8].

Due to their unique characteristics such as their porosity and ion-exchange properties, as well as their abundance, zeolites have generated worldwide interest for their use in a wide range of applications. Natural zeolites have been broadly used in industry, as catalysts, ion exchangers, absorbents for oil and spills, separation agents and agents for the removal of heavy metals from wastewaters [5,9–13]. Due to the International Agency for Research on Cancer (IARC) and Food and Drug Administration (FDA) classification of zeolites as, respectively, "non-toxic" and safe for human consumption, they are extensively used for agricultural and horticultural purposes, among others, as fertilizers, slow releasing carriers of fertilizers and insecticides, feed additives, in soil amendment, water purification and gas absorption [14,15].

The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), is the most harmful pest of olives worldwide causing serious damage to olive production [16]. It is a monophagous pest, as its larva feeds exclusively on olive fruit [17]. Adult females lay their eggs inside the olive fruit, and hatching larvae feed in the mesocarp causing fruit damage and olive oil quality deterioration [18,19], as well as premature fruit drop [20]. In Mediterranean regions, there may be more than five overlapping generations from late summer through the winter and early spring if fruits are present on the trees and field temperatures are suitable for development [21–23].

The olive fly's behavior is strongly dependent on its host fruit and is related to various physical and chemical plant stimuli. The olive fruit favors the ovarian maturation of the females due either to contact or volatile stimuli, as well as to bacteria that are present on its surface [21,24–26]. In addition, olive fruit, due to its volatile and contact chemical stimuli, favors mating success and egg production of the olive fly [23,27]. Certain olive fruit's organic volatile compounds such as n-octane and αpinene, as well as a mixture of n-octane, α-pinene, limonene, ethyl hexanol, nonanal, n-dodecane, decanal and n–tetradecane favored successful reproduction of the olive fruit fly [27].

The control of the olive fly is mainly based on the extensive use of chemical insecticides, which usually results in the development of olive fly populations with high levels of resistance and failure of effective control, and the presence of chemical residues in olives and olive oil [17]. It is, therefore, of crucial importance to develop innovative and environmentally safe methods and products for the control of the olive fly.

Previous studies have shown that inert dusts i.e., kaolin, diatomaceous earth (DE), and natural zeolites have an insecticidal efficacy with partially removing an insect's outer cuticle (epicuticule) through abrasion by hard non-sorptive particles or by disrupting the epicuticle via absorption of epicuticular lipids to sorptive particles [28]. Both processes induce rapid water loss from the insect's body and cause death by dehydration [29–31]. Natural zeolite was found to have a high insecticidal effect, when applied as dust, to wheat seeds infested with the rice weevil *Sitophilus oryzae* and the red flour beetle *Tribolium castaneaum*, and found high mortality percentages [31]. Zeolites are also toxic against other insect pests that infest stored products, such as *Tribolium confusum*, *Oryzaephilus surinamensis* the maize weevil *Sitophilus zeamais* and three other beetle pests in stored wheat [32–34].

In a recent preliminary study carried out by our research group, a high-quality Greek natural zeolite was found to have a high insecticidal effect for adults of the bean weevil *Acanthoscelides obtectus* [29]. This high-quality natural zeolite contained 92 wt. % HEU-type (clinoptilolite), 3 wt. % Mica + Clay minerals, 3 wt. % Quartz and 2 wt. % Feldspars, and has a high insecticidal effect under various temperature and humidity conditions [29].

The use of alternative to chemical insecticides products, such as inert dusts, with olive fly deterrent/repellent effects may contribute to overcome the negative consequences of the use of pesticides and efficiently protect olive production [17]. The use of oviposition repellent products, such as the earth inert dusts (kaolin, natural zeolites), appeared to be promising non-chemical methods for the control of insect pests. Kaolin is an inert dust which has been extensively used commercially with the trade name Surround® for the control of the olive fly [35]. Visual and chemical stimuli lead the female olive fly to oviposit into olive fruits [23,27,36–38]. Kaolin is commercially used against the olive fly, deterring egg laying, as the presence of the particles of the product on the fruit surface could be an obstacle for the olive fruit recognition by the females [39,40].

The aim of the present study is to characterize and test the insecticidal effect of a novel Greek natural zeolite product. We determined the particular mineralogical and chemical composition of a high quality Greek natural zeolitic rock, and evaluated its oviposition deterrent effect for the olive fruit fly *B. oleae* under different temperature and RH conditions.

2. Materials and Methods

2.1. Stock colony and experimental flies

Our laboratory colony of *B. oleae* was established with adults emerged from field-collected olive fruits in October 2021 from the area of Chalkidiki, northern Greece. The stock colony flies were maintained in wooden cages (30 x 30 x 30 cm) with wire screen sides in a climatic room at 25°C, relative humidity (RH) of 65% and a 16L:8D photoperiod. The experimental flies were second-generation descendants of the stock colony. They were reared from egg to pupae in olive fruits which were collected in early August and since then, have been maintained at 5 ± 1°C for 2–3 months before being offered to the stock flies for oviposition. For the experiments, stock females oviposited in olive fruit of the variety Megaritiki. After oviposition, the infested olives were placed in plastic cups, covered with wet hessian fabric, and kept in an incubator at a constant temperature of 20 °C, 70% relative humidity (RH) and 16L:8D photoperiod. The newly formed pupae were collected daily and placed in Petri dishes. Emerging females and males were maintained in wooden cages like those used for the stock colony at 25°C, 70% RH and 16L:8D photoperiod, and used in the experiments when they were ten days old. Stock and experimental flies were provided ad libitum with a protein liquid diet of water, yeast hydrolysate and sugar (v: w: w 5: 4: 1).

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2.2. Natural zeolite characterization

The natural zeolite sample (ZeotP) used in our experiments was collected from specific and continuous layers of zeolite-rich volcaniclastic tuffs in the surrounding area of Petrota, Evros region, northern Greece. Petrographic investigation of ZeotP was performed on a thin section by polarized microscope. The mineralogical composition was determined by X-Ray diffraction method (XRD), with a Philips PW1710 diffractometer equipped with Ni-filtered CuKα radiation on randomly oriented sample, from 3° to 63° 2θ at a scanning speed of 1.2°/ min. Chemical composition of ZeotP was performed using the ICP-MS method. The cation exchange capacity (sorption ability) was measured by the ammonium acetate saturation (AMAS) method [41].

2.3. Oviposition deterrent test effect

A portion of ZeotP with the appropriate grain size distribution (<63 μm) was prepared for the experiments. ZeotP was pulverized in a mechanical agate mortar for approximately 10 minutes, and then sieved to a size <63 μm in a mechanical sieve shaker, for 15 minutes. The procedure was repeated until the entire amount of natural zeolite was sieved, and the processed dust was collected.

ZeotP dust suspended in water (5 g dust to 100 ml water) with or without the addition of the adjuvant NU-FILM-P® (1,5 %). For the experiments, olive fruits were immersed in ZeotP suspensions in glass jars for 20 seconds or as control in water. After immersion, the fruits were allowed to dry on the surface of a metal sieve, and then were transferred to the base of a plexiglass cage (20 X 20 X 20 cm). In each cage, 5 adult females of *B. oleae* and 5 olives were transferred. To evaluate the oviposition deterrent effect of the tested natural zeolites, we scored under a stereoscope (Zeiss Stemi 305®), the number of oviposition holes on the olive fruits after 3 and 6 days of olives' exposure to the egg-laying females. In each treatment, there were four replicates (cages with olives and adult females). The experiments were carried out in a climatic room with a temperature of 25°C and a 16L:8D photoperiod.

2.4. Effect of temperature

The oviposition deterrent effect of ZeotP with the adjuvant NU-FILM-P® was evaluated at four constant temperatures using the same experimental procedure explained in the above paragraph. The plexiglass cages with the treated olive fruits and the experimental females were maintained in climatic incubators at a series of constant temperatures (17°, 20°, 25° and 30 °C) with a 16L:8D photoperiod. In each tested temperature, there were four replicates, i.e., plexiglass cages with 5 adult females and 5 olive fruits.

2.5. Effect of relative humidity (RH)

In this series of experiments, the oviposition deterrent effect of ZeotP was evaluated under 5 different relative humidity (RH) levels. For the experiments, the female flies with ZeotP treated olives were maintained at different RH levels (23, 33, 55, 75 and 94%), at 25 °C and a 16L:8D photoperiod. To maintain the flies at different RHs, we used sealed plastic containers (30 × 40 × 30 cm) with an appropriate saturated water salt solution at their bottom (5 cm deep) [42,43]. A metal net was placed approximately 3 cm above the surface of the salt solution and served as the floor for the cages with experimental flies and olives. These pot cages were modified cylindrical cups of 430 cm^3 volume (6.5 × 9.5 × 10 cm, diameter top × diameter base × height) with two circular openings (diameter: 2 cm) covered with nylon mesh to allow adequate ventilation. The cups were placed upside down on a petri dish bearing five equidistant circular holes (diameter: 1.5 cm) where the treated olive fruits were placed. The pot cages with the *B. oleae* females and the treated olives were maintained for 3 and 6 days at the different RH levels and subsequently, the number of oviposition holes on the fruits was scored. The saturated salt solutions used in the experiments and the respective RHs inside the cages are shown in Table 1. In each RH level, there were four pot cages (replicates) housing five *B. oleae* females and five olive fruits.

Table 1. Saturated salt solutions used with the corresponding RHs and saturation deficit.

Solutions	Relative humidity (%)	Saturation deficit (kPa)
$LiCl.H_2O$	23	2.96
$MgCl_2.6H_2O$	33	2.25
$Mg(NO_3)_2.6H_2O$	55	1.51
NaCl	75	0.84
KNO_3	94	0.17

2.6. Residual oviposition deterrent effect of ZeotP on olives after water spraying

We determined the residual oviposition deterrent effect of ZeotP after spraying the treated olives with water. For the experiments, olives were immersed in ZeotP, with and without the NU-FILM-P® adjuvant, as described in the aforementioned paragraph. After ~1 h of the ZeotP application, the treated olives were placed in glass petri dishes 9 cm in diameter in the basement a potter precision spraying tower (Buckard Manufacturing Co Ltd. Rickmansworth, Hertfordshire, UK) calibrated at 0,68 atm pressure, and sprayed with two constant quantities, 5 ml or 15 ml of water/min. As control, we used olives treated with the insecticide Decis® with or without NU-FILM-P® adjuvant. After water spraying, the olive fruits were transferred to plexiglass cages with *B. oleae* adult females, and the number of oviposition holes on the olives was scored after 3, 6 and 9 days. In each treatment, there were four replicates, i.e., plexiglass cages with 5 adult females and 5 olive fruits. The experiments were carried out at a climatic room with a temperature of 25°C and a 16L:8D photoperiod.

2.7. Statistical analysis

A one-way ANOVA was performed to detect whether temperature, relative humidity and water spraying affect the oviposition deterrent effect of the tested natural zeolite. Subsequent significant differences ($P < 0.05$) of means were further separated with Student–Newman–Keul's test using the IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA). Before data analysis, Kolmogorov–Smirnov's and Levene's tests were used to confirm normality and homogeneity of variances, respectively. In cases in which heterogeneity of variances was significant, appropriate data transformation (log transformation) was conducted before the analysis. In those cases where the transformations failed to satisfy the criteria for parametric analysis, the Kruskal–Wallis non-parametric ANOVA followed by the Mann-Whitney U test for all possible pairwise comparisons, was conducted.

3. Results

3.1. Natural zeolite characterization

Semi-quantitative estimates of the abundance of the mineral phases were derived from the XRD data using the intensity (counts) of certain reflections, as well as the density and mass absorption coefficient for CuKa radiation of the minerals present [11]. As shown in Table 2, ZeotP consists of 70 wt% HEU-type zeolite (clinoptilolite), 1 wt% Quartz, 4 wt% Cristobalite, 7 wt% Feldspars (K-feldspars and plagioclase), and 18 wt% amorphous material. The natural zeolite sample can be characterized as clinoptilolitic zeolitic rock. The cation exchange capacity (sorption ability) was measured 193 meq/100g.

Table 2. Semi-quantative mineralogical composition (wt.%) and ion exchange capacity (meq/100g) of Petrota natural zeolite sample ZeotP.

Sample	ZeotP
HEU-type zeolite (clinoptilolite)	70
Quartz	1
Cristobalite	4

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Feldspars	7
Amorphous material	18
Total	100
Ion exchange capacity (meq/100g)	193

Regarding the chemical composition (Table 3), ZeotP contains 68.43 wt.% SiO_2 and 11.60 wt.% Al_2O_3. The content of CaO is 3.80 wt.%, K_2O is 2.12 wt.%, whereas MgO content is 0.96 wt.%. Low amounts of Na_2O, Fe_2O_{3T} and MnO were measured.

The mineralogical composition aligns both with the chemical composition and cation exchange capacity. Except SiO_2 and Al_2O_3, ZeotP contains sufficient amounts of CaO and K_2O (Table 3).

Table 3. Chemical composition of Petrota natural zeolite sample ZeotP.

Sample	SiO_2	Al_2O_3	Fe_2O_{3tot}	MnO	MgO	CaO	SrO	BaO	Na_2O	K_2O	H_2O	Total
ZeotP	68.43	11.60	bdl*	bdl	0.96	3.80	bdl	bdl	0.03	2.12	13.03	99.97

* bdl: below detection limit.

3.2. ZeotP oviposition deterrent effect

The application on olives of an aqueous solution of ZeotP with and without NU-FILM-P® resulted in a significant decrease in the number of eggs laid on them. As shown in Table 4, when the female flies had access to olive fruits for 3 and 6 days, a mean number of 107.0 and 168.5 eggs respectively were laid in the non-treated olives whereas no eggs were laid in olives treated with a mixture of ZeotP and NU-FILM-P®. An intermediate mean number of 16.2 and 59.0 eggs were laid in olives sprayed with ZeotP without NU-FILM-P®. These results show that the addition of NU-FILM-P® adjuvant significantly increased the oviposition deterrent effect of ZeotP.

Table 4. Mean (±SE) number of *B. oleae* oviposition holes in olives immersed to 5% ZeotP aqueous solution, with and without NU-FILM-P® adjuvant. ZeotP treated and non-treated (control) olives were maintained in cages with 5 *B. oleae* females and the number of oviposition holes was determined after 3 and 6 days at a temperature of 25°C and a 16L:8D photoperiod.

Product	Mean (±SE) number of eggs (oviposition holes) after 3 days	Mean (±SE) number of eggs (oviposition holes) after 6 days
Control	107.0 ± 8.4a*	168.5 ± 12.2a
ZeotP without NU-FILM-P®	16.2 ± 6.4ab	59.0 ± 15.7ab
ZeotP with NU-FILM-P®	0.0 ± 0.0b	0.0 ± 0.0b

* Means in a column followed by the same letter are not significantly different ($P > 0.05$).

3.3. Effect of temperature

As shown in Table 5, at all the tested temperatures, there was a significant oviposition deterrent effect of ZeotP (with or without the addition of NU-FILM-P®). The number of eggs laid in olives, after the application of ZeotP with or without NU-FILM-P®) was very low to zero, in all the tested temperatures. These results show that, irrespective of temperature conditions, the oviposition deterrent effect of ZeotP is high and in particular when applied in combination with the adjuvant NU-FILM-P®.

Table 5. Mean (±SE) number of *B. oleae* oviposition holes on olives immersed to 5%/ ZeotP aqueous solution, with NU-FILM-P® adjuvant, at four (17, 20, 25, 30°C) constant temperatures. ZeotP treated and non-treated (control) olives were maintained in cages with 5 *B. oleae* females and the number of oviposition holes was determined after 3 and 6 days at a 16L:8D photoperiod.

Temperature	Mean (±SE) number of eggs (oviposition holes) after 3 days		6 days	
	Control	ZeotP with NU-FILM-P®	Control	ZeotP with NU-FILM-P®
17°C	13.5 ± 7.7ab*	0.0 ± 0.0e	35.0 ± 17.0ac	0.2 ± 0.2e
20°C	9.7 ± 2.9b	0.0 ± 0.0e	37.5 ± 10.5ac	0.0 ± 0.0e
25°C	81.5 ± 30.8d	0.2 ± 0.2e	129.0 ± 28.7b	0.7 ± 0.7e
30°C	49.2 ± 14.9cd	0.2 ± 0.2e	100.7 ± 22.2bd	1.2 ± 1.2ef

* Means in two consecutive columns followed by the same letter are not significantly different (P > 0.05).

3.4. Effect of relative humidity (RH)

As shown in Table 6, in all RHs tested, there was a significant oviposition deterrent effect of ZeotP. The number of eggs laid in olives after the application of ZeotP was significantly lower compared to the control, at all levels of relative humidity (23, 33, 55, 75 and 94% RH).

Table 6. Mean (±SE) number of *B. oleae* oviposition holes in olives immersed to 5% ZeotP aqueous solution, with NU-FILM-P® adjuvant, at five different RH levels (23, 33, 55, 75 and 94%). ZeotP treated and non-treated (control) olives were maintained in cages with 5 *B. oleae* females and the number of oviposition holes was determined after 3 and 6 days, at a temperature of 25°C and a 16L:8D photoperiod.

RH %	Mean (±SE number of eggs (oviposition holes) after 3 days		6 days	
	Control	ZeotP with NU-FILM-P®	Control	ZeotP with NU-FILM-P®
23	33.2 ± 17.0ac*	4.7 ± 2.7d	47.5 ± 21.4ab	9.5 ± 0.6d
33	34.5 ± 10.4ab	9.2 ± 1.1df	69.5 ± 8.3bc	10.0 ± 1.1de
55	73.2 ± 10.2c	4.2 ± 1.9d	84.2 ± 5.6bc	7.2 ± 3.1d
75	60.7 ± 8.4ce	3.0 ± 2.3d	90.5 ± 2.7c	6.5 ± 3.1d
94	27.0 ± 3.3bd	7.0 ± 1.1df	43.0 ± 3.4a	11.0 ± 1.1de

*Means in two consecutive columns followed by the same letter are not significantly different (P > 0.05).

3.5. Residual oviposition deterrent effect of ZeotP on olives after water spraying

As shown in Table 7, after spraying with water the ZeotP treated olives, the oviposition deterrent effect of ZeotP, with or without NU-FILM-P®, was remained high, and the number of eggs laid in the fruits was significantly lower than the respective number of eggs laid in the control (non-treated olives). However, in almost all the cases, the addition of NU-FILM-P® in ZeotP suspension, resulted in a higher residual oviposition deterrent effect after water spraying. The residual ZeotP oviposition deterrent effect after water spraying was similar to the respective effect of the pyrethroid insecticide Decis®.

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Table 7. Residual oviposition deterrent effect of ZeotP after spraying with water the treated olive fruit. Olives were immersed to 5% ZeotP with or without NU-FILM-P® and subsequently sprayed with 5 and 15 ml of water. Then, the olives were maintained in cages (5 olives with 5 *B. oleae* females) and the number of oviposition holes was determined after 3, 6 and 9 days. Temperature 25°C, 16L:8D photoperiod.

Product	Water Volume (ml)	Mean (±SE) number of eggs (oviposition holes) after 3 days		6		9	
		With NU-FILM-P®	Without NU-FILM-P®	With NU-FILM-P®	Without NU-FILM-P®	With NU-FILM-P®	Without NU-FILM-P®
ZeotP	0	0.0 ± 0.0a*	7.5 ± 4.1ab	0.0 ± 0.0a	39.7 ± 12.8ac	0.0 ± 0.0a	56.5 ± 14.1a
	5	1.0 ± 0.7a	10.5 ± 6.1b	1.7 ± 0.5a	34.2 ± 19.9a	3.0 ± 1.1ab	42.3 ± 14.6b
	15	0.0 ± 0.0a	13.8 ± 5.5b	0.0 ± 0.0a	41.8 ± 8.0b	1.8 ± 1.4a	60.8 ± 20.5bc
Decis®	0	2.8 ± 0.9ab	4.5 ± 1.8ab	3.0 ± 1.1ab	16.5 ± 7.0ab	4.7 ± 2.1b	32.2 ± 18.0b
	5	1.0 ± 1.0a	13.5 ± 6.2b	1.5 ± 1.2a	36.2 ± 11.6b	6.2 ± 0.9c	66.2 ± 18.7c
	15	3.0 ± 1.3a	0.8 ± 0.8a	4.5 ± 0.5b	4.5 ± 4.5d	4.5 ± 2.4bc	22.5 ± 10.7a

* Means in a column followed by the same letter are not significantly different (P > 0.05).

4. Discussion and conclusions

Inert dusts such as kaolin, diatomaceous earth (DE) and natural zeolites have an insecticidal efficacy which is mainly due to disruption of the insect's outer cuticle (epicuticule) through abrasion by hard non-sorptive particles or by adsorption of epicuticular lipids to sorptive particles [28]. Both processes induce rapid water loss from the insect's body and cause death by dehydration [29]. The physical properties of natural zeolites such as particle size, shape, surface area etc., as well as the chemical and thermal stability that depends on the Si/Al ratios (i.e., the least stable natural zeolites have low Si/Al ratios) affect their activity [29]. Here, we used a high quality natural zeolite with unique characteristics such as high content of clinoptilolite and microporous minerals, high cation exchange capacity (sorption ability) and content of K and Ca.

Zeolites, are highly toxic against a number of insect pests that infest stored products, such as *Sitophilus oryzae* and *Tribolium castaneaum* [30], *Tribolium confusum* and *Oryzaephilus surinamensis* [31], *Sitophilus zeamais* [32,33] and bean weevils *Acanthoscelides obtectus* [29]. Among the earth's inert dusts, kaolin has been extensively used commercially with the trade name Surround® for the control of the olive fly [34]. Our present results show that ZeotP, after appropriate processing, and application in the form of aqueous suspension on the surface of the olive fruit, can significantly prevent the egg laying of the olive fruit fly and therefore protect the olives' injury. In addition, the adjuvant NU-FILM-P®, mixed with ZeotP significantly increases the oviposition deterrent effect. The XRD analysis of ZeotP has shown that quartz is in very low quantities (1 wt. %), and fibrous minerals are completely absent which makes ZeotP safe for human health.

Previous studies of our group have shown that a high-quality Greek zeolite has a high insecticidal activity for the bruchid *Acanthoscelides obtectus*, under different temperature and RH conditions [29]. Here, we found that ZeotP, mixed with the adjuvant NU-FILM-P® and applied on olives, has a high oviposition deterrent effect, under different temperature and RH conditions. This high oviposition deterrent effect of ZeotP with NU-FILM-P® is maintained even after spraying with water the ZeotP treated olives, and is similar to the respective effect of the pyrethroid insecticide Decis® (deltamethrine), which is extensively used in the field against the olive fly.

In conclusion, the studied high quality zeolitic rock, rich in HEU-type zeolite, has a high oviposition deterrent effect against the olive fly *B. oleae*, particularly in preventing adult females from laying their eggs on olive fruits. This prevention of oviposition may be due to the creation of a thin layer of natural zeolite on the surface of the olives which seems to mechanically prevent females to adhere to the surface of the fruit and lay their eggs. Adult females lay their eggs inside the olive fruit, and the hatching larvae feed in the mesocarp causing fruit damage and olive oil quality deterioration [18,19], as well as premature fruit drop [20]. The thin layer of ZeotP formed on the olive surface prevents the oviposition process and fruit injury at a range of different temperatures and RH levels. The combination of ZeotP with an adjuvant (NU-FILM-P®) increased the oviposition deterrent effect and is a promising approach for the olive fly control.

The use of alternatives to pesticides methods, such as natural zeolite products with olive fly oviposition deterrent effect, may contribute to overcome the negative consequences of the use of chemical pesticides and efficiently protect olive production [17]. Field experiments with ZeotP, according the requirements of European legislation for the specific applications, are required to confirm its oviposition deterrent effect and efficacy for the control of the olive fly.

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