

Article

Biocontrol Potential of Microfighter: A Zeolite-Based Product Enriched with *Pseudomonas synxantha* DSL65

Elena Cudazzo ^{1,2}, Lucia Morrone ^{2,*} , Giacomo Ferretti ³ , Barbara Faccini ¹ , Daniele Mirandola ⁴, Luca Fagioli ⁴ and Annalisa Rotondi ² 

¹ Department of Environmental and Prevention Sciences, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy; elena.cudazzo@unife.it (E.C.); barbara.faccini@unife.it (B.F.)

² Institute of BioEconomy, National Research Council (IBE-CNR), Via Gobetti 101, 40129 Bologna, Italy; annalisa.rotondi@ibe.cnr.it

³ Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy; giacomo.ferretti@unife.it

⁴ Experimental Centre of the Agricultural Consortium of Ravenna, Via Madonna di Genova 39, 48033 Cotignola, Italy; mirandola@consorzioagrarioravenna.it (D.M.); fagioli@consorzioagrarioravenna.it (L.F.)

* Correspondence: lucia.morrone@ibe.cnr.it

Abstract

Particle film technology is an environmentally sustainable crop protection method, offering an alternative to chemical pesticides for disease control. Copper-based compounds have long been central to the management of bacterial and fungal diseases, particularly in organic agriculture. However, due to their environmental persistence, their use has been increasingly restricted by European regulations, making the management of widespread diseases such as Olive Knot (*Pseudomonas savastanoi* pv. *savastanoi*) and Downy Mildew (*Plasmopara viticola*) more difficult. The LIFE Microfighter project addresses this problem by testing a novel Zeo-Biopesticide (ZBp), in which natural zeolite serves as a carrier for the beneficial bacterium *Pseudomonas synxantha* DLS65. Field trials conducted in high-rainfall areas of Emilia-Romagna (Italy) evaluated the product's distribution and persistence on olive and grape leaves through ESEM (Environmental Scanning Electron Microscopy) observations, its ability to retain the microorganism, and its effectiveness for disease control. Results showed that ZBp significantly reduced Olive Knot incidence compared to both the untreated control and Cu-based treatments ($p < 0.05$), supporting its potential as an alternative for bacterial disease management, while showing no statistically significant difference compared to the control in either the incidence or severity of Downy Mildew ($p > 0.05$). Its persistence and adherence to plant surfaces, which could influence its overall field performance, were affected by environmental conditions, particularly rainfall.

Keywords: olive; grapevine; zeolite; biocontrol



check for updates

Academic Editor: Guodong Lu

Received: 30 May 2025

Revised: 20 June 2025

Accepted: 25 June 2025

Published: 27 June 2025

Citation: Cudazzo, E.; Morrone, L.; Ferretti, G.; Faccini, B.; Mirandola, D.; Fagioli, L.; Rotondi, A. Biocontrol Potential of Microfighter: A Zeolite-Based Product Enriched with *Pseudomonas synxantha* DSL65.

Agronomy **2025**, *15*, 1563. <https://doi.org/10.3390/agronomy15071563>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Particle film technology consists of aqueous formulations containing mineral particles, capable of forming a protective film to coat agricultural and horticultural crops [1]. It offers a sustainable and environmentally friendly alternative to chemical pesticides for pest management and control of abiotic stresses [2].

Kaolin and zeolites are common natural minerals used for this purpose. Their insecticidal properties have been extensively documented [3–6]. Glenn et al. [7] proved that insect behavior is significantly altered when insects interact with kaolin-coated plants due to a

physical barrier that impairs their ability to detect and identify host plants. Similar results have been documented by Daniel et al. [8] who used kaolin for treatments against *Cacopsylla pyri* L. during its initial flight period, leading to a significant decrease in oviposition when compared to the untreated control. Andric et al. [9] assessed the insecticidal properties of two natural zeolites on adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst): the study reported up to 100% insect mortality after 21 days of exposure to wheat treated with zeolites which damage the epicuticle causing desiccation. These desiccant properties are also advantageous for controlling fungal or bacterial pathogens. Percival et al. [10] identified zeolites as an effective preventive measure in disease management of apple scab, caused by *Venturia inaequalis*, through a mechanism analogous to that of kaolin. Their study highlighted the capacity of zeolites to absorb dew on plant surfaces, thereby disrupting the formation of the thin aqueous film critical for the germination and proliferation of many pathogenic microorganisms.

Mineral particles can be advantageous both independently and as carriers for synthetic insecticides or microbial agents [2]. Kvachantiradze et al. [11] utilized zeolites as a photostabilizer in combination with *Bacillus thuringiensis* for treatments against flour moth larvae, achieving a 100% mortality rate with the application of the complex. Kefalogianni et al. [12] observed that treating aubergine with *Bacillus* SP10 in combination with zeolite significantly reduced the severity of *Verticillium dahliae* infection compared to both the control and the treatment with *Bacillus* SP10 alone. However, the use of zeolite alone was equally effective in reducing disease severity.

Mineral particle films, such as natural zeolites, require optimal coverage, since they are non-systemic products that only exert toxic effects on treated portions of the leaf surface. Newly developed canopy also remains unprotected, so it needs further applications [13]. To achieve optimal performance, both surfaces of the leaf should be coated with the particle film [2]. Achieving a uniform particle distribution across the canopy remains technically challenging. Existing application methods often result in unequal deposition, with significant differences in coverage between leaves and between their upper (adaxial) and lower (abaxial) surfaces. This heterogeneity in particle coverage may result in differential physiological responses at the leaf level, which could have substantial implications for plant performance and the effectiveness of agricultural practices relying on particle-based treatments. Deposition patterns depend on several variables, including particle size, spray droplet size, droplet contact angle, method of application, product concentration, and cuticular characteristics of the treated surface like wettability or surface micro-roughness [13–15]. Low rainfall regions are better suited for this technology due to the reduced leaching risk, which in turn lowers treatment costs [16].

This technology represents a promising alternative for pest management [13], particularly in organic farming, where strict regulations limit the use of many conventional products. Among these, copper-based pesticides have historically played a crucial role in fungal and bacterial disease control within organic agricultural systems [17]. However, due to their environmental persistence and accumulation, their use has been progressively restricted under European legislation since 1991 [18]. This regulatory shift has made it increasingly difficult to manage diseases such as Olive Knot (*Pseudomonas savastanoi* pv. *savastanoi*) in olive orchards and Downy Mildew (*Plasmopara viticola*) in vineyards, both of which have traditionally relied on high copper inputs [19,20]. Olive Knot is a widespread bacterial disease that considerably reduces olive yield by inducing the formation of galls, which in turn affect tree vigor and overall production. The bacterium enters the host plant through wounds or natural openings and thrives in conditions of high humidity, making it challenging to control [20,21], particularly under organic farming constraints. Similarly, Downy Mildew (*Plasmopara viticola*) is one of the most devastating grapevine diseases,

induced by an obligate oomycete and infecting leaves, and having the potential to cause rapid defoliation, reduced crop yields, and lower fruit quality, especially under humid conditions [19]. Current restrictions [22] highlight the urgent need for more sustainable and effective alternatives to copper-based formulations.

The LIFE Microfighter project was developed to bridge this gap by evaluating the efficacy of a novel Zeo-Biopesticide (ZBp). This formulation employs natural zeolite as a carrier for the microorganism *Pseudomonas synxantha* DLS65, selected by Tontou et al. [23]. The present field study was conducted in high-rainfall provinces of the Emilia-Romagna region (Italy) and aimed to (i) examine the distribution of zeolite combined with the microorganism on olive and grape leaves via ESEM (Environmental Scanning Electron Microscopy) observations; (ii) quantify leaf coverage and assess the effects of rainfall-induced leaching; (iii) determine zeolite's function as a carrier using SEM-FEG analysis (Field Emission Gun Scanning Electron Microscopy); and finally, (iv) monitor the onset and progression of each disease in both crops. Zeolite enriched with *Pseudomonas* indicated potential in decreasing olive knot symptoms, while no visible impact was observed on *Plasmopara viticola* in grapevine.

2. Materials and Methods

2.1. Zeo-Biopesticide Description

The product consists of micronized Italian natural K-chabazite zeolite, with a zeolite content above 50%, consequently defining the tuffs as zeolitite. In the present study, chabazite is the predominant constituent ($70\% \pm 5\%$), characterizing the material as a chabazitic-zeolitite. The zeolitite is marketed by Verdi S.p.A. (Castelnovo di Sotto, Reggio Emilia, Italy), and its mineralogical composition determined by X-ray diffraction is given in Supplementary Material Table S1. In addition, the product contains *Pseudomonas synxantha* DLS65 [23]. Symbiagro S.r.l. (Via dell'Artigianato 1/A, 25030 Roncadelle, Brescia, Italy) carried out a survivability test, included in Supplementary Table S2, to validate the compatibility between these two components and finalized the development of the product formulation using its patented proprietary methodology. The results, shown in Supplementary Table S2, indicated that bacterial concentration was higher during the first 14 days (7.4×10^9 CFU/g) and remained relatively stable after up to 12 months of storage (1.5×10^8 CFU/g).

2.2. Site Description, Product Applications and Sample Collections

For each crop, the testing area has been treated as follows: 0.1 ha, no treatment of copper to check the severity of the disease (Cu0); 0.4 ha with conventional copper treatment (Cu_100); and 0.1 ha with Zeo-Biopesticide, without any addition of copper.

2.2.1. Olive

Field trials were carried out in Santarcangelo di Romagna (Province of Rimini, Italy; $44^{\circ}2'52.73''$ N, $12^{\circ}23'4.14''$ E). Climatic data from 1991 to 2020 indicate a mean annual precipitation (MAP) of 766.8 mm and a mean annual temperature (MAT) of approximately 13.9°C [24]. The olive orchard (cv. Correggiolo), planted in 2006 and rainfed, was naturally infected by *P. savastanoi* pv. *savastanoi*. Spacing was 6×6 m, resulting in a density of 278 trees/ha. The three treatments were as follows: (1) untreated negative control (Cu0), no application of Cu and particle film (only foliar applications were applied with variable water spray volume of 400 to 800 L/ha); (2) Zeo-Biopesticide treatment (ZBp), no application of Cu + application of ZBp 6 kg/1000 L per hectare and (3) positive control, application of conventional copper treatment (Cu_100) with Ossiclor 35 WG (copper oxychloride

35 g) at 2.1 kg/ha. The machinery used was a tractor equipped with a mounted sprayer (Idromeccanica Bertolini S.p.A., Via Cafiero 20, 42124 Reggio Emilia, Italy).

Rainfall data related to washout were obtained from data provided by the meteorological station of the Emilia-Romagna region in Santarcangelo di Romagna [25].

The Environmental Scanning Electron Microscopy (ESEM) allows for imaging of conductive materials or hydrated specimens in changing humidity and low-vacuum conditions, without a need for conductive coating. It is particularly suited for imaging biological samples in close-to-native conditions [26,27]. ESEM analysis was performed using a ZEISS EVO LS 10 microscope equipped with a LaB₆ thermionic source and coupled with a Bruker Quantax System for microanalysis (Esprit 2.0 software; Carl Zeiss AG, Oberkochen, Germany; Bruker Nano GmbH, Berlin, Germany). After the treatment, two sampling campaigns were conducted for ESEM distribution analyses: one on 8 May 2023 and one on 18 July 2023. At each sampling, five plants per treatment were randomly selected, and three leaves per plant were randomly collected by hand-picking and placed in plastic bags, following Rotondi et al. [16]. The same procedure was followed for ESEM leaching analysis to assess the washing effect of natural rainfall (18 July 2023 and 13 December 2023). The leaves were collected after 255.4 mm (18 July 2023) and 132.4 mm (13 December 2023) of precipitation, respectively. Sampling was scheduled according to rainfall events occurring during the treatment period.

ESEM distribution analyses were conducted on the days corresponding to the field treatments, while ESEM leaching analyses were performed after a period determined by the amount of rainfall to better simulate real field conditions.

While ESEM offers close-to-native imaging conditions, its resolution may be lower compared to conventional high-vacuum systems. Hence, the *P. synxantha* DLS65 was subjected to a high-resolution study on a Field Emission Gun Scanning Electron Microscope (SEM-FEG) that provides higher surface details required for accurate morphological identification [26,27]. For this purpose, a ZEISS LEO 1530 FEG microscope (Carl Zeiss AG, Oberkochen, Germany) was used to obtain high-resolution images of the bacterial surface.

2.2.2. Grapevine

The Fusignano experimental field is in an organic farm located within the Ravenna province (Italy). The coordinates of this site are 44.4895°, 11.9375°. The MAP (mean annual precipitation), calculated based on data from the period 1991 to 2020, is 651.1 mm. The MAT (mean annual temperature) from the same period is approximately 14 °C [24]. The grapevine orchard has the tolerant to *Plasmopara viticola* Merlot Khorus cv with a single cordon training system and a planting distance 3.0 × 1.25 m. Three foliar treatments were tested: (1) untreated negative control (Cu0) with no application of Cu and particle film (only foliar applications were applied with variable water spray volume of 400 to 800 L/ha); (2) Zeo-Biopesticide treatment (ZBp) with no application of Cu + application of ZBp 6 kg/1000 L per hectare; and (3) positive control with application of conventional copper treatment (Cu_100) with GRIFON 280 (a.i. Copper 272 g/L SC: Cu hydroxide 136 g/L + Cu oxychloride 136 g/L) at 1.5 kg/ha. At treatment time, the machinery used was a conventional tractor equipped with Compact Twin 1000 sprayer (Agricolmeccanica Friuli S.r.l., Cervignano del Friuli, Udine, Italy). After the treatment, one sampling campaign was conducted on 21 June 2023 for ESEM distribution analyses. Five plants per treatment were randomly selected, and three leaves—either healthy or diseased—were randomly collected from each plant by hand-picking and placed in plastic bags, following Rotondi et al. [16].

The Imola experimental field is an organic farm located within the Bologna province (Italy). The coordinates of this site are 44.3004°, 11.6382°. The MAP (mean annual precipitation), calculated based on data from the period 1991 to 2020, is 729.3 mm. The MAT (mean

annual temperature) from the same period is approximately 14 °C [24]. The grapevine orchard has the Downy Mildew susceptible Trebbiano Romagnolo cv with a duplex training system and a planting distance 4.0 × 1.6 m. Three foliar treatments were tested: (1) untreated negative control (Cu0) with no application of Cu and particle film (only foliar applications were applied with variable water spray volume of 400 to 800 L/ha); (2) Zeo-Biopesticide treatment (ZBp) with no application of Cu + application of ZBp 6 kg/1000 L per hectare; and (3) positive control with the application of conventional copper treatment (Cu_100) with GRIFON 280 (a.i. Copper 272 g/L SC: Cu hydroxide 136 g/L + Cu oxychloride 136 g/L) at 1.5 kg/ha. Machinery used for applications was a conventional tractor equipped with Compact Twin 1000 sprayer (Agricolmeccanica Friuli S.r.l., Cervignano del Friuli, Udine, Italy). After the treatment, one sampling campaign was conducted on 9 June 2023 for ESEM distribution analyses. Five plants per treatment were randomly selected, and three leaves, both healthy and diseased, per plant were randomly collected by hand-picking and placed in plastic bags, following Rotondi et al. [16].

2.3. ESEM (*Environmental Scanning Electron Microscopy*) Analysis

Samples of olive and grapevine leaves were stored in a refrigerator to prevent dehydration until analysis. To prepare samples for microscopic examination, an area of approximately 5.0 × 5.0 mm was cut from 15 leaves, which were used to observe the adaxial and abaxial surfaces, with the area to be examined being in the central part of the leaf, excluding the central leaf vein. Leaf pieces were placed on aluminum stubs using double-sided carbon adhesive tabs. Five areas for each leaf were observed by ESEM Zeiss EVO LS 10 (Oberkochen, Germany). All sampled olive leaves were healthy, as the pathogen rarely induces foliar symptoms. In grapevine, both diseased and healthy portions of leaves were equally observed.

Product Coverage Analyses and Its Leaching on Olive Leaf Surface

The micrographs were used to observe crystal distribution and to perform image analysis aimed at quantifying product leaf coverage across the different treatments. The product quantification was defined with ImageJ software (version 1.54k; National Institutes of Health, Bethesda, MD, USA). Five micrographs were taken at random points per each of the 5 leaf sections using High-Density Back Scattered Electrons under Low Vacuum at 450× without conductive coating. Micrographs were taken maintaining working distance, contrast and brightness as consistent as possible to keep a relatively constant area of interest and avoid difficulties when using ImageJ auto-threshold.

ImageJ was used to determine the percentage ratio between the surface covered, calculated using the automatic threshold, and the total surface area of the micrographic sample. Twenty-five coverage percentage values were extrapolated from images obtained for each date, both for leaf samples taken at treatment time and washed ones.

2.4. SEM Analysis

Sample preparation was the same as that followed with ESEM. Leaf sections were mounted on aluminum stubs using double-sided carbon adhesive tabs. The samples were coated by depositing a 10 nm gold coating using a sputter coater. A ZEISS LEO 1530 field emission scanning electron microscope (FEG-SEM) was used.

2.5. Phytopathometric Measures

Olive knot monitoring was carried out on 5 trees per treatment, 10 shoots per tree: only those without galls was chosen and marked (8 May 2023). The number of galls present on each marked shoots were counted and used as indicator of disease infection presence [21]. Observations were conducted monthly during the period from May to December 2023.

Downy Mildew symptomatology was evaluated according to the EPPO PP1/31(3) guidelines [28]. Incidence was determined based on 100 units per subplot (with 4 subplots per treatment), while severity was assessed using a 7-class scale, where each class represents the proportion of the area affected by pathogen lesions [28]. Data on incidence and severity determined in the bunches of the two grapevine cultivars can be seen in Supplementary Tables S3 and S4. In both cultivars, the copper treatment significantly reduced disease incidence and severity compared to both the Zeo-Biopesticide and the untreated control.

2.6. Statistical Analysis

Data collected from Olive Knot and Downy Mildew were elaborated using Microsoft® Excel 2007/XLSTAT© (Version 2009.3.02, Addinsoft, Inc., Brooklyn, NY, USA). Prior to conducting the analysis of variance, the Shapiro–Wilk test was performed to assess normality. Significant differences among means at $p = 0.05$ level were determined by 1-Way ANOVA followed by Tukey Honestly Significant Difference (HSD) post hoc test.

3. Results

3.1. Olive

3.1.1. ESEM (Environmental Scanning Electron Microscopy)

ESEM imaging of olive leaves treated with Zeo-Biopesticide (ZBp) revealed a uniform distribution of zeolite particles on the adaxial surface, in contrast to untreated samples from both spring and summer samplings (Figure 1).

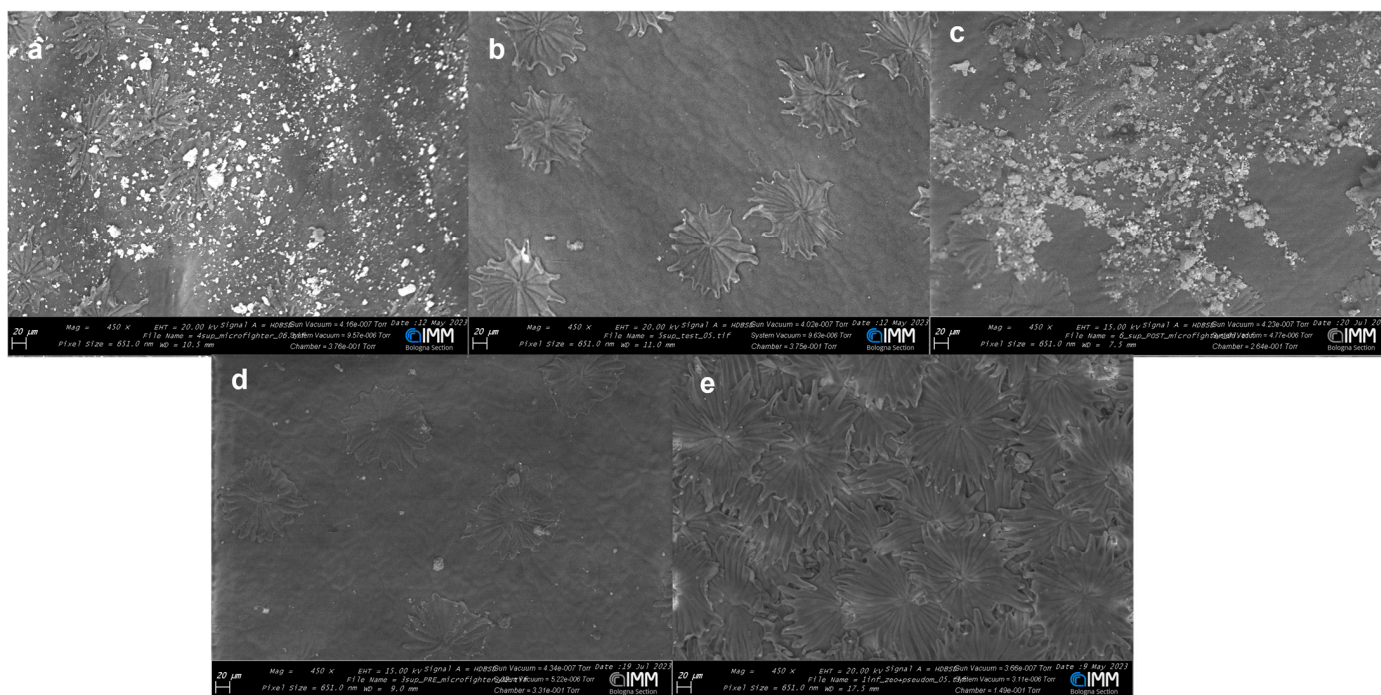


Figure 1. Environmental Scanning Electron Microscopy observations of olive leaves samplings. (a) Adaxial leaf blade treated in May with Zeo-Biopesticide with uniform dispersion of ZBp particles; (b) untreated adaxial leaf blade (May), revealing natural surface structures such as peltate trichomes; (c) adaxial leaf blade treated in July with ZBp, showing particle aggregation in the micrograph lower part; (d) untreated adaxial leaf blade (July), used as a reference control; (e) and abaxial leaf blade treated with ZBp (May), where no visible product adhesion is detected.

The presence of *P. synxantha* within mineral crystals did not appear to affect distribution. Additionally, the irregular presence of trichomes did not interfere with product adherence. Even if the new Zeo-Biopesticide is uniformly dispersed, the quantity that

is deposited on the leaf surface is variable, resulting in varying adhesion in the canopy. No adhesion of the product can be observed on the abaxial leaf blade, but the abundant presence of peltate trichomes can be noted (Figure 1).

In Figure 2A, the frequency distribution of coverage classes following the second treatment was largely concentrated in the range 10–25%, with values around 20% occurring most frequently and very low percentages scarcely represented. In the subsequent assessment (Figure 2B—third treatment), the distribution became more constrained, with a central tendency included in the 5–15% range, indicating a moderate and less variable coverage level. On average, coverage levels were below 30%, indicating limited adhesion at the time of application.

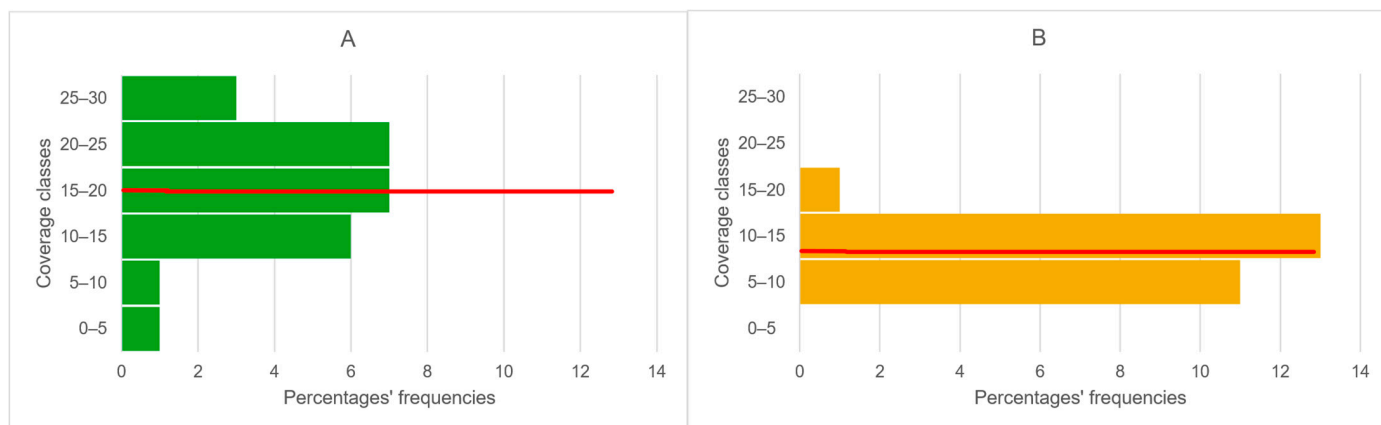


Figure 2. Histograms showing the frequency distributions of leaf coverage percentages calculated from Environmental Scanning Electron Microscopy micrographs of samples collected immediately after the (A) second (8 May 2023) and (B) third (18 July 2023) Zeo-Biopesticide-treatments. The horizontal line in each panel indicates the mean coverage value at the respective treatment time.

Following rainfall events (Figure 3A,B), Zeo-Biopesticide coverage further declined. After 255.4 mm (July) and 132.4 mm (December) of cumulative precipitation, coverage dropped to nearly 0–2% in most observations, indicating that product retention was low under high rainfall.

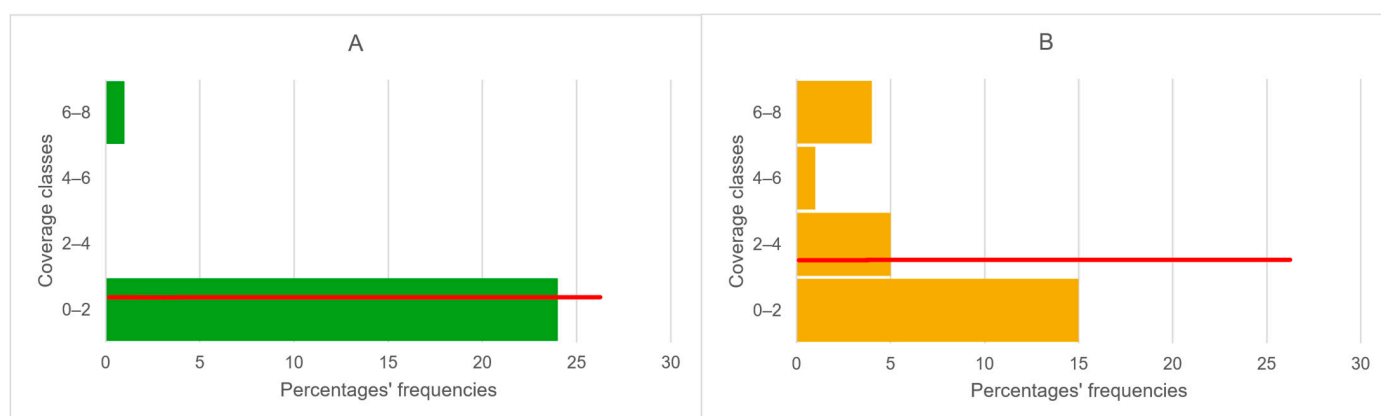


Figure 3. Histograms showing frequencies of leaf coverage percentages calculated from Environmental Scanning Electron Microscopy micrographs of samples collected after cumulative rainfall of (A) 255.4 mm (18 July 2023, following the second treatment with the Zeo-Biopesticide (ZBp)) and (B) 132.4 mm (13 December 2023, following the third treatment). Horizontal lines indicate the mean coverage values.

SEM micrographs (Figure 4) of leaves subjected to intense leaching revealed the presence of *P. synxantha* DLS65. The bacterium remained associated with zeolite particles and was also observed colonizing adjacent areas of the leaf blade.

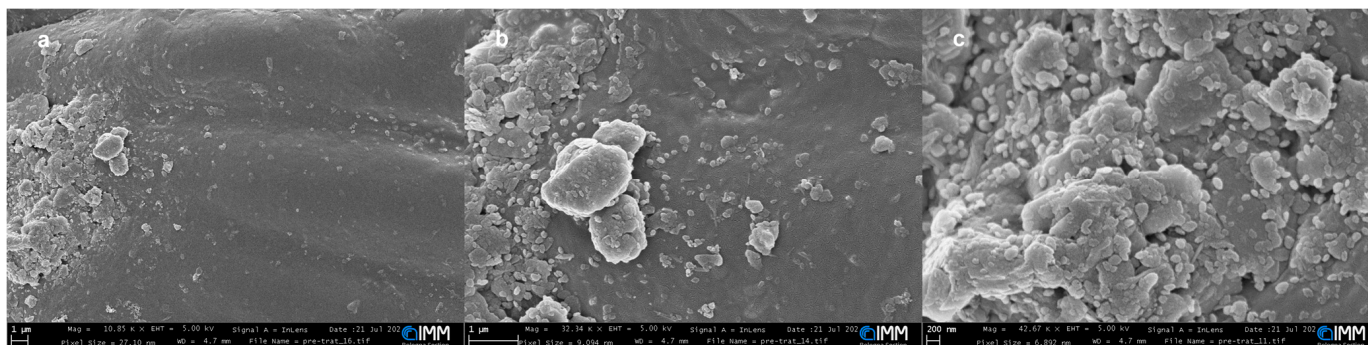


Figure 4. *Pseudomonas synxantha* DLS65 on olive leaf surface. (a) colonization of the leaf surface; (b) round-shaped bacteria surrounding a zeolite crystal; and (c) bacterial proliferation at the interface with the zeolite particle.

3.1.2. Phytopathometric Measures

Analysis of gall formation induced by the pathogen, as presented in Table 1, reveals that the control exhibited a higher incidence compared to the Zeo-Biopesticide (ZBp) treatment, which consistently maintained a statistically significant ($p < 0.05$) low rate of new galls after the third field survey, indicating a reduced incidence of infection. Furthermore, the results obtained with the new product, ZBp, were statistically better ($p < 0.05$) in terms of treatment efficacy against the disease, compared to those achieved with Cu_100, as ZBp consistently led to a significantly lower number of galls.

Table 1. Results of means of gall counts conducted on 50 shoots per treatment at each date. Zeo-Biopesticide treatment (ZBp), copper treatment (Cu_100), and negative control (Cu0).

Trt	Galls Total Number				
	8 May 2023	18 July 2023	13 September 2023	17 October 2023	13 December 2023
Cu0	0	0.10 a	0.88 a	0.94 a	1.22 a
ZBp	0	0.04 a	0.10 b	0.12 b	0.30 b
Cu_100	0	0.08 b	0.46 ab	0.48 ab	0.62 ab

Identical letters indicate no significant differences ($p > 0.05$) based on ANOVA and TUKEY's (HSD) tests.

3.2. Grapevine

3.2.1. ESEM (Environmental Scanning Electron Microscopy)

An extensive deposition of Zeo-Biopesticide has been observed on the upper epidermis of Merlot leaves (Figure 5).

ESEM images (Figure 5) revealed that, on treated adaxial surfaces, ZBp formed a continuous and uniform layer, under which most leaf structures were no longer distinguishable. However, the outlines of the polygonal epidermal cells remained clearly visible, likely due to the absence of trichomes. The microorganism's presence did not affect the distribution of the formulation.

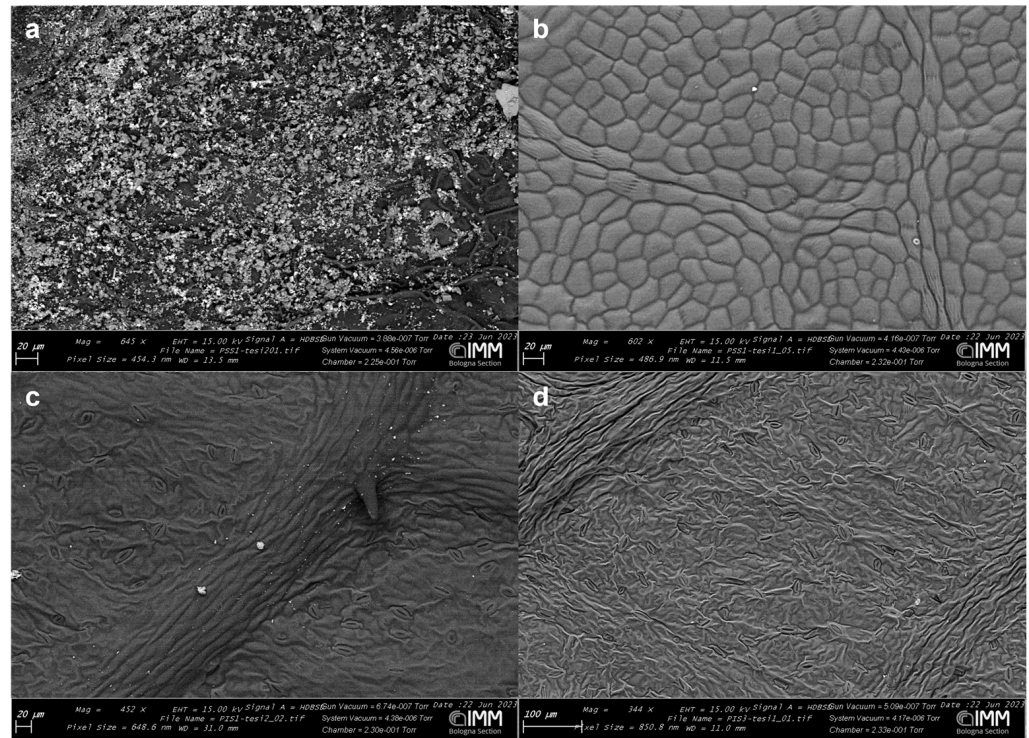


Figure 5. ESEM (Environmental Scanning Electron Microscopy) observations of Merlot Khorus leaf surfaces with and without Zeo-Biopesticide treatment. (a) ZBp-treated adaxial surface showing a continuous film of ZBp; (b) untreated adaxial surface with exposed epidermal structures; (c) ZBp-treated abaxial surface with limited deposition of ZBp; (d) untreated abaxial surface. Polygons epidermal cells are visible in both (c,d) panels.

In contrast, only a limited Zeo-Biopesticide (ZBp) deposition was observed on the abaxial surface of treated leaves, where stomata and epidermal structures remained visible in both treated and untreated samples. The images confirm the near-complete absence of trichomes on both leaf surfaces of Merlot cv (Figure 5). Preliminary stages of the disease were detected on the abaxial surface of untreated leaves (Figure 6), where *Plasmopara viticola*, during the second phase of its infection cycle, released zoospores that attached and encysted upon reaching the stomatal openings [29]. ESEM images show germinated zoospores (Figure 6a,b) and emerging sporangiophores from stomata (Figure 6c).



Figure 6. ESEM (Environmental Scanning Electron Microscopy) observations of untreated Merlot Khorus leaves. (a,b) germination of encysted zoospores of *Plasmopara viticola* during early infection stages; (c) emergence of sporangiophores through stomatal openings.

Advanced stages of the disease (Figure 7) were observed in both untreated samples and ZBp-treated leaves. The abaxial surfaces appeared fully covered with sporangiophores containing desiccated sporangia, suggesting a prior release of zoospores. Additionally, signs of cell wall degradation—likely associated with cell-wall-degrading secretions—were detected on both leaf sides and under both treatments [30].

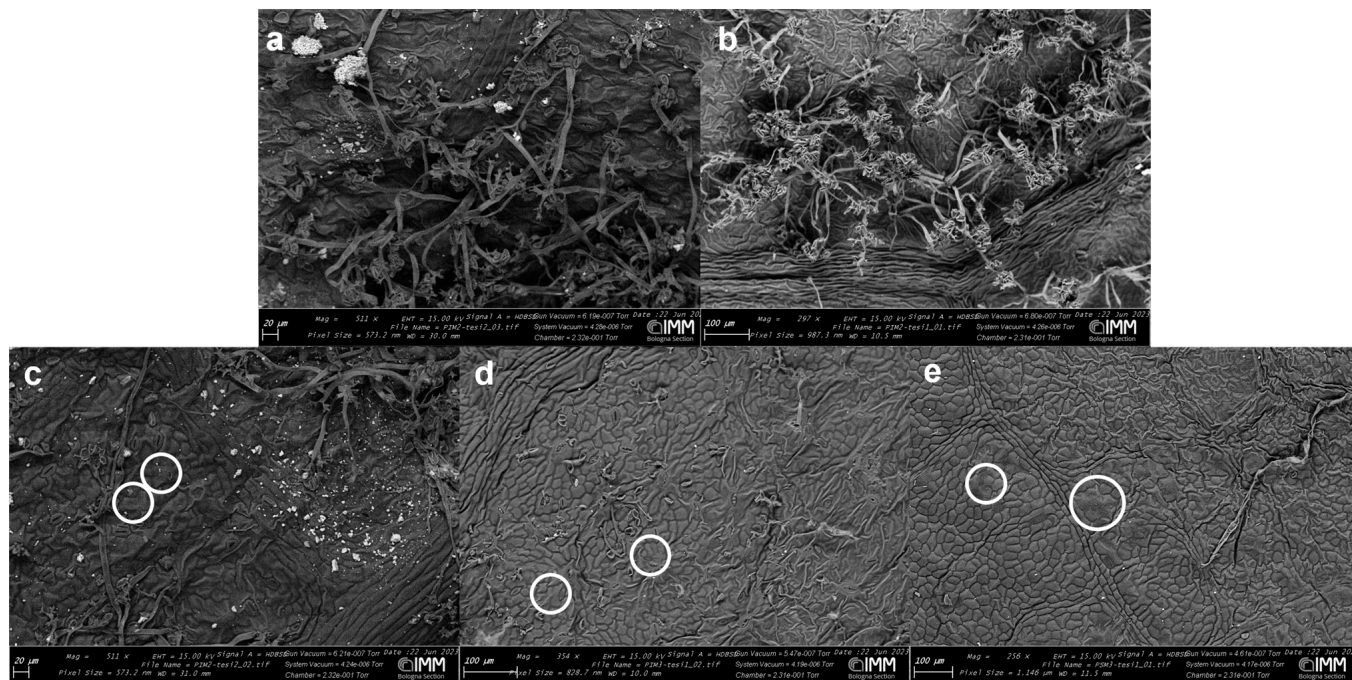


Figure 7. ESEM (Environmental Scanning Electron Microscopy) observations of Merlot Khorus leaves samplings. (a) Abaxial surface of a Zeo-Biopesticide-treated leaf covered with fully developed sporangiophores; (b) abaxial surface of untreated leaf with similar sporangiophore development; (c) abaxial surface of ZBp-treated leaf with visible cell wall degradation (circled); (d) abaxial surface of untreated leaf with degraded cell wall (in circle); (e) adaxial surface of untreated leaf showing degraded cell wall (in circle).

Particles distribution on the adaxial leaf blade of the *Plasmopara viticola* sensible Trebbiano cv was not as uniform as in Merlot (Figure 8).

In both healthy and diseased treated leaves, particles tended to accumulate near leaf hairs rather than forming a continuous layer. The untreated control was characterized by the absence of mineral residues, allowing clear visualization of erect and reclining hairs along with epidermal cells. As observed in Merlot, the abaxial surface retained minimal amounts of product, with few zeolite particles and abundant reclining hairs clearly identifiable. Advanced stages by the oomycete were detected in Trebbiano diseased leaves, too. Figure 8 shows plenty of Downy Mildew fully developed sporangiophores twisted around reclining hairs on the abaxial leaf side. Additionally, signs of cell wall degradation likely caused by enzymatic activity were also observed in this cultivar.

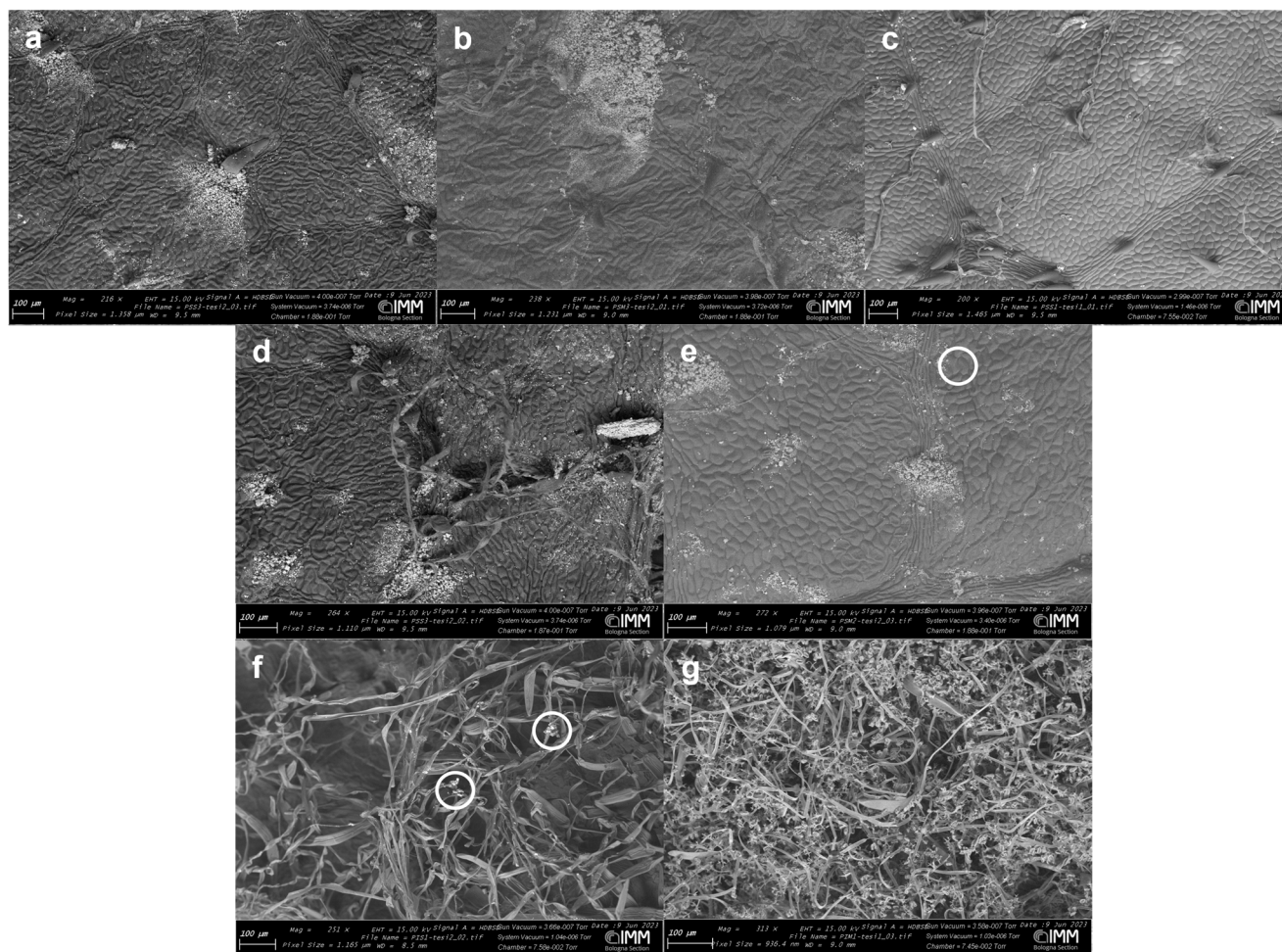


Figure 8. ESEM (Environmental Scanning Electron Microscopy) observations of Trebbiano Romagnolo leaves samplings. (a) Zeo-Biopesticide-treated adaxial surface of a healthy leaf, with product accumulation near erect hairs; (b) ZBp-treated adaxial surface of a diseased leaf; (c) untreated adaxial surface showing reclining and erect hairs; (d) ZBp-treated adaxial surface with ZBp concentrated near reclining hairs; (e) ZBp-treated adaxial surface with degraded cell wall (in circle); (f) ZBp-treated abaxial surface with product particles near reclining hairs (in circle); (g) ZBp-treated abaxial surface fully covered by sporangiophores.

3.2.2. Phytopathometric Measures

In accordance with ESEM (Environmental Scanning Electron Microscopy) observations of the leaves, Table 2 reports disease incidence and severity in Merlot Khorus, showing no statistical differences ($p > 0.05$) between the untreated control and the ZBp treatment from the initial sampling. Therefore, the new Zeo-Biopesticide did not demonstrate efficacy.

Table 2. Results of Merlot Khorus mean percentages of leaves incidence and severity. Zeo-Biopesticide treatment (ZBp), copper treatment (Cu_100) and negative control (Cu0).

Trt	Merlot Khorus Leaves Incidence				Trt	Merlot Khorus Leaves Severity			
	14 June 2023	30 June 2023	17 July 2023	2 August 2023		14 June 2023	30 June 2023	17 July 2023	2 August 2023
Cu0	4.40 a	39.80 a	55.40 a	73.40 a	Cu0	0.32 a	9.41 a	17.08 a	25.78 a
ZBp	4.00 a	38.40 a	54.40 a	72.20 a	ZBp	0.24 a	8.90 a	16.55 a	23.13 a
Cu_100	0.40 b	7.20 b	23.20 b	38.40 b	Cu_100	0.01 b	0.34 b	2.62 b	6.86 b

Identical letters indicate no significant differences ($p > 0.05$) based on ANOVA and TUKEY (HSD) tests.

A clear onset of infection, similar to that observed in Merlot Khorus, was also discovered in Trebbiano Romagnolo leaves. This trend became evident from the second observation, as demonstrated by the lack of statistical differences ($p > 0.05$) in disease incidence and severity between the untreated control and the ZBp, as shown in Table 3.

Table 3. Results of Trebbiano Romagnolo mean percentages of leaves incidence and severity at each date. Zeo-Biopesticide treatment (ZBp), copper treatment (Cu_100) and negative control (Cu0).

Trebbiano Romagnolo Leaves Incidence						Trebbiano Romagnolo Leaves Severity					
Trt	15 May 2023	1 June 2023	21 June 2023	10 July 2023	3 August 2023	Trt	15 May 2023	1 June 2023	21 June 2023	10 July 2023	3 August 2023
Cu0	12.00 a	52.40 a	100.00 a	80.20 a	61.80 a	Cu0	1.61 a	14.76 a	49.36 a	38.84 a	34.43 a
ZBp	3.00 b	52.00 a	100.00 a	80.40 a	61.60 a	ZBp	0.34 b	13.12 a	48.70 b	38.21 a	32.64 a
Cu_100	0.00 c	5.20 b	67.80 b	41.00 b	22.60 b	Cu_100	0.00 c	0.30 b	8.29 c	6.78 b	5.16 b

Identical letters indicate no significant differences ($p > 0.05$) based on ANOVA and TUKEY'S (HSD) tests.

4. Discussion

The distribution of the new Zeo-Biopesticide on the adaxial surface of olive leaves was uniform and was not influenced by the presence of trichomes. The adhesion of the product immediately after the two treatments observed on the adaxial leaf surfaces was low, with average coverage of 17.38% and 10.46%, respectively. Although the particle distribution is uniform, the low coverage is due to the discontinuous nature of the ZBp film, as observed with zeolite alone by Rotondi et al. [16]. This depends not only on spray volume and tree structure [31] but also on leaf morphological characteristics such as size, surface roughness, and wettability [15,32]. Since the adaxial surfaces of olive leaves are primarily covered with epicuticular waxes [33], this can create a more or less hydrophobic cuticular layer, which affects leaves wettability and consequently the deposition capacity and subsequent adhesion of the product. A reduced percentage of covered area (about 21%) on the adaxial leaf surface of *Prunus laurocerasus* treated with kaolin was observed by Salerno et al. [34] due to the presence of the epicuticular wax film. On the abaxial surfaces of treated leaves, the product adhered minimally. This could be related to the presence of trichomes, which make the surface difficult to wet [31], to field conditions like temperature, humidity, and wind exposure to which treated foliage is subjected, or treatment conditions (product application technique or formulation used) [14].

Adherence was also minimal in samples tested for leaching, with average coverage values of 1.43% and 2.53%. The first percentage refers to sampling performed in the summer, after a precipitation in May that alone exceeded three times the expected monthly average [35]. On one hand, the high levels of rainfall significantly reduced the amount of minerals retained on the leaf, but on the other hand, they created conditions favorable for *Pseudomonas synxantha* strain DLS65 [23] to grow on the leaf surface. Water availability is indeed a critical factor for microbial growth [36,37]. Furthermore, a preliminary characterization of *Pseudomonas synxantha* DLS65 by Bellameche et al. [38] indicated its antimicrobial activity against *Pseudomonas savastanoi* pv. *savastanoi*, which is associated with the production of volatile organic compounds (VOCs). In our study, the antagonistic bacterium was observed associating with zeolite particles and also began colonizing the leaf surface. A *in vitro* study by Modica et al. [39] revealed that the *P. synxantha*–zeolite formulation allowed the bacterial antagonist to survive and remain bioactive for over six months. While the porous structure of zeolites can create a favorable microenvironment for microbial growth [40], adhesion likely occurred on the external surface, where ion exchange processes may influence nutrient availability. Therefore, zeolite may serve as an effective substrate, facilitating the attachment and proliferation of microorganisms. Moreover, zeolite could function both as a carrier for novel biopesticides in foliar treatments for

pest management and as a biofertilizer. Other minerals have already been used for this purpose: an example of successful microbial inoculant is vermiculite, which in a laboratory experiment by Subhashini et al. [41] has extended the shelf life of *Rhizobium*, *Azospirillum lipoferum*, and *B. megaterium* for about 8 months.

In grapevine, particle distribution on the adaxial surface varied between cultivars. In Merlot Chorus cv, which is tolerant to *Plasmopara viticola*, the mineral particles completely covered the upper leaf surface. In contrast, in the susceptible Trebbiano Romagnolo, which had leaf hairs, the particles were mostly localized around them. In fact, the surface of leaves shows many forms of roughness that can impact their wetting properties. Among these features, trichomes are particularly significant [42]. This variability is reflected in the resulting zeolite deposition. To the best of our knowledge, the ultrastructural characteristics of the epidermis of both cultivars under study have not yet been described. Gaskin et al. [15] conducted a study on characterizing plant surfaces for spray adhesion and retention in which they found that Grape foliage cv. Cabernet Sauvignon is moderately difficult-to-wet on the upper, and very-difficult-to-wet on the lower surface. In our study, ESEM observations have revealed how the product did not remain adherent to the lower leaf surface, regardless of the presence (Trebbiano) or absence (Merlot) of trichomes. Boso et al. [43] reported that stomata in *Vitis* species are present only in the lower epidermis of the leaf. When an oomycete zoospore encounters a stoma, it attaches, encysts, and subsequently forms a germ tube that penetrates the substomatal cavity, initiating the infection process [29]. The absence of zeolite film left the stomata unprotected, so advanced stages of the oomycete were seen in treated leaves of both cvs. These results contrast with previous studies where chabazite–zeolites were effective against Downy Mildew [44], likely due to differences in active ingredient, cultivar response, or field conditions.

5. Conclusions

Organic agriculture, in a context where copper-based pesticides are progressively banned due to their environmental persistence, has a direct need for alternative pest control practices. In the present work, it was shown that the novel Zeo-Biopesticide represented an adequate substitute for copper-based products. The new product significantly reduced the incidence of Olive Knot in olive orchard, whereas its application on grapevines did not effectively control Downy Mildew. These findings suggest that ZBp may be more suitable for managing bacterial diseases than oomycete pathogens under field conditions. The detected absence of efficacy may be attributed to essential ecological and biological distinctions between our bacterial-based treatment and the oomycete pathogen. In fact, intra-genus competition arises when organisms share similar ecological niches, while *Plasmopara viticola*, being an oomycete, presents distinct biological characteristics that may limit its susceptibility to *Pseudomonas synxantha* DLS65 formulation.

The research further demonstrated that ZBp dispersion and adhesion were affected by environmental conditions, including rainfall, and by plant cultivar and species characteristics. Field conditions like rain caused humidity and temperature variations, while not affecting the intrinsic interaction between the pathogen and the formulation, may have impacted *Plasmopara viticola* development. Adhesion could be improved by reformulation, which would increase the economic and ecological sustainability of the product by promoting more efficient colonization of plant surfaces. Future work should focus on optimizing formulation properties for a range of crops and climatic scenarios. Additionally, assessing the ultrastructural characteristics of different cultivars and developing predictive indices for foliar retention could further support the effective use of biopesticides in integrated and organic management programs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15071563/s1>, Table S1: Mineralogical composition of the zeolite provided by Verdi s.p.a., Table S2: Variation in *Pseudomonas* sp. DLS65 colony concentration in zeolite crystals over time, Table S3: Merlot Khorus bunches' incidence and severity, Table S4: Trebbiano Romagnolo bunches' incidence and severity.

Author Contributions: Conceptualization, A.R., L.M., B.F. and L.F.; methodology, A.R., L.M., E.C. and L.F.; software, E.C.; investigation, A.R., L.M., E.C., L.F. and D.M.; data curation, L.M., E.C., D.M. and L.F.; writing—original draft preparation, E.C.; writing—review and editing, A.R., L.M., B.F. and G.F.; supervision, A.R.; project administration, A.R., L.M. and B.F.; funding acquisition, A.R., L.M. and B.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union under the LIFE Programme (LIFE-2021-SAP-ENV-ENVIRONMENT), grant number 101074218.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The authors gratefully really thank Franco Corticelli for ESEM and SEM analysis support; Matteo Mari, Ivan Neri, Enrico Nardini, Edoardo Tamburini, Antonio Allegri, Federica Manucci and Amadei Paolo for technical support; Symbiagro S.r.l. and Eleonora Pegoiani for experimental support.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Sharma, R.R.; Reddy, S.V.R.; Datta, S.C. Particle Films and Their Applications in Horticultural Crops. *Appl. Clay Sci.* **2015**, *116*–117, 54–68. [[CrossRef](#)]
2. Glenn, D.M.; Puterka, G.J. *Particle Films: A New Technology for Agriculture*; CABI: Wallingford, UK, 2010; Volume 31, ISBN 978-0-470-65088-2.
3. Bush, D.S.; Demkovich, M.; Aldunate, M.; Siegel, J.; Berenbaum, M.R. Kaolin as a Management Alternative for Insecticide-Resistant Navel Orangeworm (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **2023**, *116*, 2095–2103. [[CrossRef](#)]
4. Salerno, G.; Rebora, M.; Kovalev, A.; Gorb, E.; Gorb, S. Kaolin Nano-Powder Effect on Insect Attachment Ability. *J. Pest Sci.* **2020**, *93*, 315–327. [[CrossRef](#)]
5. Rumbos, C.I.; Sakka, M.; Berillis, P.; Athanassiou, C.G. Insecticidal Potential of Zeolite Formulations against Three Stored-Grain Insects, Particle Size Effect, Adherence to Kernels and Influence on Test Weight of Grains. *J. Stored Prod. Res.* **2016**, *68*, 93–101. [[CrossRef](#)]
6. Walters, D.R. Disguising the Leaf Surface: The Use of Leaf Coatings for Plant Disease Control. *Eur. J. Plant Pathol.* **2006**, *114*, 255–260. [[CrossRef](#)]
7. Glenn, D.M.; Puterka, G.J.; Vanderzwet, T.; Byers, R.E.; Feldhake, C. Hydrophobic Particle Films: A New Paradigm for Suppression of Arthropod Pests and Plant Diseases. *J. Econ. Entomol.* **1999**, *92*, 759–771. [[CrossRef](#)]
8. Daniel, C.; Pfammatter, W.; Kehrl, P.; Wyss, E. Processed Kaolin as an Alternative Insecticide against the European Pear Sucker, *Cacopsylla pyri* (L.). *J. Appl. Entomol.* **2005**, *129*, 363–367. [[CrossRef](#)]
9. Andrić, G.G.; Marković, M.M.; Adamović, M.; Daković, A.; Golić, M.P.; Kljajić, P.J. Insecticidal Potential of Natural Zeolite and Diatomaceous Earth Formulations against Rice Weevil (Coleoptera: Curculionidae) and Red Flour Beetle (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **2012**, *105*, 670–678. [[CrossRef](#)]
10. Percival, G.C.; Boyle, S. Evaluation of film forming polymers to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions. *Crop Prot.* **2009**, *28*, 30–35. [[CrossRef](#)]
11. Kvachantiradze, M.; Tvalchrelidze, E.; Kotetishvili, M.; Tsitsishvili, T. Application of clinoptilolite as an additive for the photostabilization of the *Bacillus thuringiensis* formulation. In *Studies in Surface Science and Catalysis*; Kiricsi, I., Pál-Borbély, G., Nagy, J.B., Karge, H.G., Eds.; Elsevier: Amsterdam, The Netherlands, 1999; Volume 125, pp. 731–735, ISBN 978-0-444-50244-5. [[CrossRef](#)]
12. Kefalogianni, I.; Gkizi, D.; Pappa, E.; Dulaj, L.; Tjamos, S.E.; Chatzipavlidis, I. Combined Use of Biocontrol Agents and Zeolite as a Management Strategy against Fusarium and Verticillium Wilt. *BioControl* **2017**, *62*, 139–150. [[CrossRef](#)]

13. De Smedt, C.; Someus, E.; Spanoghe, P. Potential and Actual Uses of Zeolites in Crop Protection. *Pest Manag. Sci.* **2015**, *71*, 1355–1367. [CrossRef] [PubMed]
14. Privé, J.-P.; Russell, L.; LeBlanc, A. Gas Exchange of Apple and Blackberry Leaves Treated with a Kaolin Particle Film on Adaxial, Abaxial, or Both Leaf Surfaces. *HortScience* **2007**, *42*, 1177–1182. [CrossRef]
15. Gaskin, R.E.; Steele, K.D.; Forster, W.A. Characterising Plant Surfaces for Spray Adhesion and Retention. *N. Z. Plant Prot.* **2005**, *58*, 179–183. [CrossRef]
16. Rotondi, A.; Morrone, L.; Facini, O.; Faccini, B.; Ferretti, G.; Coltorti, M. Distinct Particle Films Impacts on Olive Leaf Optical Properties and Plant Physiology. *Foods* **2021**, *10*, 1291. [CrossRef]
17. La Torre, A.; Iovino, V.; Caradonia, F. Copper in Plant Protection: Current Situation and Prospects. *Phytopathol. Mediterr.* **2018**, *57*, 201–236.
18. Council of the European Communities. Council Regulation (EEC) No 2092/91 of 24 June 1991 on Organic Production of Agricultural Products and Indications Referring Thereto on Agricultural Products and Foodstuffs. *Off. J. Eur. Commun.* **1991**, *198*, 7.
19. Roda, R.; Prats-Llinàs, M.T.; Forcadell, S.; Mazzieri, M.; Calvo-Garrido, C.; Nadal, M.; de Lamo, S.; Ferrer-Gallego, R. The Effect of Copper Reduction on the Control of Downy Mildew in Mediterranean Grapevines. *Eur. J. Plant Pathol.* **2024**, *169*, 529–542. [CrossRef]
20. Nguyen, K.A.; Förster, H.; Adaskaveg, J.E. Efficacy of Copper and New Bactericides for Managing Olive Knot in California. *Plant Dis.* **2018**, *102*, 892–898. [CrossRef]
21. Teviotdale, B.L.; Krueger, W.H. Effects of Timing of Copper Sprays, Defoliation, Rainfall, and Inoculum Concentration on Incidence of Olive Knot Disease. *Plant Dis.* **2004**, *88*, 131–135. [CrossRef]
22. European Commission. Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018 Renewing the Approval of Copper Compounds as Active Substances Under Regulation (EC) No 1107/2009 of the European Parliament and of the Council, and Amending the Annex to Commission Implementing Regulation (EU) No 540/2011. *Off. J. Eur. Union.* **2018**, *317*, 16–20.
23. Tontou, R.; Gaggia, F.; Baffoni, L.; Devescovi, G.; Venturi, V.; Giovanardi, D.; Stefani, E. Molecular characterisation of an endophyte showing a strong antagonistic activity against *Pseudomonas syringae* pv. *actinidiae*. *Plant Soil* **2016**, *109*, 97–106. [CrossRef]
24. Arpae Emilia-Romagna Tabelle Climatiche. Available online: <https://www.arpae.it/it/temi-ambientali/clima/dati-e-indicatori/tabelle-climatiche> (accessed on 18 February 2024).
25. ARPAE Emilia-Romagna. DEXT3R—Dati Agrometeorologici in Emilia-Romagna. Available online: <https://simc.arpae.it/dext3r/> (accessed on 27 June 2023).
26. Danilatos, G.D. Foundations of Environmental Scanning Electron Microscopy. In *Advances in Electronics and Electron Physics*; Hawkes, P.W., Ed.; Academic Press: San Diego, CA, USA, 1988; Volume 71, pp. 109–250. [CrossRef]
27. Donald, A.M. The Use of Environmental Scanning Electron Microscopy for Imaging Wet and Insulating Materials. *Nat. Mater.* **2003**, *2*, 511–516. [CrossRef]
28. European and Mediterranean Plant Protection Organization (EPPO). *EPPO Standard PP1/031(3): Plasmopara viticola*; EPPO: Paris, France, 2000. Available online: <https://pp1.eppo.int/standards/PP1-031-3> (accessed on 10 April 2024).
29. Yin, X.; Liu, R.Q.; Su, H.; Su, L.; Guo, Y.R.; Wang, Z.J.; Du, W.; Li, M.J.; Zhang, X.; Wang, Y.J.; et al. Pathogen Development and Host Responses to *Plasmopara viticola* in Resistant and Susceptible Grapevines: An Ultrastructural Study. *Hortic. Res.* **2017**, *4*, 17033. [CrossRef]
30. Díez-Navajas, A.M.; Wiedemann-Merdinoglu, S.; Greif, C.; Merdinoglu, D. Nonhost versus Host Resistance to the Grapevine Downy Mildew, *Plasmopara viticola*, studied at the tissue level. *Phytopathology* **2008**, *98*, 776–780. [CrossRef] [PubMed]
31. Bukovac, M.J.; Cooper, J.A.; Whitmoyer, R.E.; Brazee, R.D. Spray Application Plays a Determining Role in Performance of Systemic Compounds Applied to the Foliage of Fruit Plants. *Acta Hort.* **2002**, *594*, 65–75. [CrossRef]
32. Yuri, J.A.; Palma, M.; Sepúlveda, Á.; Moya, M. Water Retention on the Surface of Apples and Sweet Cherry Leaves and Fruits. *J. Plant Prot. Res.* **2022**, *62*, 136–144. [CrossRef]
33. Fernández, V.; Almonte, L.; Bahamonde, H.A.; Galindo-Bernabeu, A.; Sáenz-Arce, G.; Colchero, J. Chemical and Structural Heterogeneity of Olive Leaves and Their Trichomes. *Commun. Biol.* **2024**, *7*, 60. [CrossRef] [PubMed]
34. Salerno, G.; Reborá, M.; Piersanti, S.; Saitta, V.; Kovalev, A.; Gorb, E.; Gorb, S. Reduction in Insect Attachment Caused by Different Nanomaterials Used as Particle Films (Kaolin, Zeolite, Calcium Carbonate). *Sustainability* **2021**, *13*, 8250. [CrossRef]
35. Arpae Emilia-Romagna. Rapporto IdroMeteoClima 2023—Online il Video Riepilogativo. Available online: <https://www.arpae.it/it/notizie/rapporto-idro-meteo-clima-2023-online-il-video-riepilogativo> (accessed on 31 May 2024).
36. Scott, W.J. Water Relations of *Staphylococcus aureus* at 30 °C. *Aust. J. Biol. Sci.* **1953**, *6*, 549–564. [CrossRef]
37. Scott, W.J. Water relations of food spoilage microorganisms. In *Advances in Food Research*; Mrak, E.M., Stewart, G.F., Eds.; Academic Press: New York, NY, USA, 1957; Volume 7, pp. 83–127, ISBN 978-0-12-016407-3. [CrossRef]
38. Bellameche, F.; Modica, F.; Fagioli, L.; Giovanardi, D.; Stefani, E. Preliminary Characterization and Mode Action of *Pseudomonas synxantha* DLS65 as the active ingredient of Microfighter, an innovative biopesticide. *J. Plant Pathol.* **2024**, *106*, 1423–1534.

39. Modica, F.; Fagioli, L.; Cortiello, M.; Giovanardi, D.; Reyes, F.; Stefani, E. Reduction of Copper Inputs in the Management of Key Diseases of Grapevine, Olive and Tomato by an Innovative Zeo-Biopesticide. In Proceedings of the XXVIII Congress of the Italian Phytopathological Society (SIPaV), Naples, Italy, 15 November 2023; Springer: Cham, Switzerland, 2023; Volume 105, p. 166.
40. Lin, H.; Ma, R.; Lin, J.; Sun, S.; Liu, X.; Zhang, P. Positive Effects of Zeolite Powder on Aerobic Granulation: Nitrogen and Phosphorus Removal and Insights into the Interaction Mechanisms. *Environ. Res.* **2020**, *191*, 110098. [[CrossRef](#)] [[PubMed](#)]
41. Subhashini, R. Suitability of Amended Vermiculite as a Carrier for Bacterial inoculants. *Res. Crops* **2008**, *9*, 707–723.
42. Holloway, P.J. Surface Factors Affecting the Wetting of Leaves. *Pestic. Sci.* **1970**, *1*, 156–163. [[CrossRef](#)]
43. Boso, S.; Gago, P.; Alonso-Villaverde, V.; Santiago, J.L.; Mendez, J.; Pazos, I.; Martínez, M.C. Variability at the Electron Microscopic Level in Leaves of Members of the Genus *Vitis*. *Sci. Hortic.* **2011**, *128*, 228–238. [[CrossRef](#)]
44. Calzarano, F.; Seghetti, L.; Pagnani, G.; Metruccio, E.G.; Di Marco, S. Control of Grapevine Downy Mildew by an Italian Copper Chabasite-Rich Zeolitite. *Agronomy* **2022**, *12*, 1528. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.