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Effectiveness of Low Copper-Containing Chemicals against Olive Leaf Spot Disease Caused by *Venturia oleaginea*

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Abstract: The high level of copper (Cu) accumulation in the soil, the risk of surface water contamination, and the potential public health problems due to Cu entering the food chain have raised concerns on the use of Cu compounds in agriculture, including olive growing. As a consequence, there is worldwide regulatory pressure on agricultural systems to limit the use of Cu compounds. Hence, a field trial was carried out to evaluate the effectiveness of low copper containing chemicals to control olive leaf spot (OLS) disease caused by *Venturia oleaginea*. The trial was conducted in 2021 in an olive (cv. Nabali Baladi) grove in Palestine. Copper complexed with lignosulphonate and gluconate (Disper Cu Max®) and the self-defense inducer Disper Broton GS® were evaluated and compared to dodine and the traditionally and frequently used copper hydroxide. In addition, untreated trees were used as the control. Treatments were made in March, July, and August. In March 2021, leaves grown in 2020 were present and 100% infected. *V. oleaginea* infections caused defoliation in untreated and treated olive trees with varying degrees of intensity: the Control had the most defoliation, followed by copper hydroxide and Disper Cu Max®, whereas dodine and, in particular, Disper Broton GS® had the least. All treatments reduced symptomatic leaves but their efficacy varied significantly: copper hydroxide was the least effective, Disper Cu Max® was intermediate, dodine and, mainly, Disper Broton GS® were the most effective. Overall, the results are promising since Disper Cu Max® and Disper Broton GS® were able to significantly reduce OLS damage and the amount of copper used for treatments.

Keywords: copper fungicides; dodine; *Olea europaea* L.; induced resistance; olive peacock eye disease; self-defense inducers

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1. Introduction

Olive (*Olea europaea* L.), the largest fruit crop in the Mediterranean area, is cultivated on about 9.5 million hectares, contributing to 95 per cent of the total land used for olive cultivation worldwide. In fact, about 98% of the olive oil and 80% of the table olive production worldwide are from Mediterranean countries [1]. Olive cultivation plays a significant role on the economic and social life of communities where it is cultivated. Not only it is essential for providing olive oil and table olives, but also for creating, conserving, and shaping the landscape and improving human nutrition and health [2].

In Palestine, olive is the leading fruit tree, covering approximately 45% of the farmland and 51% in the West Bank [3,4]. Around 94% of the olives produced are used for oil extraction, while the remaining 6% are used for pickling and soap [5]. Olive production accounts for 13% of the annual agricultural production and is one of the principal sources of income [3,6].

Olive yields can be reduced due to adverse environmental conditions, poor harvesting techniques, alternate bearing cycles, ageing trees, and inadequate disease and pest management. The olive tree can be adversely affected by many harmful pests and serious diseases that endanger production [3]. The number and occurrence of pests and diseases has increased markedly in recent years [7], currently comprising over 255 species. Among these species, new organisms, such as mites, nematodes, and pathogenic microorganisms are especially increasing. In most cases, fungi, bacteria, and viruses are responsible for the outbreak of olive diseases, but only in limited cases do they cause economic loss.

In Palestine, the predominant and extremely damaging olive diseases and pests are olive leaf spot (OLS) caused by *Venturia oleaginea* (Castagne) Rossman & Crous, Verticillium wilt, the olive fruit fly, *Bactrocera oleae* (Rossi), and the olive moth, *Prays oleae* (Bernard) [3]. The presence of *V. oleaginea* has been recorded for over a century in the Mediterranean regions and has caused decreased growth and yield in olives, especially in environments that are favorable to the disease [3,8,9], including Palestine [6].

OLS disease is mainly controlled by using copper-based fungicides, though severe infections are difficult to control, and can lead to significant yield loss. Repeated applications of copper compounds can protect orchards from the disease, but increase the risk of chemical residues and environmental pollution [6]. Being a heavy metal, there is increasing concern about the use of copper. In fact, in the European Union the use of copper compounds has been reduced to an average of 4 kg of metallic copper/ha/year over a 7-year period. Hence, in order to reduce or eliminate the use of copper, it is important to find alternatives to conventional copper fungicides [10,11].

There is growing evidence that cytotropic-translaminar (dodine and strobilurins) and systemic (triazoles) copper-free organic fungicides protect olive trees against *V. oleaginea* attacks. In particular, difenoconazole + azoxystrobin were found to be slightly better than copper fungicides, and tebuconazole + trifloxystrobin were similar to copper fungicides [12,13]. Sometimes dodine gave better results than copper fungicides [14] and in other cases responded similarly [15,16].

Recently, interesting results were obtained with a low copper formulation based on copper complexed with lignosulphonic and gluconic acids [12] and with copper nanoparticles [17].

In potted olive trees, promising results in controlling OLS disease were obtained with several inducers of systemic resistance such as salicylic acid, chitosan, 3-amino butyric acid, FoliR-Fos 400, acibenzolar-S-methyl, and potassium silicate [10,18]. The exploitation of induced resistance opens possibilities for a new and environmentally sustainable approach for the control of OLS. In this regard, it is imperative to test resistance inducers under field conditions.

The aim of this study was to evaluate, in open-field, the efficacy of a copper complexed with lignosulphonic and gluconic acids (Disper Cu Max®) and a self-defense inducer (Disper Broton GS®) in controlling OLS disease. The two chemicals, which contain low levels of copper, were compared with two frequently used fungicides in controlling OLS: copper hydroxide and dodine. The latter fungicide has never been used in Palestine.

2. Materials and Methods

2.1. Field Trial and Experimental Design

The trial was carried out in Asira (north of the West Bank of Palestine), in an olive orchard of adult olive trees of the cultivar Nabali Baladi, which is the prevalent cultivar in Palestine and is highly susceptible to OLS disease. This region is the major site of Palestinian olive production and its climatic conditions favor the growth and spread of olive leaf spot disease. Conventional Palestinian cultivation techniques were used: (i) soil tilling 3–4 times per year; (ii) fertilizing with animal manure; (iii) pruning every two years.

A completely randomized block design with 5 treatments and 3 blocks (replicates) was established in the olive orchard. Each block consisted of 5 adjacent rows and each treatment, consisting of 4 trees, was assigned to a randomly chosen row.

Treatments included:

(1) Copper complexed with lignosulphonic acid and gluconic acid (Copper complexed with LS and G acid) (Disper Cu Max®-Eden Modern Agriculture S.L-Spain), which contains 14% Cu and was applied at the dose of 150 g/100 L of water;
(2) Self-defense inducer (Disper Broton GS®-Eden Modern Agriculture S.L-Spain) which contains 3.5% complexed Cu as well as 1.5% complexed Mn, 1% complexed Zn, 10% nitrogen (Ureic N); it was applied at the dose of 300 g/100 L of water;
(3) Copper hydroxide (Fugoran®-Agri-Estrella-Mexico), which contains 70% Cu and was applied at the dose of 150 g/100 L of water;
(4) Dodine (Syllit 544 SC), which was applied at the dose of 165 mL/100 L of water.
(5) Control (untreated trees).

The level of metallic Cu contained in the treatments is reported in Table 1.

Table 1. Amount of metallic copper per treatment, considering the use of 1000 L of solution per hectare.

Treatment	Metallic Copper (g/ha)
Control	0
Copper complexed with LS and G acid (Disper Cu Max®)	210
Dodine	0
Self-defense inducer (Disper Broton GS®)	105
Copper hydroxide	1050

Treatments were applied at the end of March, mid-July, and August 2021. For all treatments and all times, applications were carried out with a hand-held sprayer (CKS 110P, Idromeccanica Bertolini, Reggio Emilia, Italy) connected to a tractor, providing uniform spraying and distribution throughout the full tree canopy. After each treatment, the sprayer was cleaned with water to ensure there was no contamination or sediment from the previous treatment.

2.2. Defoliation and Infection Evaluations

At the beginning of the trial (January 2021), eight branches in the lower canopy of each tree were labelled. For each labelled branch, the number of nodes and leaves were counted approximately every 20 days, both on old shoots and on new ones formed during the trial. This permitted the determination of defoliation, which is defined as the number of leaves fallen from the affected trees. Furthermore, the number of infected symptomatic leaves on the same branches was counted. Symptomatic leaves and defoliation were calculated as a percentage with respect to the leaves on the shoots at each date of sampling and to the leaves which were on the shoots at the beginning of the trial, respectively.

Asymptomatic leaf infections were also determined using the method described by Salman et al. [6]. At the time of each measurement, samples of old (grown in 2020) and new (grown in 2021) asymptomatic leaves (about 100 old or new leaves/tree) were randomly collected from branches near the labelled ones. The asymptomatic sampled leaves were soaked in a 5% NaOH solution for 5 min and the number of those with spots caused by the pathogen were recorded.

2.3. Agronomic Evaluations

The number of inflorescences was determined on the labelled branches. Subsequently, the number of fruits was counted. These determinations were used to calculate fruit-set (starting, approximately, one month after full bloom—50% of open flowers—and then periodically). Fruit set is expressed as the number of fruits/inflorescences.

At the end of the experiment, 100 fruits/tree were collected to determine both fresh and dry weights. The latter was determined by drying the fruit samples in an oven at 105 °C for 4 days.

2.4. Statistical Analysis

Data are presented as means ± standard errors. Data were statistically analyzed by ANOVA, according to a completely randomized block design, and averages were compared using the Student–Newman–Keuls Test. The relationships between some of the parameters were evaluated by calculating the coefficients of determination (R^2).

3. Results

3.1. Effect of Treatments on Defoliation

Defoliation increased progressively in all treatments, but with varying levels of intensity (Figure 1). The control showed the most intense defoliation, followed by copper hydroxide and Disper Cu Max®, while dodine and, especially, Disper Broton GS® gave the lowest values.

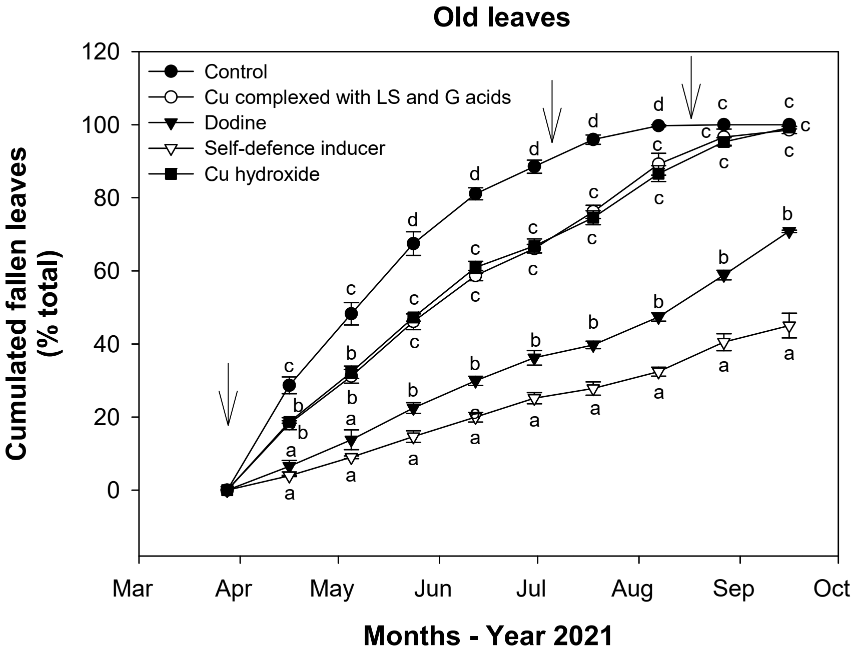

Figure 1. Effects of chemical treatments on the percentage of defoliation of old olive leaves (developed in 2020 and still present in 2021). The arrows indicate the dates of fungicide application. The bars represent the standard error. For each date, means followed by different letters are significantly different at $p \leq 0.05$.

Moreover, it is worth noting that at the end of the experiment, the self-defense inducer (Disper Broton GS®)-treated trees still had about 60% of the leaves that were present at the beginning of the experiment, while trees treated with dodine maintained about 30% of the leaves. With respect to the control, copper hydroxide and Cu Complex (Disper Cu Max®)-treated trees slowed the drop of leaves, but at the end of the experiment (September) they were not statistically different from the control.

3.2. Effect of Treatments on Fungal Infections

At the beginning of the trial, the symptomatic leaves grown in 2020 were 72–90% of the total, whereas the asymptomatic but infected leaves ranged from 10 to 28%, as shown in Table 2. This means that all leaves (100%) grown in 2020 were infected.

Table 2. Symptomatic and asymptomatic infected leaves at the beginning of the experiment (March 2021), on the trees where the different treatments were applied.

Treatment	**Symptomatic Leaves (% Total)**	**Asymptomatic Infected Leaves (% Total)**	**Symptomatic + Asymptomatic Infected (% Total)**
Control	90.4 a	9.6 a	100.0 a
Copper complexed with LS and G acid	78.0 a	22.0 a	100.0 a
Dodine	74.1 a	25.9 a	100.0 a
Self-defense inducer	72.4 a	27.6 a	100.0 a
Copper hydroxide	77.6 a	22.4 a	100.0 a

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$.

The effect of treatments on the % of symptomatic old leaves are reported in Figure 2. The Control showed the highest values, followed by copper hydroxide. All the other treatments showed lower values. The number of symptomatic leaves decreased throughout the season and did not differ at the end of the experiment, most probably because of the gradual drop of infected leaves.

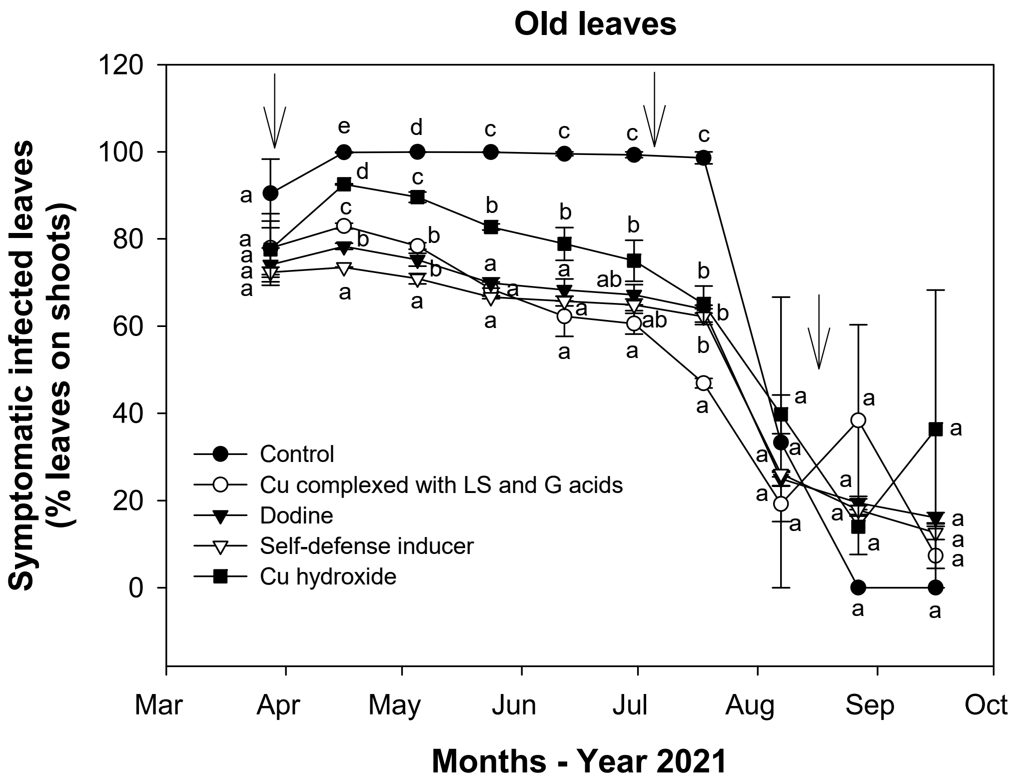

Figure 2. Effect of chemical treatments on symptomatic *Venturia oleaginea* infection in old leaves (grown in 2020). Arrows indicate the dates of fungicide application. Bars represent the standard error. For each date, means followed by different letters are significantly different at $p \leq 0.05$.

The effect of treatments on the % of asymptomatic infected old leaves is reported in Figure 3. It should be noted that as both symptomatic and asymptomatic infected leaves are the total presence of leaves on the shoots, their percentages are complementary. Thus, Control and Cu hydroxide-treated trees had a lower percentage of asymptomatic infected leaves due to a higher percentage of symptomatic leaves.

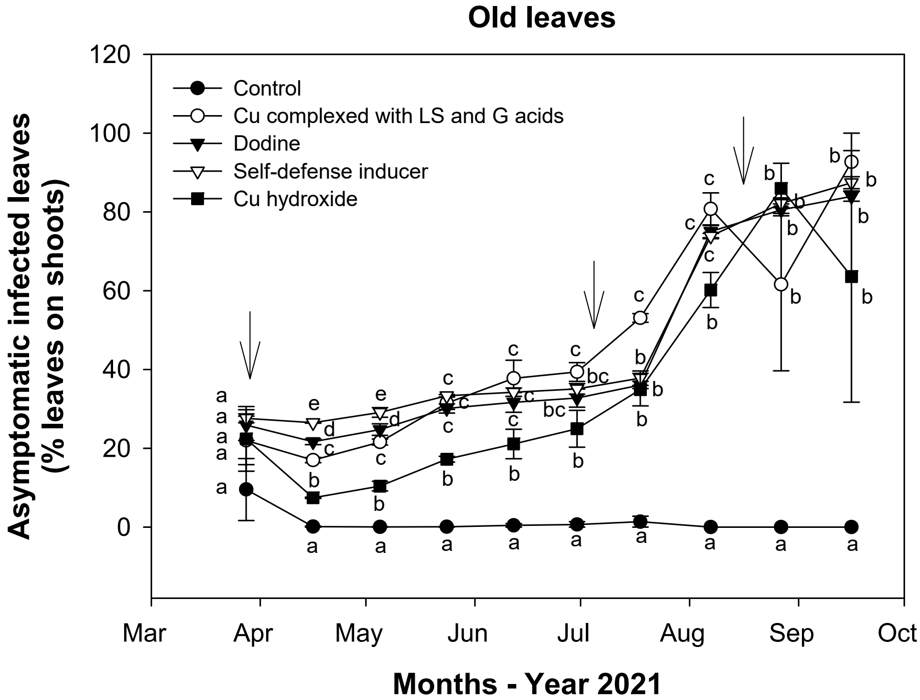

Figure 3. Effect of chemical treatments on asymptomatic *Venturia oleaginea* infection in old leaves (grown in 2020). Arrows indicate the dates of fungicide application. Bars represent the standard error. For each date, means followed by different are significantly different at $p \leq 0.05$.

The % of symptomatic infected new leaves, reported in Figure 4, shows that the control had the highest values of symptomatic leaves, followed by copper hydroxide, while the other treatments, particularly those treated with dodine and the self-defense inducer (Disper Broton GS®), had lower values of symptomatic leaves.

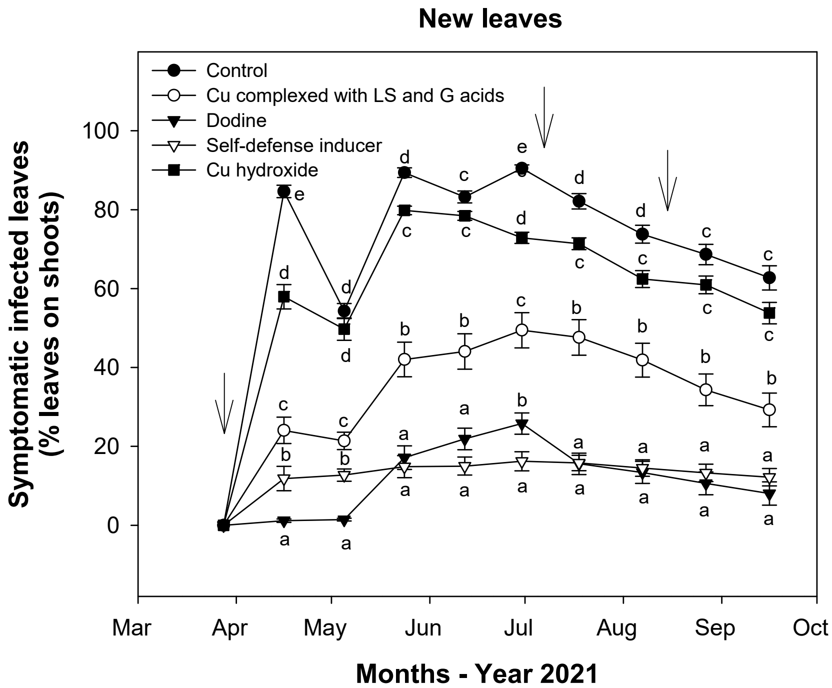

Figure 4. Effect of chemical treatments on symptomatic *Venturia oleaginea* infection in new leaves (grown in 2021). Arrows indicate the dates of fungicide application. Bars represent the standard error. For each date, means followed by different letters are significantly different at $p \leq 0.05$.

In new young leaves, Control and copper-treated trees had a lower percentage of asymptomatic infected leaves because they had a higher percentage of symptomatic ones (Figure 5). Likewise, all other treatments had higher values of asymptomatic infected leaves.

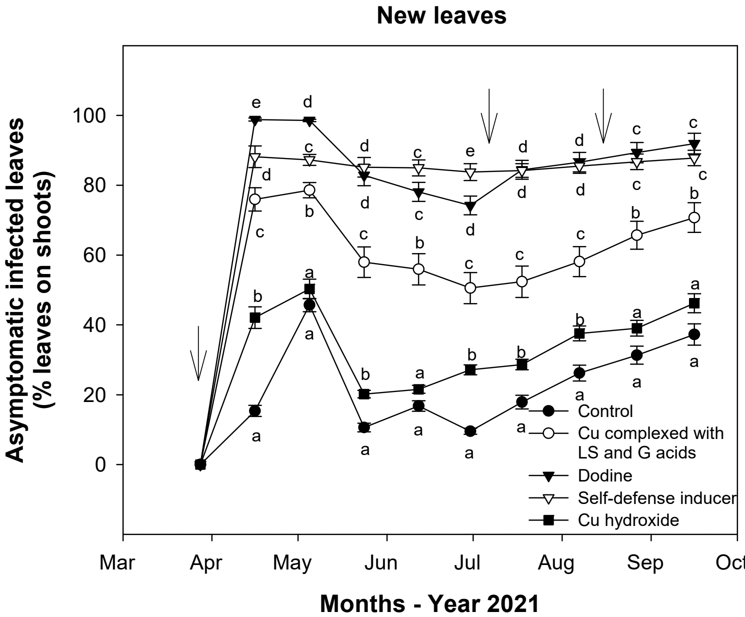

Figure 5. Effect of chemical treatments on asymptomatic *Venturia oleaginea* infection in new leaves (grown in 2021). The arrows indicate the dates of fungicide application. The bars represent the standard error. For each date, means followed by different are significantly different at $p \leq 0.05$.

3.3. *Effects of Fungal Infections on Fruit-Set and Fruit Dry Weight*

Fruit-set, expressed as the number of fruits/inflorescence and determined about one month after full bloom and then up to September, tended to be higher in trees treated with dodine and the self-defense inducer (Disper Broton GS®), followed by those treated with Cu complex (Disper Cu Max®), copper hydroxide, and the Control, respectively (Figure 6).

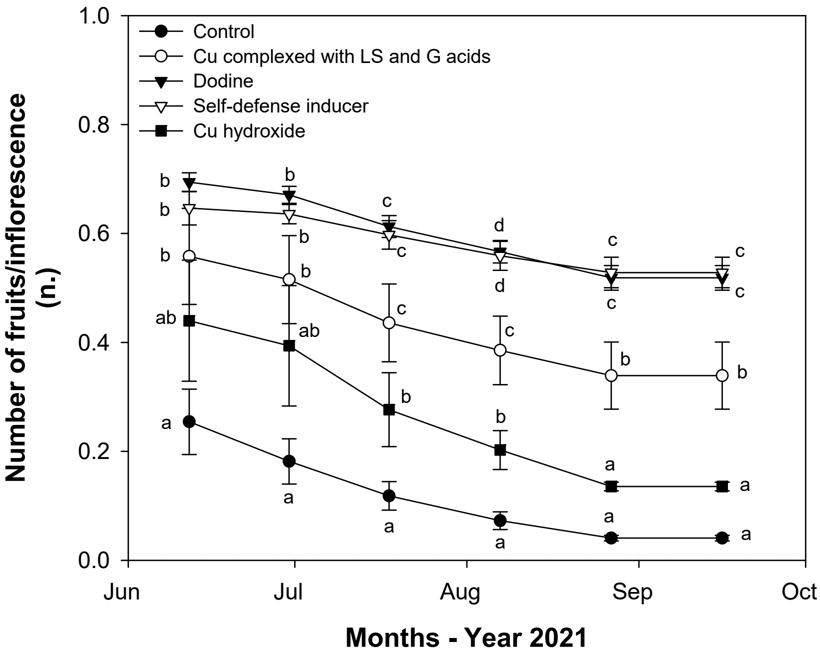

Figure 6. Effect of chemical treatments on olive fruit-set determined about one month after full bloom and up to September. The bars represent the standard error. For each date, means followed by different letters are significantly different at $p \leq 0.05$.

The weight of fruits on dodine and self-defense inducer (Disper Broton GS®) treated trees tended to be higher with respect to those of the other treatments, but differences were not statistically significant.

Correlations between defoliation and fruit-set showed a strong negative relationship between the average defoliation of old leaves from March to mid-June and the number of fruits/inflorescence (fruit set) (Figure 7). This indicates that there was an indirect effect of *V. oleaginea* on fruit-set.

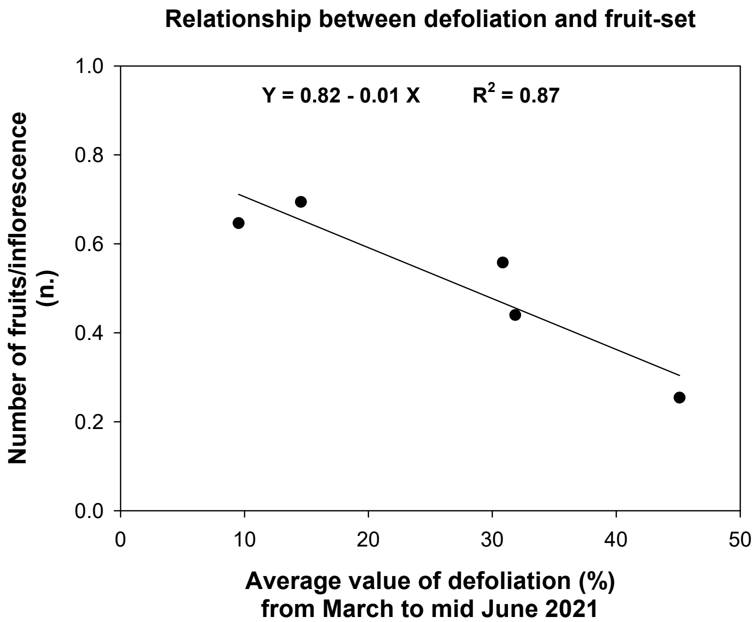

Figure 7. Relationship between the number of fruits/inflorescence formed in 2021 and the average defoliation of old leaves from March to mid-June.

Furthermore, fruit weight (g/100 fruits) was negatively correlated to the average percentage of defoliation of old leaves from mid-June to mid-September, as shown in Figure 8. This indicates that there was an indirect effect of *V. oleaginea* on the fruit dry weight.

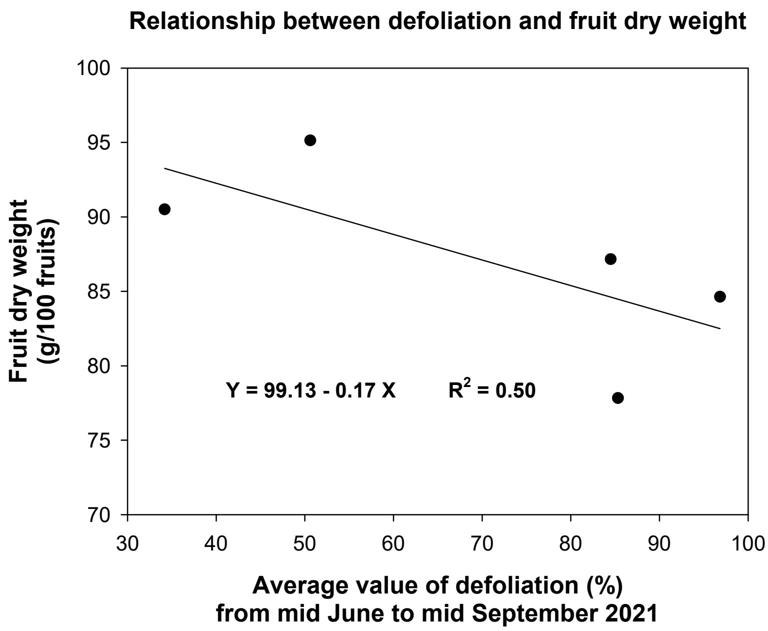

Figure 8. Relationship between the fruit dry weight in 2021 and the average defoliation of old leaves from mid-June to mid-September.

4. Discussion

Control of OLS disease is mainly based on the application of copper (Cu) fungicides in all countries where olive is grown, and especially in organic olive groves [19,20]. When the disease is severe and the level of total *V. oleaginea* inoculum is high, several applications of copper fungicides are necessary in one year [21]. However, the high level of Cu accumulation in the soil, and the risk of surface and subsurface water contamination and potential public health problems due to Cu entering the food chain, have raised concerns on the use of Cu in agriculture [22]. As a consequence, there is worldwide regulatory pressure on agriculture in general, especially in organic production, to restrict the use of these compounds. For example, the European Commission has included Cu compounds in the list of substances as candidates for substitution. At the present time, a total application of a maximum of 28 kg of metallic copper per hectare over a period of 7 years is authorized. Some researchers already predict that the use of Copper compounds may be banned in agriculture in the near future [22]. Hence, given this situation, it is urgent to test chemical compounds with a low Cu content and to find alternatives to copper compounds. Thus, this study was designed to evaluate the use of two chemicals to control OLS disease: Disper Cu Max® and Disper Broton GS®. They have a metallic Cu content five and ten times lower than copper hydroxide, respectively (Table 1).

The olive grove chosen for the present study was optimal, as: (i) severe *V. oleaginea* infection was present; (ii) the location is very favorable to OLS disease development [12,23–25]; (iii) the cultivar present (Nabali Baladi) is highly susceptible to OLS disease [6,12,23,26].

It is known that OLS disease provokes intense defoliation [15,16], and that reduction in defoliation in fungicide-treated olive trees is likely and essentially due to the reduction in the degree of infection.

All the treatments applied from March to September compared to the Control tended to reduce the defoliation of old leaves, especially those with dodine (as a curative fungicide) and, particularly, the self-defense inducer (Disper Broton GS®), which had the lowest defoliation values. The reduction in defoliation rate in all trees treated with the Cu complex (Disper Cu Max®) and, in particular, with dodine and the self-defense inducer (Disper Broton GS®) may be due to the fact that treatments penetrated the leaves, providing a curative effect and longer persistence, as they were not washed out by rainfall.

Regarding the new vegetation in 2021, new shoots continued to grow until July and the new leaves had relatively high percentages of symptomatic infection, notably in the Control and Cu hydroxide-treated trees. The percentage of asymptomatic infected new leaves in 2021 was significantly lower in Control trees and Cu hydroxide-treated trees, largely due to a higher percentage of symptomatic leaves. All other treatments (Cu complex, dodine, and mainly the self-defense inducer) had higher values of asymptomatic infected leaves because they had lower levels of symptomatic ones. The infection patterns can be explained by considering that the percentages of symptomatic and asymptomatic infected leaves are expressed as a percentage of the total leaves on the shoot. Therefore, they are complementary: if one increases the other decreases. The higher percentages of symptomatic leaves on the Control and Cu hydroxide-treated trees indicate an infection at a highly advanced and severe stage. Therefore, treatments with Disper Cu Max® and, especially, with dodine and the self-defense inducer (Disper Broton GS®), seem to block the disease at the first stages of development.

The increase in fruit-set on trees treated with dodine, the self-defense inducer Disper Broton GS® and the Cu complex (Disper Cu Max®) may be a direct result of less defoliation in the early part of the 2021 season. Possibly, the permanence of old leaves enabled greater formation of assimilates through photosynthesis, which resulted in more fruits per inflorescence. In spite of the fact that severe infections of olive leaf spot in fall and winter (like in the present investigation) resulted in severe defoliation in the following spring, trees treated with the self-defense inducer (Disper Broton GS®) and dodine were able to retain the majority of their old leaves until the new ones had formed (end of June–beginning of July). This may have improved fruit-set, as also demonstrated by the negative relationship between the average percent of defoliation from March to mid-June and the number of fruits/inflorescence. Moreover, the same treatments were able to maintain a higher percentage of old leaves until the end of the experiment than Control and Cu hydroxide. Indeed, at the end of the trial, about 30% of the leaves remained on dodine-treated trees, and about 60% on the self-defense inducer (Disper Broton GS®)-treated trees, whereas Control, Cu hydroxide, and the Cu complex (Disper Cu Max®)-treated trees had 0% leaves. The longer permanence of old leaves on trees treated with dodine and the self-defense inducer (Disper Broton GS®) also explains the negative relationships between the average percentage of defoliation from mid-June to mid-September and the fruit dry weight evaluated at the end of the experiment. A negative relationship between defoliation and fruit-set was also observed by Issa et al. [18,23], although in this case the different degrees of defoliation were not due to the effects of treatments with fungicides but to different environmental conditions.

The results indicate that the treatments had a remarkable impact on decreasing the intensity of infections and defoliation. Among the compounds tested, dodine and, especially, the self-defense inducer (Disper Broton GS®) were the most effective, resulting in the least defoliation of old leaves, the lowest intensity of the disease on young leaves, and the highest level of fruit-set. With respect to copper hydroxide, Cu complexed with LS and G acid had an intermediate efficacy, resulting in a lower intensity of the disease and a higher fruit set. The greater effectiveness of these compounds may be attributed to their mode of action, as both dodine and the Cu complex (Disper Cu Max®) penetrate the leaf tissue and likely provide a curative effect. The self-defense inducer (Disper Broton GS®) in turn is likely able to trigger the plant's self-defense mechanisms and, consequently, shows a curative effect.

The good results obtained with the Cu complex (Disper Cu Max®) and, especially, dodine agree with previous studies in which preliminary positive results were obtained with Disper Cu Max® [12], and dodine was shown to be very effective in trees highly infected by OLS disease as in the present investigation [14]. The results obtained with the self-defense inducer Disper Broton GS® are completely novel. Its protective effect is probably due to a direct antimicrobial activity exerted by copper as well as zinc, which is known to have antifungal activity [27], as well as by an indirect effect due to the activation of induced resistance [28,29]. Therefore, this opens a new horizon for the control of OLS disease by exploiting the natural defense system of the tree.

All the compounds tested made it possible to significantly reduce the use of copper, as dodine does not contain any copper and Cu complexed with LS and G acid and the self-defense inducer contain low amounts. This is crucial for minimizing the use of copper to comply with European regulations, which specify an average maximum of 4 kg/hectare per year. It is important to note that using Cu complexed with LS and G acid gives a five-fold decrease in the amount of copper required for each treatment compared to copper hydroxide, whereas the self-defense inducer gives a ten-fold reduction (Table 1).

To develop an effective approach for olive leaf spot management, the NaOH test should be used to monitor disease incidence. It is critical to maintain a low percentage of infected leaves. A threshold of 20% could be used, as studies evaluating artificial defoliation have shown that a loss of such percentage of leaf surface can be compensated by an increase in the photosynthetic activity of the remaining leaves [30]. Thus, when the percentage of symptomatic + asymptomatic infected leaves exceeds 20%, treatments are necessary to control the disease. All of the fungicides evaluated may be used for this purpose. In this regard, it is important to emphasize that disease control is easier if treatments are applied annually [31].

5. Conclusions

In a field trial carried out in Palestine in a severely *Venturia oleaginea*-infected olive grove, it was demonstrated that the applications of copper complexed with lignosulphonate and gluconate (Disper Cu Max®) or the self-defense inducer chemicals Disper Broton GS®, which contain levels of metallic copper 5–10 times lower than traditionally used copper fungicides, significantly protected olive trees against the fungal attacks. Protection was comparable to that obtained with applications of copper hydroxide for Disper Cu Max® and dodine for Disper Broton GS®. In addition, the protective effect was associated with a reduction in symptomatic infections, especially in new leaves, and the fall of old leaves.

All treatments, compared to the untreated control, were also able to maintain higher reproductive activity in the trees. Indeed, fruit-set and fruit dry weight were negatively related to defoliation, which was higher for most of the period in control trees.

Further studies are necessary to optimize treatment timing in order to further improve the effectiveness of the chemicals investigated, and to better understand the mode of action of the self-defense inducer using a molecular approach.

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