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A. La Torre^a, C. Mandalà^a, L. Pezza^a, F. Caradonia^a & V. Battaglia^a

^a Consiglio per la Ricerca e la sperimentazione in Agricoltura - Centro di ricerca per la patologia vegetale, Rome, Italy

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RESEARCH ARTICLE

Evaluation of essential plant oils for the control of *Plasmopara viticola*

A. La Torre*, C. Mandalà, L. Pezza, F. Caradonia and V. Battaglia

Consiglio per la Ricerca e la sperimentazione in Agricoltura - Centro di ricerca per la patologia vegetale, Rome, Italy

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The aim of this study was to test the effectiveness of various concentrations of tea tree oil and clove oil against *Plasmopara viticola*. *In vitro* tests entailed assessing the development of *P. viticola* on leaf disks and calculating sporangial germination. In a field trial, formulated products containing 23.8% tea tree oil (BM-608) and 15% clove oil (Sporatec) were tested in an organic vineyard to control downy mildew. The efficacy of pure and formulated essential oils was compared with a water control and a reference product (Airone, containing copper oxychloride 50% and copper hydroxide 50%). Laboratory experiments revealed the inhibitory activity of essential oils, although the effectiveness was lower than that of the reference product. The field data demonstrated that BM-608 and Sporatec were able to control the development of downy mildew although they were not as effective as the reference product. This study suggests the application of essential oils in order to reduce the use of synthetic pesticides in agriculture, in accordance with European laws, and to avoid the environmental pollution of copper in organic farming.

Keywords: natural products; tea tree oil; clove oil; grape downy mildew; organic farming

1. Introduction

Awareness of the importance of environmental protection and the food safety has increased noticeably over the last years. According to EU legislation on the sustainable use of pesticides (Directive 2009/128/EC), member states should take all necessary measures to reduce the risks and impacts of pesticide use on human health and the environment. In addition, Commission Regulation (EC) No. 473/2002 aimed to reduce the annual amount of copper used in organic farming. Since the use of copper as a fungicide may have long-term consequences due to its accumulation in the soil, research efforts should be increased in order to find appropriate alternative solutions.

A large number of investigations have focused on natural products able to reduce or replace copper (1–7). However, copper is still essential for the cultivation of a wide range of plants, especially for the control of *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni on grapevine. These considerations led us to carry out research in order to control grape downy mildew in an environmentally friendly way by identifying the effectiveness of essential oils: tea tree oil and clove oil.

Tea tree oil (TTO) is obtained by the steam distillation or hydro-distillation of leaves and terminal branches of Australian bush *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae) (8–10). It is a complex mixture of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated

alcohols (11). Although the composition is variable, it is regulated by an international standard for ‘Oil of *Melaleuca* – terpinen-4-ol type’, which sets the maxima and/or minima of fourteen components of the oil, which were selected for a variety of reasons, including biological activities. According to ISO 4730:2004, the major components are as follows: terpinen-4-ol ($\geq 30\%$), γ -terpinen (10–28%), α -terpinen (5–13%) and 1.8-cineole ($\leq 15\%$) (8, 10, 12).

TTO has been found to have some of the strongest antimicrobial properties ever discovered in a plant (13). The antimicrobial activity is attributed mainly to terpinen-4-ol, which is the major component of the oil (11, 14). TTO is effective as an antiseptic, fungicide and bactericide (15). It has been re-evaluated in recent years as an alternative agent to antibiotics (10, 16). The mode of action of this oil, which has been studied in bacteria and fungi, involves both the loss of membrane integrity and permeability, accompanied by the release of intracellular material and the inhibition of cellular respiration, with the consequent inability to maintain homeostasis associated with changes in cell morphology (10, 11, 17).

Cloves (*Syzygium aromaticum* (L.) Merr. & L.M. Perry, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of the tree family Myrtaceae (18, 19). Clove oil (CO) is a steam distillation product obtained from clove leaves or buds, which contain phenylpropanoids such as eugenol,

*Corresponding author. Email: anna.latorre@entecra.it

thymol, carvacrol and cinnamaldehyde (19). The major component is eugenol (84–88%) and further distillation of CO produces a refined product containing almost pure eugenol (i.e. > 95%) (8). Several studies have demonstrated the potent antifungal (20–22), antiviral (19) and antibacterial effects of cloves (23–26). Cloves are used in Ayurveda, Chinese medicine and Western herbalism (27). Cloves are antimutagenic (28), anti-inflammatory (29), antioxidant (19), antiulcerogenic (24), antithrombotic (18) and antiparasitic (30). The main antifungal action of phenolic compounds, such as eugenol, appears to be exerted on the cellular membrane (11, 17).

TTO and CO have recently been included in EU Regulation No. 540/2011 (the list of active substances approved for use in plant protection products) and are authorized for use in many European countries. To date, commercial formulations have not been authorized against *P. viticola*. TTO has been authorized as a fungicide to control early blight on potato, and powdery mildew on carrot, herbs, cucumber, watermelon, tomato, pepper and ornamentals, and CO has been authorized for post-harvest use on apples and pears against *Gloeosporium* spp. and *Penicillium* spp..

Our research thus aimed to evaluate the efficacy of these essential oils for the control of *P. viticola*, a key pathogen in grapes. The activity of pure and formulated essential oils was evaluated both *in vitro* and *in vivo*.

2. Experimental

2.1. Laboratory experiments

2.1.1. Tested products

TTO and CO essential oils (pure and formulated products) were used for this study. The investigated products containing TTO were: (i) extract from TTO (main components: terpinen-4-ol 40.7% and gamma terpinene 20.3%), which was purchased from SOVIMPEX (France) and certified by FARMALABOR in terms of component content in accordance with ISO 4730:2004 (E-TTO); (ii) terpinen-4-ol (Fluka code 86477 ≥ 99%) (T-4-ol) and (iii) BM-608, a commercial formulation containing 23.8% (w/w) of TTO, developed by Biomor Israel Ltd. (BM-608).

The investigated formulated products containing CO were: (i) BIOXEDA containing 20% (w/w) of CO (the main component is eugenol with a content/minimum purity of 800 g/kg), which was provided by Xeda International S.A. (France) and (ii) SPORATEC containing 18% rosemary oil, 15% CO and 5% thyme oil, developed by Brandt Consolidated, Inc. (USA).

2.1.2. Pathogen

Plasmopara viticola was obtained from naturally infected vines in an organic vineyard cv. Malvasia di

Candia located near Rome, Italy. Infected grapevine leaves showing the symptoms of the disease were used as the source of sporangial inoculum of oomycete.

2.1.3. Leaf disk assay and inoculation with *P. viticola*

Leaf disks were prepared according to Boso and Kassemeyer (31). Untreated leaves of *Vitis vinifera* L. cv. Malvasia di Candia, were used to obtain 18-mm diameter leaf disks, which were punched out with a cork borer. Ten leaf disks, obtained from ten healthy leaves, were prepared for each treatment and, after surface sterilization, were immersed for 20 minutes in solutions of different concentrations (ranging from 0.0125% to 1%) of the tested products. The immersion provided a uniform distribution of products on all parts of the leaf disk. In order to overcome limited solubility in aqueous media, E-TTO and T-4-ol were emulsified with 0.05% Tween 20. Distilled sterile water was used as a control. A control solution containing 0.05% Tween 20 was also considered in order to examine the effects of the solubilizing agent. A standard (Airone, marketed by ISAGRO S.p.A. – copper oxychloride 50% and copper hydroxide 50%) was used as a positive control in the dosage prescribed on the label.

Leaf disks were dried aseptically and then placed in Petri dishes (90 mm) containing 1% water agar and 30 mg/L benzimidazole. The leaf disks were placed with the abaxial side up and after 24 hours each disk was administered one drop of 30 µL inoculum by means of a micropipette. The inoculum was prepared by rinsing infected leaves with distilled sterile water and adjusting to 1.3×10^5 sporangia/mL using a hemocytometer. Each experiment was repeated twice, with two Petri dishes for each treatment. Petri dishes were incubated at $20 \pm 1^\circ\text{C}$ overnight in darkness (to induce sporulation) and then kept in a thermostatic chamber ($20 \pm 1^\circ\text{C}$, 12-hour photoperiod). Leaf disks were examined daily to verify the presence of phytotoxic effects. Ten days after inoculation, disease severity was measured as the percentage of leaf disk area with sporulating colonies, determined using a binocular microscope at $32\times$ magnification. We used the following 0–5 scale: 0=no visible downy mildew development, 1=0–5%; 2=6–25%; 3=26–50%; 4=51–75%; 5=>76% leaf area affected. The Percentage Disease Index (PDI) was calculated according to McKinney's formula (32):

$$PDI = \left[\sum (f \times v) \right] / (N \times X)$$

where f is the infection class frequency, v is the number of leaf disks of each class, N is the total number of leaf disks observed and X is the highest value of the evaluation scale. The effectiveness in % (Eff %) of test products was computed using Abbott's formula (33):

$$\text{Effectiveness \%} = [(I_c - I_t)] \times 100$$

where I_c is the percentage of disease on the untreated leaf disks and I_t is the percentage of disease on the treated leaf disks.

2.1.4. Sporangial discharge

The percentage of sporangia-releasing zoospores (i.e. empty sporangia) was counted under a light microscope in order to evaluate the effectiveness of the products on sporulation. Glass slides (Thermo scientific code 289303041) were used with three wells sized 14 mm. Each slide was placed in a Petri dish: one slide for each Petri dish and one Petri dish for each concentration of products. To facilitate sporulation of the oomycete, a moistened tissue paper was placed in the bottom of each Petri dish. A total of 100 μL of sporangial suspension of *P. viticola* (0.9×10^5 sporangia/mL) was pipetted into each slide well, and 100 μL of the products was added at concentrations ranging from 0.0125% to 1% for TTO and from 0.0125% to 0.1% for CO.

Two controls were considered: one control consisted of 100 μL of sporangial suspension with 100 μL of sterile distilled water added, and the other consisted of 100 μL of sporangial suspension and 100 μL of Tween 20 (0.5%) in order to evaluate the effect of the emulsifying agent. A formulation containing copper (Airone) was used as a positive control in the dosage prescribed on the label. One hundred sporangia were counted for each slide well (for a total of 300 sporangia/slide) for each concentration, after 16 and 40 hours of incubation at $20 \pm 1^\circ\text{C}$. Sporangia were observed microscopically ($40 \times$ magnification). The percentage of germinated (empty) sporangia was calculated by dividing the number of empty sporangia by the total number of sporangia. The average percentage germination was calculated from the three replicates/slide.

2.2. Field trial

2.2.1. Experimental conditions

A field trial was carried out in 2010 in an organic vineyard near Rome (Italy) (41.4°N , 12.3°E , 180 m a.s.l.). Grapevines of *V. vinifera* cv. Malvasia di Candia were used to study the effect of BM-608 and Sporatec against *P. viticola* compared with an untreated control and a reference product (standard) containing copper. Cuproxat® SDI (tribasic copper sulphate) and Bentoram® (copper hydroxide) were used as standards in the dosages prescribed on the labels. BM-608 was applied at a concentration of 0.75% of spray volume, and Sporatec at a rate of 1%. The rootstock (forty-four years old) employed was Kober 5BB (*V. berlandieri* \times *V. riparia*). The training system was 'tendone', consist-

ing of a continuous overhead canopy from which the bunches of grapes hang (34). Plots were prepared, each containing twelve vine plants and repeated four times in randomized blocks. The distance between the vines was 2.5 m \times 2.5 m. In order to avoid drift of product from one plot to another, each plot was separated from its neighbor by a row of untreated plants. Cultural conditions were uniform in the experimental site and conformed to organic viticulture practices.

The products were sprayed until near run-off at a pressure of 1.5 bar, with sufficient coverage of the lower and upper surfaces of the leaves. For all the products, spraying was performed with a pulled sprayer (Martignani K.W.H. electrostatic sprayer system). The commercial products were used according to the manufacturer's instructions. The first application was carried out before the appearance of disease symptoms, and the interval between the treatments varied between five to eight days, depending on the disease pressure. The trial was carried out according to the EPPO/OEPP PP1/31 (3) guidelines (35).

2.2.2. Meteorological data

Because of the importance of climatic conditions for the epidemiology of grape downy mildew, a weather station (Davis Instruments, model wireless) was placed in the trial site to record precipitation, air temperature, soil moisture at a depth of 20 and 40 cm, leaf wetness, solar radiation, relative humidity, soil temperature, and wind speed and direction. Data were acquired through a GSM modem, which was on board the weather station for remote transmission to a data management WeatherLink software program.

2.2.3. Disease assessment

The assessments were made at intervals of seven days starting from the first symptom of disease until harvest. Phenology was described according to the Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale (36), in which grapevine phenological development stages are described by Lorenz et al. (37). We scored 100 leaves and 100 bunches picked randomly from ten central plants of each plot and estimated the percentage of affected organs (disease incidence) and the percentage areas of leaves and bunches showing disease symptoms (disease severity). Disease severity was computed using a scale of nine classes (0–8) McKinney's formula (32).

The effectiveness of the products was calculated using Abbott's formula (33).

Observations regarding phytotoxicity and other side effects were recorded.

2.3. Statistical analysis

Statistics were performed using GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) and differences between treatments were considered significant at $p < 0.05$. All percentages were transformed by arcsin square root before analysis and were back-transformed to percentages for presentation in tables and figures. The data obtained were subjected to analysis of variance (ANOVA) for quantitative variables, and means were separated using Tukey's test. The non-parametric Kruskal–Wallis test was used to analyze ordinal variable results, the values were separated by Dunn's *post hoc* test.

3. Results

3.1. Laboratory experiments

3.1.1. Effect of essential oils on leaf disk bioassay and sporangial discharge

The results obtained by testing TTO on inoculated leaf disks and on sporulation of *P. viticola* are reported in Table 1. E-TTO completely inhibited the development of *P. viticola* on leaf disks at 0.025% and showed the same effectiveness as the standard. Phytotoxic effects were observed, starting from a concentration of 0.05%. Phytotoxicity symptoms consisted in complete or partial necrosis of leaf tissues. There was no significant difference in disease severity between leaf disks treated with 0.05% Tween 20 and the water control. T-4-ol showed a reduction in the development of *P. viticola* compared with the control amended with Tween 20 at non-phytotoxic concentrations of 0.0125% and 0.025%. T-4-ol showed no growth at 0.05%. Phytotoxicity symptoms, consisting of complete or partial necrosis of leaf tissues, were observed starting from 0.125%. BM-608 showed a reduction in the development of *P. viticola* on leaf disks compared with the water control at all the concentrations that were not phytotoxic. At 0.25% and 0.5%, BM-608 differed significantly from the water control. BM-608 showed phytotoxicity symptoms at the two highest concentrations (0.75% and 1%), although the effects were less severe than those obtained using the other two products. The effectiveness of TTO on grapevine leaf disks are shown in Figure 1.

Only those concentrations that did not show phytotoxicity effects during *in vitro* leaf disk bioassay were examined for sporangial discharge. The inhibitory activity of the copper standard was also evaluated. The percentages of empty sporangia slightly increased as the duration of the sporulation period increased (Table 1). The results obtained testing E-TTO at a concentration of 0.0125% showed that after 40 hours of incubation,

the percentage of empty sporangia was statistically different from the percentage of empty sporangia in the control amended with Tween 20. E-TTO at 0.025% like the standard was statistically different from the control amended with Tween 20 after incubation for 16 and 40 hours. T-4-ol showed inhibitory activity at all the tested concentrations (0.0125–0.025% and 0.05%); however, the inhibitory activity was lower than the standard. The inhibitory activity of BM-608 increased with increasing concentrations. BM-608 reduced the percentage of empty sporangia at all concentrations after both 16 and 40 hours of incubation. After 16 hours of incubation, at the highest tested concentrations (0.25% and 0.5%), the percentage of empty sporangia was not statistically different from the percentage of empty sporangia of the standard. After 40 hours of incubation at 0.5%, BM-608 was as effective as the standard in reducing the percentage of sporulation.

Table 2 shows the results obtained by testing CO products (Bioxeda and Sporatec) on inoculated leaf disks and on sporangial discharge. At a non-phytotoxic concentration (0.0125%), Bioxeda showed a reduction in the development of *P. viticola* compared with the water control. Phytotoxicity symptoms were observed starting from a concentration of 0.025%. Symptoms of phytotoxicity consisted of complete or partial necrosis of leaf tissues. Sporatec formulation, like the standard, completely inhibited the development of *P. viticola* on leaf disks at 0.0125%. Sporatec showed less severe phytotoxicity symptoms than those obtained using Bioxeda at the same concentration. The effects of CO products on grapevine leaf disks are shown in Figure 2.

Only those concentrations that did not show phytotoxicity effects during *in vitro* leaf disk bioassay were examined for sporangial discharge. The percentages of empty sporangia slightly increased as the duration of the sporulation period increased. The best inhibitory activity was obtained with Sporatec at 0.0125%. Sporatec formulation showed a higher sporangial discharge than the positive control (standard), but both Sporatec and standard were statistically different from the water control. Sporatec reduced the percentage of empty sporangia after both 16 and 40 hours of incubation. Bioxeda also showed inhibitory activity at tested concentration (0.0125%); however, compared with the untreated control, this inhibition was only statistically significant after 40 hours of incubation.

3.2. Field trial

3.2.1. Effects of tested products on disease incidence and disease severity

Meteorological data (precipitation, air temperature, relative humidity and leaf wetness) recorded during the field trial are reported in Figure 3.

Table 1. Effectiveness of various concentrations of tea tree oil (TTO) on *Plasmopara viticola* development and sporangial germination.

Concentration (%)	Compound													
	E-TTO						T-4-ol						BM-608	
	PDI (%) ^a	Eff % ^b	% Empty sporangia ^c		PDI (%)	Eff %	% Empty sporangia		PDI (%)	Eff %	% Empty sporangia			
		At 16 hours	At 40 hours			At 16 hours	At 40 hours			At 16 hours	At 40 hours			
0.0125	6 ± 0.0ab	75.0	10.0 ± 2.2bc	10.7 ± 1.3b	8 ± 0.0ab	62.5	10.0 ± 3.4bc	12.0 ± 1.1b	16 ± 0.0ab	0.0	12.0 ± 1.0ab	14.0 ± 1.0b		
0.025	0 ± 0.0b	100.0	6.7 ± 2.0cd	8.0 ± 1.2b	6 ± 1.4ab	75.0	9.4 ± 1.2c	10.7 ± 2.4b	14 ± 1abc	0.0	11.1 ± 2.0b	12.4 ± 1.3b		
0.05	p.e. ^d	—	—	—	0 ± 0.0b	100.0	8.7 ± 2.7bc	11.3 ± 1.5b	8 ± 0.0abc	0.0	9.3 ± 0.7b	11.3 ± 0.6b		
0.125	p.e.	—	—	—	p.e.	—	—	—	8 ± 0.0abc	50.0	10.0 ± 1.0b	10.7 ± 0.6b		
0.25	p.e.	—	—	—	p.e.	—	—	—	4 ± 4.7bc	75.0	7.3 ± 2.1bc	11.3 ± 1.1b		
0.5	p.e.	—	—	—	p.e.	—	—	—	2 ± 3bc	75.0	1.3 ± 2.7d	2.7 ± 1.1c		
0.75	p.e.	—	—	—	p.e.	—	—	—	p.e.	—	—	—		
1	p.e.	—	—	—	p.e.	—	—	—	p.e.	—	—	—		
Control water	30 ± 2.3ab	—	24.0 ± 1.2a	31.3 ± 2.3a	30 ± 2.3ab	—	24.0 ± 1.2a	31.3 ± 2.3a	30 ± 2.3a	—	24.0 ± 1.2a	31.3 ± 2.3a		
Control Tween 20	36 ± 2.0a	—	20.7 ± 1.2ab	25.3 ± 1.2a	36 ± 2.0a	—	20.7 ± 1.2ab	25.3 ± 1.2a	—	—	—	—		
Standard ^e	0 ± 0.0b	100.0	2.0 ± 3.4d	2.7 ± 1.1c	0 ± 0.0b	100.0	2.0 ± 3.4d	2.7 ± 1.1c	0 ± 0.0c	100.0	2.0 ± 3.4cd	2.7 ± 1.1c		

Notes: ^aPercentage Disease Index was calculated with McKinney's formula. Values within columns followed by same letter are not significantly different by Dunn's *post hoc* test ($p < 0.05$).

^bPercentage effectiveness was obtained by using Abbott's formula.

^cThe data represent the mean values of three replicates ± SE. Values within columns followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

^dp.e., phytotoxic effect.

^eStandard: Airone (copper oxychloride 50% and copper hydroxide 50%) used at the dosage prescribed on the label.

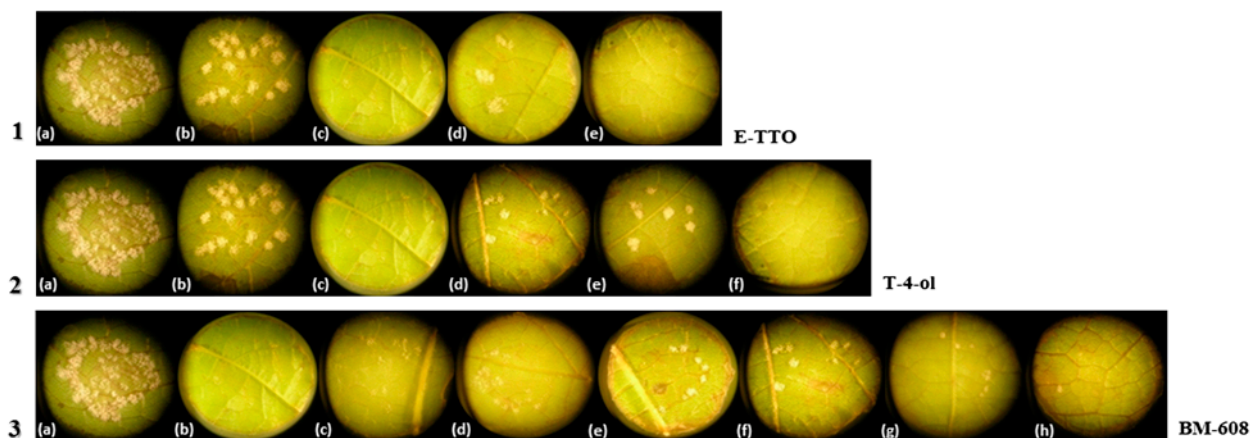


Figure 1. Development of *Plasmopara viticola* ten days after inoculation on grapevine leaf disks treated with: water 1 (a), Tween 20 1 (b), standard 1 (c), extract from TTO (E-TTO) at 0.0125% 1 (d), 0.025% 1 (e); water 2 (a), Tween 20 2 (b), standard 2 (c), terpinen-4-ol (T-4-ol) at 0.0125% 2 (d), 0.025% 2 (e), 0.05% 2 (f); water 3 (a), standard 3 (b), BM-608 at 0.0125% 3 (c), 0.025% 3 (d), 0.05% 3 (e), 0.125% 3 (f), 0.25% 3 (g), 0.5% 3 (h).

Table 2. Effectiveness of various concentrations of Bioxeda and Sporatec on *Plasmopara viticola* development and sporangial germination.

Concentration (%)	Compound							
	Bioxeda				Sporatec			
	PDI (%) ^a	Eff % ^b	% Empty sporangia ^c		PDI (%)	Eff %	% Empty sporangia	
At 16 hours			At 40 hours	At 16 hours			At 40 hours	
0.0125	14.0 ± 0.0ab	27.0	12.0 ± 1.0a	14.7 ± 1.4b	0 ± 0.0b	100.0	10.0 ± 1.1b	12.7 ± 1.5b
0.025	p.e. ^d	–	–	–	p.e.	–	–	–
0.05	p.e.	–	–	–	p.e.	–	–	–
0.1	p.e.	–	–	–	p.e.	–	–	–
Control water	36.0 ± 0.0a	–	24.0 ± 1.2a	31.3 ± 2.3a	36.0 ± 0.0a	–	24.0 ± 1.2a	31.3 ± 2.3a
Standard ^e	0 ± 0.0b	100.0	2.0 ± 3.4b	2.7 ± 1.1c	0 ± 0.0b	100.0	2.0 ± 3.4c	2.7 ± 1.1c

Notes: ^aPercentage Disease Index was calculated with McKinney's formula. Values within columns followed by same letter are not significantly different by Dunn's *post hoc* test ($p < 0.05$).

^bPercentage effectiveness was obtained by using Abbott's formula.

^cThe data represent the mean values of three replicates ± SE. Values within columns followed by same letter are not significantly different according to Tukey's test ($p < 0.05$).

^dp.e.: phytotoxic effect. ^eStandard: Airone (copper oxychloride 50% and copper hydroxide 50%) used at the dosage prescribed on the label.

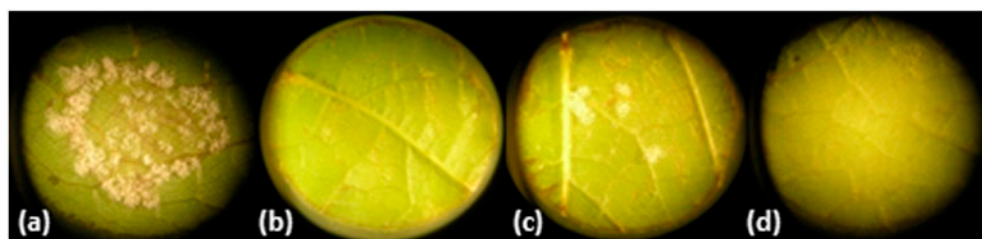


Figure 2. Development of *Plasmopara viticola* ten days after inoculation on grapevine leaf disks treated with: water (a), standard (b), Bioxeda at 0.0125% (c) and Sporatec at 0.0125% (d).

The humid conditions during the last week of May caused the appearance of oil spots on 24 May 2010 (BBCH 57 – fully developed inflorescences; flowers

separating) due to the presumed infection by the rain on 17 May 2010, which was the date of the first infection. The first symptoms of grapevine downy

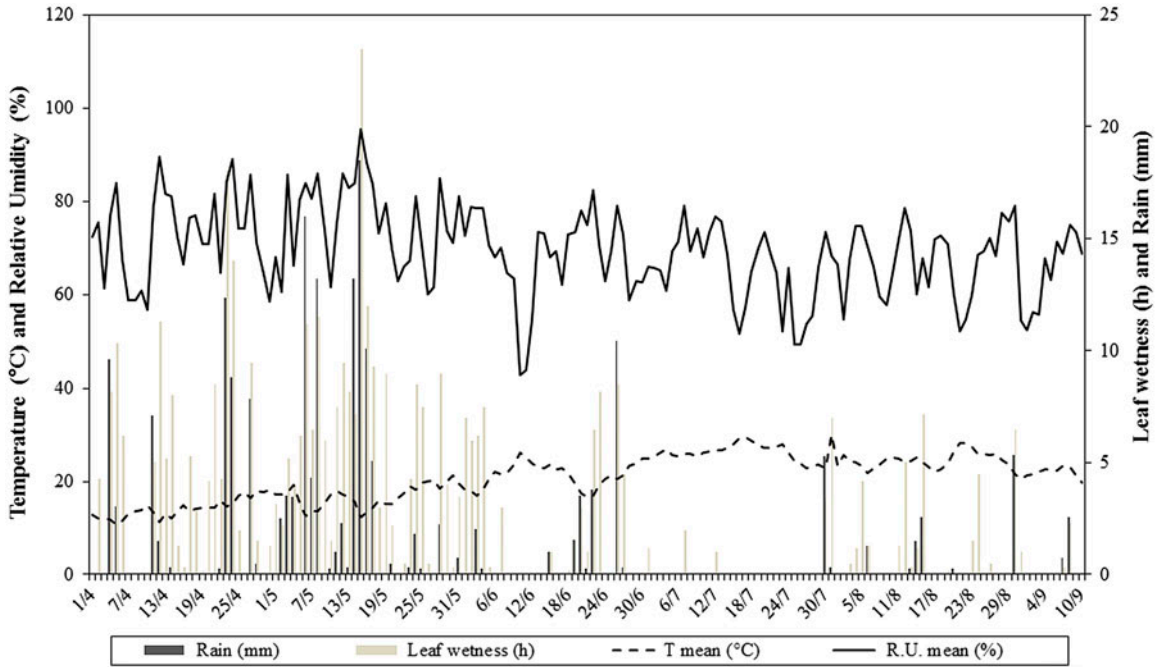


Figure 3. Meteorological data (precipitation, air temperature, relative humidity and leaf wetness) registered during the field trial.

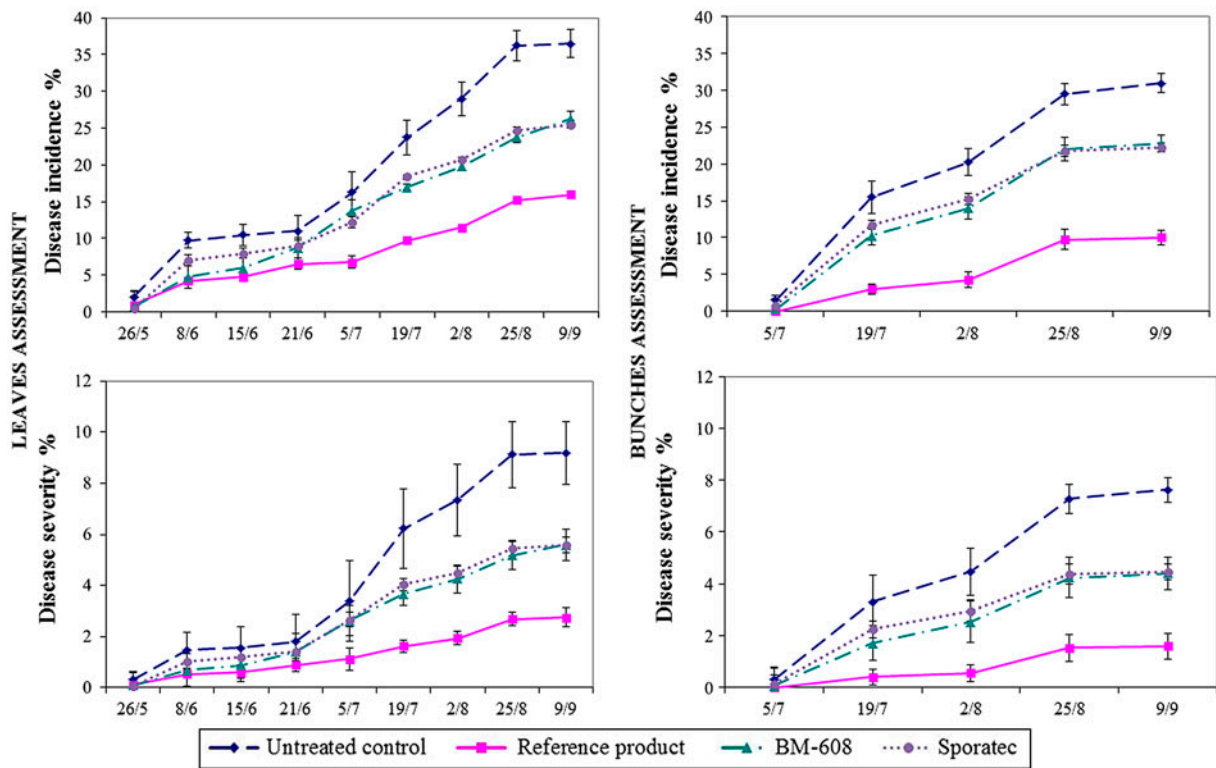


Figure 4. Effectiveness of tested products on disease incidence on leaves (a) and bunches (b), and disease severity on leaves (c) and bunches (d). The data represent the mean values of four replicates. Vertical bars indicate mean \pm SE.

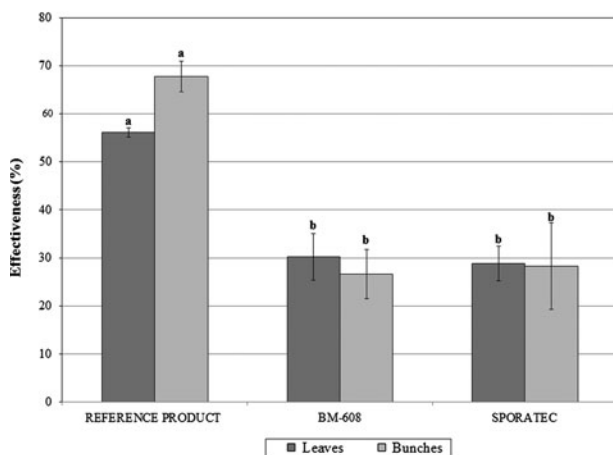


Figure 5. Effectiveness at the harvest of tested products. The data represent the mean values of four replicates. Vertical bars indicate mean \pm SE. Means with different letters on the top of each type of bar indicate significant differences between treatments according to Tukey test at $p < 0.05$.

mildew on bunches were observed on 23 June 2010 (BBCH 75 – berries pea-sized, bunches hanging). The phytosanitary situation was not particularly critical. Figure 4(a–d) shows the results of the field trial. The plants treated with BM-608 and the plants treated with Sporatec showed a higher disease incidence and disease severity than the plants treated with the standard; however, both formulations reduced disease incidence and severity in comparison with the water control. During the trial no symptoms of phytotoxicity were noted on either the leaves or the bunches.

3.2.2. Effectiveness of tested products and copper amounts provided by the treatments

Figure 5 shows the effectiveness of the tested products on the leaves and bunches at harvest. The effectiveness of BM-608 was similar to the effectiveness achieved with the Sporatec formulation. Both commercial formulations, showed an effectiveness lower than the effectiveness obtained using the cupric reference products. BM-608 provided 30% protection on leaves and 27% protection on bunches compared with the untreated control. Sporatec provided 29% protection on leaves and 28% protection on bunches compared with the untreated control. The BM-608 and Sporatec commercial formulations did not use copper, while the standard provided 6.3 kg/ha of total metallic copper.

4. Discussion

In conclusion, laboratory tests showed that TTO and CO (pure and formulated products) are able to act against *P. viticola* by inhibiting mycelial growth and

indirect sporangia germination. Field trials showed that treatments with BM-608 and Sporatec reduced disease symptoms compared with the water control, though they were not as effective as the reference products. This level of protection is acceptable especially in organic farming where only few plant protection products can be used, which also do not have the same effectiveness as synthetic pesticides. This study, in general, is in agreement with the other studies showing the antimicrobial activity of TTO (9–11, 14) and CO (19–26, 30). The results indicated that TTO is able to act as a fungicide (9–11, 15, 38) and bactericide (11, 14) both *in vitro* and *in vivo*. The antimicrobial activity was assessed in various crops in agriculture, such as tomato and barley. TTO was also evaluated against grape downy mildew in vineyards and the studies revealed a good inhibition of disease (15, 38). Our results are not in accordance with the investigation of Dagostin et al. (5), which evaluated BM-608 in field trial to control grape downy mildew; however, the level of disease control provided by this formulation, at a lower dosage than the dosage used in our research, was not statistically different from the untreated control. Few studies have examined the activity of CO on grapevine downy mildew (38). To our knowledge, most of the investigations have examined the effects of CO against human pathogens (19, 21, 22, 24–26). The prospect of using essential oils is very promising for reducing the dependency on harmful synthetic pesticides in agriculture, in line with European legislation on the sustainable use of pesticides, and also for reducing the dependency on copper in organic farming. TTO and CO do not have any harmful effects on human or animal health or on groundwater, in fact they have no negative impacts on the environment (39–40). They could thus play an important role in controlling downy mildew and other plant diseases. Essential oils could also be applied as a tool to reduce the number of chemical treatments in integrated pest management or to reduce the number of copper treatments in organic farming.

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