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POSSIBILITIES OF USING CHEMICALS IN THE PROTECTION OF LAVENDER (*Lavandula angustifolia*) AGAINST *Phytophthora citrophthora*

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Abstract. The objective of our studies was to develop effective, chemical methods of protecting narrow-leaved lavender (*Lavandula angustifolia*) against *Phytophthora citrophthora* with regard to their application. Under the laboratory conditions, activity of the tested agents was assessed on the basis of growth of *P. citrophthora* on a medium containing fungicides and the size of necrosis on the inoculated lavender shoots. In greenhouse tests growth of plants in the infested medium was evaluated. Laboratory research conducted indicated the highest effectiveness in limitation of *P. citrophthora* growth of Acrobat MZ 69 WG and Infinito 687,5 SC. In greenhouse tests, an evaluation of growth of lavender sprayed with Luna Senasation 500 SC and Python Consento 450 SC, carried out 3 weeks of cultivation, showed a significantly higher number of shoots in comparison with plants growing in the infested as well as non – infested control. All tested chemicals caused faster growth of the lavender. Change of the application method, i.e., foliar application of these products, substantially improved the growth results.

Key words: *Polyphagous pathogen*, medicinal plants, chemical agents, application method

INTRODUCTION

Phytophthora species are soilborne, polyphagous pathogens, well adapted to diverse plant hosts and environmental conditions. They are among the most dangerous pathogens of horticultural and forest plants. The name *Phytophthora* is derived from Greek

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and literally means 'plant destroyer' (phyto – plant; phthora – destroyer) [Erwin and Ribeiro 1996].

The studies that have been conducted during last years on the occurrence of *Phytophthora* spp. in Poland, indicated that not only new species of that genus have appeared, but also the host plant range has expanded [Orlikowski et. al 2012]. One of the most dangerous pathogens in the genus *Phytophthora* is *Phytophthora citrophthora* (Smith & Smith) Leonian. The pathogen was first described on rotting lemon fruits in California, initially as *Pythiacystis citrophthora* and in 1925 it was qualified to the genus *Phytophthora*. In the following years, it turned out it is the most dangerous pathogen for citrus trees [Erwin and Ribeiro 1996]. In addition to being a serious pathogen on citrus, *Phytophthora citrophthora* is also an important threat to several other crops, including ornamental plants Disease symptoms caused by *Phytophthora citrophthora* are: stem and root rot, trunk canker, fruit rot, gummossis and twig blight [Erwin and Ribeiro 1996]. The pathogen can survive in a form of vegetative mycelium, which produces sporangia. Depends on environmental conditions (temperature, humidity) sporangia may germinate directly, or differentiate to produce zoospores. Each of this structure (mycelium, sporangium or zoospores) have an infection potential, and infection process is a cycle [Erwin and Ribeiro 1996, van West et. al. 2003). Till 1996 the pathogen was described on 80 different plant species [Erwin and Ribeiro 1996]. This species brought to the country at the end of the 20th century occurs in both, forest nurseries and ornamental plants [Orlikowski et al. 2009, Orlikowski et al. 2012]. *P. citrophthora* was found for the first time in Poland on dying shoot tips of *Pieris japonica* (Thunb.) D. Don ex G. Don and *Syringa vulgaris* L. [Orlikowski and Szkuta 2001, 2005] as well as on stem bases of *Podocarpus alpinus* R.Br. ex Hook.f. [Szkuta 2004]. Currently, *P. citrophthora* is causing serious losses in the plantings of not only lilac, fetterbush but also boxwood, rhododendron, umbrella-pine, periwinkle, heuchera and lavender [Orlikowski and Szkuta 2001, Orlikowski et al. 2009, Orlikowski et al. 2010, Orlikowski and Ptaszek 2010, Ptaszek and Orlikowski 2010, Orlikowski et al. 2011, 2012]. Losses caused by *P. citrophthora*, depends on the species of cultivated plants and development stage, and may reach more than several dozen percent [Orlikowski et al. 2012, Ptaszek 2008, Ptaszek and Orlikowski 2010]. That species causes root and stem base rot, what leads to plant dying within a few weeks of their cultivation. Orlikowski and Valjuskaite [2007] report that phytophthorosis is a growing problem in lavender cultivation, where it often substantially decreases the quality of plants and leads to their total decay in the final stage.

Attention to high plant health is one of the basic tasks in the modern, intensive and economically viable production of ornamental plants. The condition to achieve satisfactory results is the proper plant protection, using elements of integrated actions, called the Integrated Plant Protection [Daughtrey and Benson 2005]. Given the constant intensification of ornamental plant production in Poland, losses caused by species of the genus *Phytophthora*, limited chemical protection possibilities and insufficient data in domestic literature on the eradication of individual species of that genus, it needs to be said that there are serious grounds to undertake research being the subject of this paper.

The objective of the undertaken research was to develop effective, chemical methods of protecting narrow-leaved lavender (*Lavandula angustifolia*) against *Phytophthora citrophthora* with regard to their application.

MATERIAL AND METHODS

The studies assessed the efficiency of 7 chemical plant protection products, listed in Table 1. In all experiments *P. citrophthora* isolate from infected lavender was used.

Table 1. List of tested plant protection products

Product trade name	Active substances and their content	Registered for ornamental plant protection/ application method
Acrobat MZ 69 WG	dimetomorf (90 g/kg), mancozeb (600 g/kg)	no
Infinito 687.5 SC	propamocarb hydrochloride (625 g/l), fluopicolide (62.5 g/l)	no
Luna Sensation 500 SC	fluopyram (250 g/l), trifloxystrobin (250 g/l)	no
Pyton Consento 450 SC	propamocarb hydrochloride (375 g/l), fenamidone (75 g/l)	no
Ridomil Gold MZ Pepite 67.8 WG	metalaxyl-M (38.8 g/kg), mancozeb (640 g/kg)	yes/watering
Mildex 711.9 WG	fosetyl-aluminium (667 g/kg) fenamidone (44 g/kg)	yes/spraying
Previcur Energy 840 SL	propamocarb hydrochloride (530 g/l), fosetyl-aluminium (310 g/l)	yes/watering

The evaluation of effectiveness of selected products for protecting narrow-leaved lavender against *P. citrophthora* was carried out in laboratory and greenhouse tests.

Laboratory assessment of the tested products' activity. The impact of listed products on linear growth of *P. citrophthora* was tested in *in vitro* conditions. 7-day-old cultures of that species, growing on a potato-dextrose agar (PDA), at a temperature of 25°C, in the dark, were used for the tests. The tested chemicals were added to flask with sterile and cooled down to 50°C PDA medium, so that the concentration of the active substance was 1, 10, 100 ppm, respectively. The control was a medium without additional fungicide. The medium was mixed with the product and then poured (25 ml) to Petri dishes with a diameter of 90 mm. The dishes were placed in a laminar air flow cabin for 24 hours, until completely solidified. After that time, 5 mm in diameter plugs of medium overgrown by *P. citrophthora* hyphae were placed in the middle part of the Petri dishes. The underside of the dishes had two perpendicular lines drawn, crossing at a right angle, in the centre of the inoculum. Dishes were placed in an incubator and incubated at 25°C. The observations of the mycelium growth were carried out after 4 and 8 days of incubation. The colony diameter was measured along plotted lines.

The effectiveness of tested products was analyzing using a simplified Abbot's formula. The percentage efficacy of the fungicides in relation to the control combinations was calculated [Abbott 1925]. For each combination, the experiment was conducted in 4 replications, each one in one dish. The experiment was repeated twice, at 2-week intervals.

The second stage of the study included the evaluation of effectiveness of tested products in inhibiting the colonisations of tissues of narrow-leaved lavender (*Lavandula angustifolia* Mill.) inoculated with *P. citrophthora*, according to the methodology given by Orlikowski and Szkuta [2001]. 7-day-old cultures of *P. citrophthora*, growing on V8 multi-vegetable agar, at a temperature of 25°C were used for inoculation of shoots. The solutions of tested plant protection products, in concentrations of 0.1, 0.15 and 0.2%, were prepared in glass vials. Narrow-leaved lavender shoot tips, about 5 cm long, were soaked for 2 minutes in prepared solutions and then placed in cuvettes (32.5 × 25.5 × 5.5 cm), lined with damp, synthetic sterile mat, covered with a plastic net so that they did not contact the wet substrate directly. Fragments of the medium, with a diameter of 3 mm, overgrown by hyphae of *P. citrophthora*, collected from edges of 7-day cultures, were applied on shoot bases. Cuvettes were covered with thin film and incubated on laboratory table at a temperature of 22–24°C. Every time, the tests included uninfected control, in which plant organs were inoculated with clean medium discs, and infected control, in which plant fragments were soaked only in distilled water and then infected with *P. citrophthora*. Observations was conducted after 3 and 5 days of incubation. The measure of impact of tested products on the pathogen was the length of necrosis on the shoots. Experimental design was completely randomized with 4 replications and 5 plant organs in each rep. The experiments were carried out twice, at a 2-week interval.

Greenhouse assessment of the tested products' activity. The cultures of *P. citrophthora* were prepared for the test using a method described by Orlikowski [1999], on the oatmeal culture medium (OM). The medium overgrown by the pathogen was homogenized with an addition of distilled water (150 ml per 1 dish) and such a uniform suspension was mixed with the substrate in the proportion: contents of 1 dish per 1 litre of moor substrate. The substrate was placed in bags and incubated in a greenhouse for 14 days. After that period, rooted narrow-leaved lavender seedlings (*L. angustifolia*) were planted in 1 litre pots filled with infected moor substrate and placed on window sills in the greenhouse.

Then, the plants were treated with the tested plant protection products (tab. 1). Two application methods were used: in the first one, the seedlings were watered with 25 ml of the working liquid/plant, in the second – they were sprayed with 0.5 l of the working liquid/10m^2. Every time, uninfested (without the pathogen) and infested (substrate infested without application of the product) control combinations were included in the studies. The plants in the control samples were sprayed with clean water only.

The plants were cultivated for a period of 3 weeks, in controlled conditions, at a temperature of 17–26°C and air humidity of 54–75%.

In the course of the experiment, after 2 and 3 weeks of cultivation, the number of shoots was counted and the average plant growth (in mm) evaluated.

The experiments were assumed in the random block system of 4 replications, 10 plants each, and repeated twice within at a 2-week interval.

After completed laboratory and greenhouse experiments, from the tissue of plants with disease symptoms and from substrate collected from under the plants, the trigger factor was reisolated and again identified to the species.

Statistical analysis. The obtained results were analyzed using the variance analysis method. The significance of differences between the means were assessed with the Duncan multiple range test, at a significance level of $\alpha = 0.05$.

RESULTS

The evaluation of biological activity of products in laboratory test showed that enriching the culture medium with only 1 µg/ml of active substances of Luna Sensation 500 SC, Previcur Energy 840 SL, Pyton Consento 450 SC and Ridomil Gold MZ Pepite 67.8 WG limited the growth of *P. citrophthora* (fig. 1). The effectiveness of mentioned fungicides after 4 days of incubation was 7; 17; 27 and 25% resepctively. Complete inhibition of the development of *P. citrophthora* at a concentration of 10 µg of the active substance/ml was observed in combinations with Acrobat MZ 69 WG and Infinito 687.5 SC. Such effectiveness in the case of Mildex 711.9 WG and Ridomil Gold MZ Pepite 67.8 WG was achieved at a concentration of 100 µg of the active substance. This effect remained over the following 4 days of incubation (fig. 2). In the case of other tested products their effectiveness was 55% for Luna Sensation 500 SC, 52% for Previcur Energy 840 SL and 57% for Pyton Consento 450 SC (fig. 2) in relation to the control combinations.

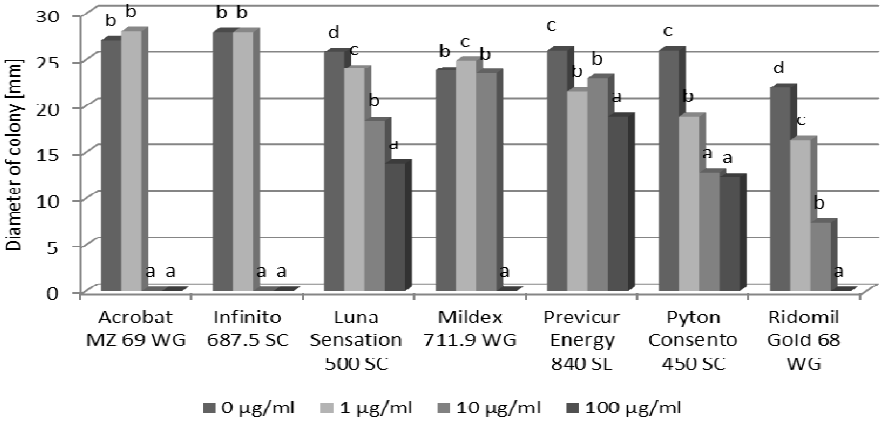

Fig. 1. Influence of the concentration of tested products on the growth of *P. citrophora* after 4 days of incubation

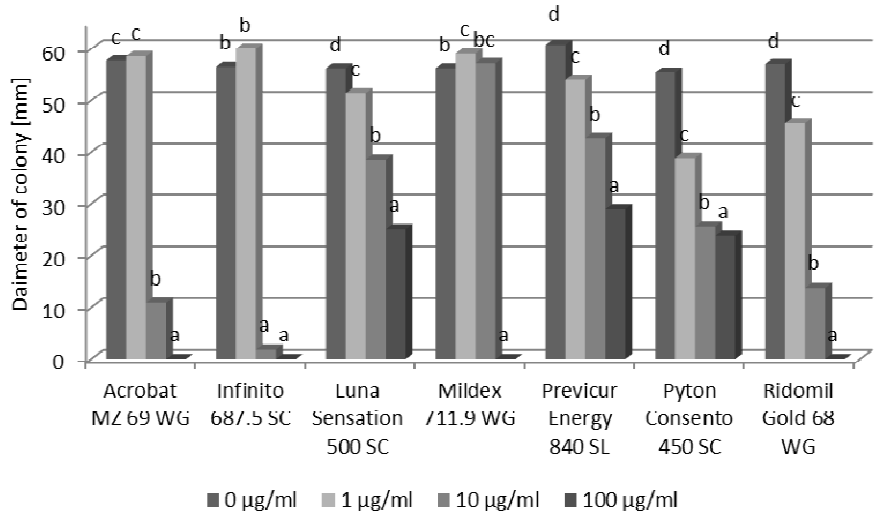

Fig. 2. Influence of the concentration of tested products on the growth of *P. citrophora* after 8 days of incubation

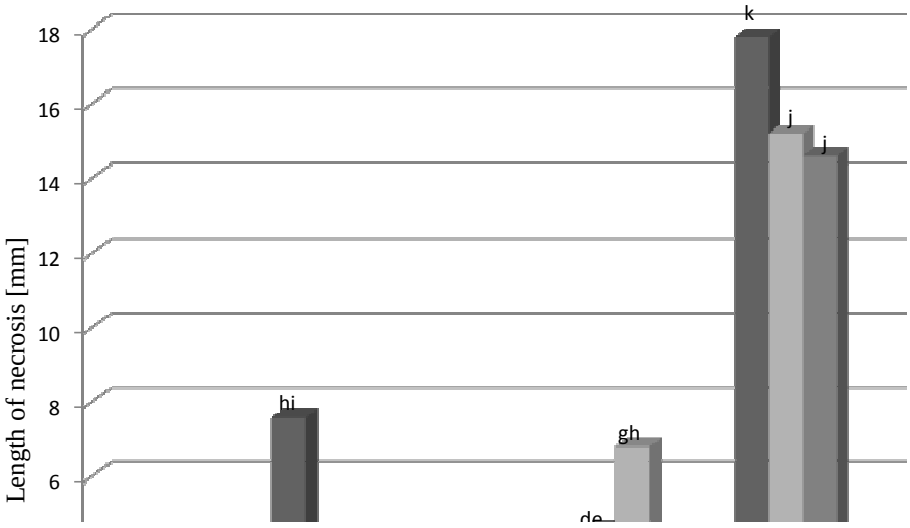

Fig. 3. Influence of the concentration of tested products on the colonization of narrow-leaved lavender shoots by *P. citrophthora*, after 3 days of incubation

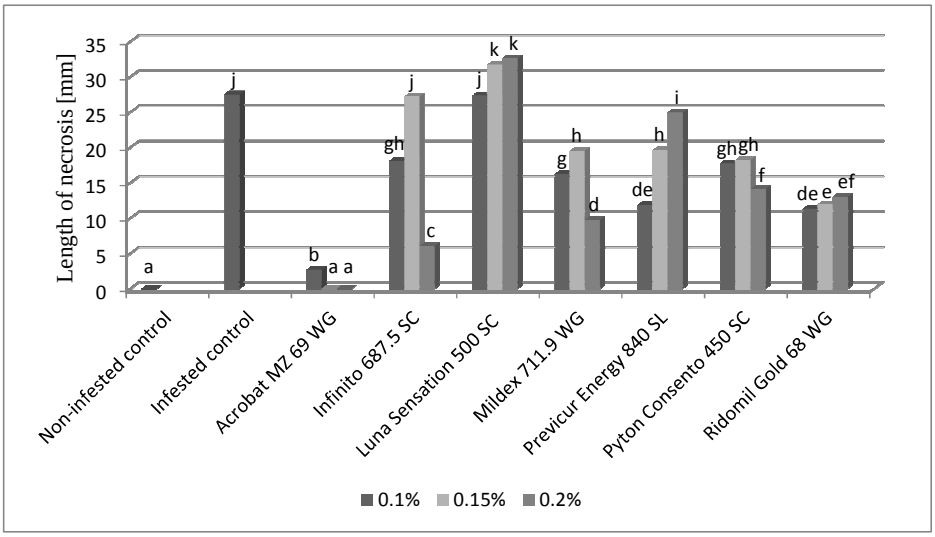

Fig. 4. Influence of the concentration of tested products on the colonization of narrow-leaved lavender shoots by *P. citrophthora*, after 5 days of incubation

Table 2. Impact of tested products on the development of lavender cultivated in a substrate infected with *P. citrophthora*

Protection products	Application method	Average number of shoots per plant, after weeks since planting		Average height of plants after weeks since planting (mm)	
		2	3	2	3
Non-infested control	–	4.7 a-e	4.3 a	97.5 de	213.6 b
Infested control	–	5.2 de	5.0 c-e	91.6 b-d	173.7 a
Acrobat MZ 69 WG	spraying	5.0 b-e	4.5 a-c	99.2 e	264.1 i
	watering	4.5 a-c	4.3 ab	75.3 a	222.9 bc
Infinito 687.5 SC	spraying	4.6 a-e	4.7 a-d	89.5 bc	250.7 f-i
	watering	5.0 b-e	4.7 a-d	94.5 c-e	240.8 d-g
Luna Sensation 500 SC	spraying	4.2 a	6.7 g	96.5 de	262.7 i
	watering	4.8 a-e	4.8 a-d	88.9 bc	228.6 b-d
Mildex 711.9 WG	spraying	4.5 a-d	4.7 a-d	94.5 c-e	257.1 hi
	watering	4.8 a-e	4.88 a-d	100.1 e	242.3 d-h
Previcur Energy 840 SL	spraying	4.6 a-e	5.5 e	95.8 de	255.0 g-i
	watering	4.5 a-d	4.8 a-d	91.8 b-d	265.2 i
Pyton Consento 450 SC	spraying	4.3 ab	6.1 f	87.5 b	237.4 c-f
	watering	4.8 a-e	4.7 a-d	99.5 e	234.3 c-e
Ridomil Gold 68 WG	spraying	4.5 a-d	5.3 de	97.2 de	254.5 g-i
	watering	5.3 e	4.88 a-d	97.5 de	237.6 c-f

Values in the columns, marked with the same letter, show no significant difference (5%) according to Duncan's test

Further research that included inoculation of lavender shoots showed that Acrobat MZ 69 WG at all used concentrations, protected the tissues against infection by *P. citrophthora* (figs 3 and 4). Only after 5 days from the inoculation, a minimal (2.7 mm) development of necrosis was observed (fig. 4). The use of Infinito 686.5 SC and Python Consento 450 SC, at a concentration of 0.2%, completely protected lavender shoots against infection, for the first 3 days of incubation. In turn, Mildex 711.9 WG and Ridomil Gold MZ Pepite 67.8 WG, at all concentrations, significantly limited the development of phytophthorosis. Luna Sensation 500 SC did not inhibit the colonisation of shoot at the used concentration (fig. 3). After 5 days of incubation, Mildex 711.9 WG, Previcur Energy 840 SL, Python Consento 450 SC and Ridomil Gold MZ Pepite 67.8 WG significantly ($\alpha < 0.05$) limited the development of necrosis on lavender shoots, in comparison to the infected control (fig. 4). Only Luna Sensation 500 SC at all concentrations and Infinito 685.5 SC at 0.15%, did not protect the plants against the pathogen (fig. 4).

In greenhouse tests, an evaluation of growth of lavender sprayed with Luna Senasation 500 SC and Python Consento 450 SC, carried out 3 weeks of cultivation, showed a significantly ($\alpha < 0.05$) higher number of shoots in comparison with plants from both controls (infested and non-infested) (tab. 2). All tested chemicals caused faster growth of the lavender ($\alpha < 0.05$). The height of the plants after three weeks of cultivation in combinations where chemicals were used, was 237.4–264.1 mm with foliar application and 222.9–265.2 mm with subsurface, while in the case of infested control, the height was only 173.7 mm ($\alpha < 0.05$) (tab. 2). Change of the application method, i.e., foliar application of these products, substantially improved the growth results. Regardless of the combination, no typical symptoms of the disease were found on the observed plants.

DISCUSSION

The conducted studies tested 7 two-component plant protection products based on different active substances. The experiments included products currently registered in Poland (as of 18/7/2014) for the protection of ornamental plants and products impacting *Phytophthora infestans* in the protection of potatoes or tomatoes. Acrobat MZ 69 WG and Infinito 6875 SC proved to be the most effective during *in vitro* tests. Complete inhibition of *P. citrophthora* development was observed even at minor concentrations of dimetomorf and mancozeb (Acrobat MZ 69 WG) or propamocarb and fluopicolid (Infinito 687.5 SC). Studies conducted on lavender shoots confirmed 100% effectiveness only in the case of Acrobat MZ 69 WG. High effectiveness of that product in the protection of ornamental plants is confirmed by the results of the test conducted by Orlikowski [2006]. However, Shin et al. [2010] during greenhouse experiments, when comparing the effect of fluopicolid with dimetomorf during preventive use, showed higher effectiveness of eradicating *Phytophthora capsici* by fluopicolid.

Both products have not been yet approved for ornamental plant protection in Poland. On the other hand, Acrobat MZ 69 WG is registered and successfully used in the protection of potatoes and tomatoes against *Phytophthora infestans* [Kapsa and Bernat 2012] and the protection of cucumbers and onions against *Pseudoperonospora cubensis*

[Ratajkiewicz and Baranowski 2007], while fluopicolid, being the active substance of Infinito 687.5 SC, is proving effective in limiting pathogens causing downy mildew and *P. infestans* on potatoes or *P. capsici* on peppers [Bardsley et al. 2006, Latorse et al. 2006]. The results obtained from these studies are a confirmation of suitability of both fungicides, also in the protection of lavender against *P. citrophthora*. Orlikowski [2006] notes that, in addition to a strong limitation of sporulation of *Phytophthora* spp., Acrobat MZ 69 WG limits the number of pathogens in the substrate and induces plant immunity. Safety of their use is not without significance. Oszako et al. [2009] stress that active substances of Infinito 687.5 SC are safe for the environment and the fosetyl-aluminium quickly decomposes into phosphoric acid.

In carried out own studies, Luna Sensation 500 SC, used in dish tests, even in the highest doses, only partially inhibited the growth of *P. citrophthora*, but was the best among the tested product, in terms of stimulating lavender growth. The research conducted by Meszka and Korzeniowski [2013] and Korzeniowski [2014] showed that Luna Sensation 500 SC very strongly limits *Botrytis cinerea* and *Sphaerotheca macularis* on strawberries, but also pathogens causing powder mildews on other species of plants, including *Podosphaera leucotricha* on apple-trees *and Sphaerotheca pannosa* var. *rosae* on roses (unpublished data, source Bayer CropScience). Lindermann and Davies [2008] conducted a study on the effectiveness of fungicides containing active substances from the group of strobilurins, i.e. azoxystrobin 50% (Heritage) and pyraclostrobin 20% (Insignia). None of the two products showed any inhibitory impact on the growth of *P. citrophthora* during subsurface and foliar application. Benson and Parker [2011] studied chemicals based on strobilurins, used for spraying gerberas, in order to protect it against *P. cryptogea*. The results confirmed limited effectiveness of products based on strobilurins.

CONCLUSIONS

Phytophthora species immunity towards commonly used fungicides is increasing. For this reason, chemicals with different active substances and a different action mechanism, including these in Acrobat MZ 69 WG or Infinito 687.5 SC should be used. That would allow rotation in an integrated ornamental plant protection.

Therefore, on the basis of the obtained results and available literature, we can come up with a thesis that Luna Sensation 500 SC shows too low effectiveness, for it to be implemented in the programmes of ornamental plants' protection against phytophthorosis. It is a new product that will be used, i.a., to protect ornamental plants against noble rot and powdery mildew and its impact on *Phytophthora* should be treated as a side-effect, while protecting against other pathogens.

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MOŻLIWOŚCI WYKORZYSTANIA ŚRODKÓW CHEMICZNYCH W OCHRONIE LAWENDY (*Lavandula angustifolia)* PRZED *Phytophthora citrophthora*

Streszczenie. Celem podjętych badań było opracowanie skutecznych chemicznych metod ochrony lawendy wąskolistnej (*Lavandula angustifolia*) przed *Phytophthora citrophthora* z uwzględnieniem sposobu ich aplikacji. W warunkach laboratoryjnych oceniano aktywność testowanych środków na podstawie wzrostu *P. citrophthora* na pożywce zawierającej fungicydy oraz wielkości nekrozy na inokulowanych pędach lawendy. W testach szklarniowych oceniano wzrost roślin w zakażonym podłożu. Na podstawie przeprowadzonych badań laboratoryjnych wnioskuje się, że największą skutecznością w ograniczaniu rozwoju *P. citrophthora* odznaczały się Acrobat MZ 69 WG i Infinito 687,5 SC. W testach szklarniowych ocena wzrostu lawendy opryskiwanej środkami Luna Sensation 500 SC i Python Consento 450 SC przeprowadzona po 3 tygodniach uprawy wykazała istotnie większą liczbę pędów w porównaniu z roślinami rosnącymi zarówno w kontroli zakażonej, jak i niezakażonej. Wszystkie testowane środki powodowały szybszy wzrost lawendy. Zmiana aplikacji, tj. nalistne zastosowanie tych środków, istotnie poprawiło wyniki wzrostu.

Słowa kluczowe: patogen polifagiczny, rośliny lecznicze, środki chemiczne, metody aplikacji

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