

EFFICACY OF FUNGICIDES AND ESSENTIAL OILS AGAINST BACTERIAL DISEASES OF FRUIT TREES

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Abstract: In the framework of the performed studies, the antibacterial activity of the following fungicides was evaluated: Miedzian 50 WG (active substance – a.s. 50% copper oxychloride), Ridomil MZ Gold 68 WG (a.s. 3.8% metalaxyl-M and 64% mancozeb), Euparen Multi 50 WG (a.s. 50% tolylfluanid), Captan 80 WG [a.s. 80% N-(captan)], Dithane Neotec 75 WG (a.s. 75% mancozeb). The evaluation also concerned the essential oils: lavender, sage, lemon balm, clove, and a preparation based on thyme oil (BioZell). Each preparation and compound was tested against the following bacterial pathogens: *Erwinia amylovora*, *Xanthomonas arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *Pseudomonas syringae* pv. *syringae*, *Agrobacterium tumefaciens* (presently *Rhizobium radiobacter*). Each preparation and compound was tested at a concentration of 1,000 ppm of active substance. Copper oxychloride was also tested at a concentration of 1,500 ppm. Among the tested fungicides, metalaxyl-M with mancozeb, mancozeb alone, and copper oxychloride inhibited all of the tested strains of pathogenic bacteria. Tolylfluanid did not inhibit any of the bacteria used. Out of the investigated essential oils, the strongest inhibitors of bacteria were: sage, cloves, and BioZell.

The protective activity of the above mentioned fungicides was also evaluated *in vivo*. They were assessed against fire blight on apple blossoms and pear fruitlets, against bacterial canker on sweet cherry fruitlets, and against crown gall on sunflower seedlings (the test plant). All fungicides were applied at the same concentrations as those in the *in vitro* tests. Only copper oxychloride was found to show protective activity against the studied diseases. This result indicates that the antibacterial properties of the other fungicides did not correspond with their activity on the plant organs used in the *in vivo* experiment.

Key words: bacterial pathogens, control, essential oils, fruit trees, fungicides

INTRODUCTION

The protection of orchards and nurseries against bacterial diseases constitutes one of the most important and difficult to solve problems in fruit production. In Poland losses of economical importance are caused mainly by fire blight (*Erwinia amylovora*), bacterial canker of stone fruits (*Pseudomonas syringae*), and crown gall (*Agrobacterium tumefaciens*, syn. *Rhizobium radiobacter*) (Sobiczewski 2009). In some regions, walnut bacterial blight (*Xanthomonas arboricola* pv. *juglandis*), and bacterial blight of hazelnut (*X. arboricola* pv. *corylina*) may be also harmful. Because of the irregular occurrence, difficulties in prediction, and the chronic course of most bacterioses, the development of effective control programs is very important and needs more research. At present, there is a very small assortment of chemical products available for growers. The compounds are almost exclusively based on copper, act only as protectants, and especially on apples can cause phytotoxic effects. In some countries, besides copper, different antibiotic preparations (Burr 2001; McManus and Stockwell 2001; Janse 2005) and fungicide based on phosethyl of aluminium are used (Psallidas and Tsiantos 2000). Clearly there is a need for development of new,

effective control compounds for fruit bacterial diseases. Among the new perspectives are products of natural origin, including biopreparations and resistance inducers (Burr 2001; Sobiczewski 2001; Kado 2010).

The aim of our study was to evaluate antibacterial activity of some fungicides and essential oils. The efficacy of fungicides against fire blight, bacterial canker of stone fruits, and crown gall was also studied.

MATERIALS AND METHODS

Antibacterial activity of fungicides and essential oils

By using the agar plate method, the following fungicides: Miedzian 50 WG (active substance – a.s. 50% copper oxychloride), Ridomil MZ Gold 68 WG (a.s. 3.8% metalaxyl-M and 64% mancozeb), Euparen Multi 50 WG (a.s. 50% tolylfluanid), Captan 80 WG [a.s. 80% N-(captan)], Dithane Neotec 75 WG (a.s. 75% mancozeb) and synthetic essential oils: lavender, sage, lemon balm, clove, and a preparation BioZell (www.biozell-2000b.com) based on thyme oil were evaluated. A fixed quantity (150 µl) of either a water suspension or a solution of each test-

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ed preparation or compound was introduced into wells (Ø 10 mm) made in King B medium in Petri dishes, the surface of which was previously seeded with a bacteria of a given strain. Each preparation and compound was tested at a concentration of 1,000 ppm of active substance. Copper oxychloride was also tested at a concentration of 1,500 ppm. After incubation for 48 h at 24°C, the width of the zones of bacterial growth inhibition were measured from the edge of the well.

Bacterial strains

From our own collection we used the following strains of bacterial pathogens which cause disease on fruit trees: Ea659 – *E. amylovora* (fire blight), RIPF-X13 *X. arboricola* pv. *corylina* (bacterial blight of hazelnut), RIPF-04 *X. arboricola* pv. *juglandis* (walnut bacterial blight), Ps110 – *P. syringae* pv. *syringae* (bacterial canker of stone fruits), and At4 – *A. tumefaciens* (syn. *Rhizobium radiobacter*) (crown gall). Bacteria were cultivated on King B medium, and after 24 h a water suspension containing 10⁸ cfu/ml was prepared. One hundred µl of the suspension were placed on the surface of King B medium in Petri dishes (Ø 9 cm) and evenly distributed using a glass rod. Next, 4 symmetrically positioned wells were made using a corkborer.

Efficacy of fungicides against fire blight on apple blossoms

Branches of the cv. Jonagold were cut in the orchard whilst in full bloom. Their cut ends were dipped into glass jars with water and placed in the greenhouse. The blossoms on each branch (6 to 7 branches per treatment) were sprayed with either a water suspension of the tested fungicide (at the same concentrations as in *in vitro* tests) or only with water (as the control). After 24 hours, blossoms were inoculated by spraying with an aqueous suspension of Ea659 at 10⁷ cfu/ml and afterwards placed in plastic box in the greenhouse. The temperature in the box was maintained at 22–28°C and the air humidity at about 90–100%.

Five and seven days after inoculation, the presence of blight symptoms on the blossoms was recorded according to a slightly modified scale of Pusey (1999): 0 – apparently healthy flower, 1 – necrosis visible on sepals or petals and/or flower bottom, 2 – necrosis in the whole ovary, 3 – necrosis additionally covering a half length of the peduncle, 4 – total necrosis of ovary and peduncle.

Efficacy of fungicides against fire blight on pear fruitlets

Pear fruitlet slices at a thickness of 7–8 mm of cv. Conference were momentarily dipped into an aqueous suspension of tested preparation and then placed in Petri dishes on moist filter paper according to the Sobiczewski and Millikan (1985) method. After 6 hours the slices were inoculated by spraying with a water suspension of Ea659 at a concentration of 10⁷ cfu/ml. The disease symptoms were evaluated after five days of incubation at room temperature. Each isolate was tested on 40–50 slices. The following five-point scale was used: 0 – no symptoms, 1 – several drops of ooze, 2 – about half of the slice covered with ooze drops and necrosis, 3 – more than half of the slice covered with necrosis and heavy ooze, 4 – ooze and necrosis of the entire slice.

Efficacy of fungicides against bacterial canker on sweet cherry fruitlets

Sweet cherry fruitlets of the cv. Napoleon were disinfected in 50% ethanol and then air dried. Two wounds of 2 mm deep on one side of each fruitlet were made using a sterile needle. The wounded fruitlets were placed on a moistened filter paper in Petri dishes. The fruitlets were immediately sprayed with a water suspension of a given preparation at the same concentration as in the *in vitro* test. After 6 hours, the fruitlets were inoculated by spraying with a suspension of strain Ps110 at a concentration of 10⁸ cfu/ml. Evaluation of the severity of the disease symptoms was performed 4 days after incubation at 24°C using a five-point scale from 0–4: 0 – no necrosis and 4 – necrosis with a diameter of four mm or more. Forty fruitlets per treatment were applied (Sobiczewski *et al.* 1980).

Efficacy of fungicides against crown gall on sunflower seedlings

The roots of 7-day-old sunflower seedlings were wounded using a sterile needle. Then, the wounded seedlings were immersed for 10 minutes in a water suspension of tested preparation or in water (the control). Afterwards they were planted in the pots where the soil was contaminated with the bacteria strain At4 (50 ml of bacterial suspension at a concentration of 10⁸ cfu/ml was put into each hole of which a seedling was planted). The presence of galls on roots was assessed after 30 days. Each treatment consisted of 24 plants (Sobiczewski and Piotrowski 1983).

Results of all the tests were subjected to ANOVA analysis of variance. For separation of means, the Newman-Keuls test at a 5% significance level was used.

RESULTS

Among the tested fungicides, metalaxyl-M with mancozeb, mancozeb alone, and copper oxychloride inhibited all of the tested strains of pathogenic bacteria (Table 1). Metalaxyl-M with mancozeb, and mancozeb alone showed the strongest activity with the largest inhibition zones for the RIPF-X13 and RIPF-04 strains. Copper oxychloride showed relatively weak activity although it was most active against the RIPF-X13 strain at a concentration of 1,500 ppm. Low activity was also observed for captan. No inhibition was seen against the RIPF-X13 and Ps110 strains. Tolyfluanid did not inhibit any of the pathogenic bacteria used (Table 1). Out of the investigated essential oils, BioZell, the oil of sage, and the oil of cloves strongly inhibited the bacteria. However, the largest zones of inhibition were observed for the At-4 strain in contact with a thyme based oil preparation (Table 1). Essential oils such as lavender and lemon balm showed the lowest inhibition potential and no activity against the Ps110 strain. Lemon balm did significantly inhibit the At-4 strain.

On cut apple branches, the most effective in protection of blossoms against fire blight was copper oxychloride at both concentrations tested (1,000 and 1,500 ppm). The activity of the remaining fungicides was not significantly different as compared to the control (Table 2).

In tests on pear fruitlets (fire blight), sweet cherry fruitlets (bacterial canker of stone fruits), and sunflower

Table 1. Growth inhibitions zones (mm) of bacterial pathogens caused by fungicides and essential oils on KingB medium after 48 h of incubation

Treatment	Strains*				
	Ea659	RIPF-X13	RIPF-04	Ps110	At-4
Control (water)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Copper oxychloride (Miedzian 50 WG)	2.0 b	3.0 b	2.0 b	2.0 c	3.0 c
Copper oxychloride (Miedzian 50 WG)**	3.0 bc	4.3 b	2.7 b	2.7 d	2.0 bc
Metalaxyl-M, mancozeb (Ridomil MZ Gold 68 WG)	3.0 bc	6.0 c	7.7 c	1.7 c	2.6 bc
Tolyfluanid (Euparen Multi 50 WG)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Captan (Captan 80 WG)	3.0 bc	0.0 a	2.0 b	0.0 a	2.0 bc
Mancozeb (Dithane Neotec 75 WG)	4.0 c	6.0 c	9.0 c	2.0 c	3.0 c
Essential oils:					
BioZell	9.0 e	12.3 d	11.0 d	1.0 b	17.6 f
Lavender	3.3 bc	1.0 a	1.7 b	0.0 a	1.7 b
Lemon balm	4.7 c	3.0 b	3.3 b	0.0 a	10.0 d
Sage	6.0 d	11.0 d	8.0 c	3.0 d	9.3 d
Clove	13.3 f	11.3 d	11.3 d	1.0 b	13.0 e

*Ea 659 – *E. amylovora* (causal agent of fire blight), RIPF-X13 – *X. arboricola* pv. *corylina* (bacterial blight of hazelnut), RIPF-04 – *X. arboricola* pv. *juglandis* (walnut bacterial blight), Ps110 – *P. syringae* pv. *syringae* (bacterial canker of stone fruits), At4 – *A. tumefaciens* (syn. *Rhizobium radiobacter*) (crown gall). Each compound was tested at a concentration of 1,000 ppm
**1,500 ppm

Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test

Table 2. Protective activity of fungicides against fire blight on apple blossoms of cv. Jonagold

Treatment	Disease severity:	
	5 DAI**	7 DAI
Control (water)	1.1 b [0]	2.3 c [0]
Copper oxychloride	0.3 a [72,7]	0.9 ab [60,8]
Copper oxychloride*	0.2 a [81,8]	0.6 a [73,9]
Metalaxyl-M, mancozeb	0.7 ab [36,4]	1.7 abc [26,1]
Tolyfluanid	0.5 ab [54,5]	1.2 abc [47,8]
Captan	0.6 ab [45,5]	1.2 abc [47,8]
Mancozeb	1.1 b [0]	1.9 bc [17,4]

Each compound was tested at a concentration of 1,000 ppm

*1,500 ppm; **DAI – days after inoculation

Values in square brackets present efficacy (degree of disease severity in comparison to control)

Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test

Table 3. Protective activity of fungicides against fire blight on pear fruitlets of cv. Conference

Treatment	Disease severity 5 DAI**
Control (water)	4.0 c
Copper oxychloride	1.0 b
Copper oxychloride*	0.4 a
Metalaxyl-M, mancozeb	4.0 c
Tolyfluanid	4.0 c
Captan	4.0 c
Mancozeb	4.0 c

Each compound was tested at a concentration of 1,000 ppm

*1,500 ppm

**DAI – days after inoculation

Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test

Table 4. Protective activity of fungicides against bacterial canker on sweet cherry fruitlets of cv. Napoleon

Combination	Disease severity 4 DAI**
Control (water)	3.3 c
Copper oxychloride	0.7 a
Copper oxychloride*	0.6 a
Metalaxyl-M, mancozeb	3.4 c
Tolyfluanid	2.3 b
Captan	3.1 c
Mancozeb	3.3 c

Each compound was tested at a concentration of 1,000 ppm

*1,500 ppm

**DAI – days after inoculation

Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test

Table 5. Protective activity of fungicides against crown gall on sunflower roots

Treatment	Percent of seedlings with tumors on roots
Control (water)	91.7 c [0]
Copper oxychloride	52.5 b [42,7]
Copper oxychloride*	21.6 a [76,4]
Metalaxyl-M, mancozeb	66.6 bc [27,4]
Tolyfluanid	95.8 c [0]
Captan	91.7 c [0]
Mancozeb	70.8 bc [22,8]

Each compound was tested at a concentration of 1,000 ppm
*1,500 ppm

Values in square brackets present efficacy (% of symptomatic plants in comparison to control)

Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test

seedlings (crown gall) only copper oxychloride showed protective activity significantly different from the control (Table 3, 4, 5). It should be noted that tolyfluanid significantly reduced bacterial canker on sweet cherry.

DISCUSSION

Out of the tested fungicides, copper oxychloride, metalaxyl-M with mancozeb, and mancozeb alone showed the greatest spectrum of activity against the used strains of bacterial pathogens *in vitro*. It was established that fungicides containing mancozeb caused the largest inhibition zones of *E. amylovora*, *X. arboricola* pv. *juglandis* and *X. corylina*. In the literature, there is almost no data on the activity of this compound against bacterial pathogens of fruit trees. Conlin and McCarter (1983) reported that mancozeb inhibited the growth of *Pseudomonas syringae* pv. *tomato* on agar medium. Adaskaveg and Hine (1985) obtained similar results in the case of *X. campestris* pv. *vesicatoria*.

Our study showed that out of the essential oils tested sage oil, and clove oil, and also the preparation BioZell showed the highest antibacterial activity against all pathogens. Other authors also demonstrated the activity of oil from *T. vulgaris* against *E. amylovora* (Scortichini and Rossi 1989; 1993; Basim *et al.* 2000; Vanneste and Boyd 2002; Kokoskova *et al.* 2011), *P. syringae* pv. *syringae* (Basim *et al.* 2000; Kokoskova *et al.* 2011), *A. tumefaciens* (El-Zemity *et al.* 2008) as well as pathogenic xanthomonads (Kotan *et al.* 2007). It was also found that essential oil from sage was more active than streptomycin (Kotan *et al.* 2007). Moreover, clove oil showed activity against both *E. amylovora* and *A. tumefaciens* (Vanneste and Boyd 2002; El-Zemity *et al.* 2008).

In our work, we documented that only copper oxychloride showed protective activity against fire blight, bacterial canker of stone fruits, and crown gall on the used plant organs. Preparations containing copper compounds are well known and have been commonly used for over 100 years as fungicides. Preparations with copper also show protective activity against bacterial diseases.

Various copper formulations are the only means registered in many countries for the control of such bacterial diseases as fire blight or bacterial canker of stone fruits. However, these formulations only show a quite high efficacy on the plant surface.

Our study showed some activity of tolyfluanid and captan against fire blight on apple blossoms. Reports about protective activity of preparations containing mancozeb are available. Aldwinckle *et al.* (2002) proved 36.2% efficacy of ManKocide 15 WP in protection of apple shoots. Additionally, in a similar experiment performed by Momol *et al.* (1999), the reduction of the disease was found (efficacy 37.6%) after the application of Manzate 200 in comparison with the untreated control. We showed that efficacy of fungicides containing mancozeb, determined 7 days after inoculation, ranged from 17.4 to 26.1% depending on the fungicide. On the other hand, in the test on pear fruitlets, only copper fungicides showed protective activity. In the case of the crown gall test on sunflower seedlings, aside from copper oxychloride, only fungicides containing mancozeb showed some protective activity. Similar results obtained by Oros (1983) indicated that after the application of mancozeb into soil contaminated with tumorigenic bacteria, the number of bacteria significantly decreased. When pH of the soil was very low but mancozeb was present, the pathogen was almost totally eliminated (Berczyński *et al.* 2002). Moreover, a study on the control of walnut bacterial blight also showed the significant efficacy of Manex – a preparation based on mancozeb and copper. It was found more effective than copper alone (Buchner *et al.* 2001).

To the best of our knowledge, in the available literature there is no information on the evaluation of the fungicides which we studied, against bacterial canker of stone fruits. Most of the published data relate to copper compounds only. At present, much attention is focused on the potential threat of copper to the environment (Merry *et al.* 1986; Renan 1994; Toselli *et al.* 2009). Products of natural origins including essential oils, show promise. There are reports indicating that they can be even more effective than chemical compounds (Nguefack *et al.* 2005; Bajpai *et al.* 2011). When a high inoculum potential is occurring in nature, such products still might not be effective enough or not effective at all.

The results of our research indicate that antibacterial activity of various compounds demonstrated on artificial medium, did not correspond with their protective activity on plants. Moreover, none of the fungicides except those containing copper, gave a satisfactory protection of plants inoculated with the used bacterial pathogens.

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