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# POSSIBILITIES OF USING CHEMICAL AGENTS FOR THE PROTECTION OF *Gerbera jamesonii* AGAINST *Phytophthora cryptogea*

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**ABSTRACT**

The objective of the study was to develop effective chemical methods of protection of *Gerbera jamesonii* against *Phytophthora cryptogea* with consideration of the way of their application. In laboratory conditions, the activity of 7 tested agents was evaluated based on growth of *P. cryptogea* on a medium containing fungicides, and size of necrosis in inoculated gerbera leaf blades. In greenhouse tests, growth of plants in infected substrate was assessed. The study showed variable susceptibility of *Phytophthora cryptogea* to the tested plant protection products. The best solution protecting gerbera crops against *P. cryptogea* turned out to be Ridomil Gold MZ Pepite 68 WG. In programs of gerbera protection against *Phytophtora*, the agent should be introduced by means of watering. Products such as Acrobat MZ 69 WG, Infinito 687.5 SC, and Mildex 711.9 WG can be considered an element of integrated protection of gerbera.

**Key words:** *Phytophtora cryptogea*, chemical agents, application method

## INTRODUCTION

*Phytophthora cryptogea* Pethybridge & Lafferty was first described in 1919 in Ireland as the causal agent of stem base rot of tomato. Since then, the pathogen has been recorded on more than 150 host plants [Olson and Benson 2013]. It is a species with a broad range of occurrence, both in geographical and climatic terms. *P. cryptogea* is currently considered one of the most dangerous pathogens of ornamental plants, particularly those cultivated in greenhouses and foil tunnels. In Poland, the discussed species was first reported in the early 1970's in plantings of gerbera (*Gerbera jamesonii*) [Orlikowski 1976/77]. In the following years, the pathogen was isolated from pot plants, including cineraria (*Senecio cruentus*), *Pachypodium lamerei* [Orlikowski 1993], and poinsettia (*Euphorbia pulcherrima*) [Orlikowski and Ptaszek 2013], and from foil tunnel cultivations of Peruvian lilly (*Alstroemeria* × *hybrida*) [Ptaszek and Skrzypczak 2008]. Until now, *P. cryptogea* has also been recorded in container ornamental plant nurseries, in cultivation of both deciduous and coniferous plants [Orlikowski and Szkuta 2008, Orlikowski and Ptaszek 2008, Orlikowski et al. 2012a]. Fast spreading of the species should be definitely associated with the international trade of plant material that has been intensifying for many years. An equally important source of *P. cryptogea* is water from natural streams and water bodies, and ponds located in ornamental plant nurseries, as well as water used for irrigation [Orlikowski et al. 2012b, Ptaszek and Orlikowski 2015].

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In the production of gerbera, *P. cryptogea* is the basic problem causing rotting of the shoot base [Orlikowski 1978]. The pathogen develops in the ground where it infects the shoot base leading to its browning and dying. Still in the 1980's, the disease was the cause of losses reaching up to 50% [Orlikowski et al. 2012a]. The development of the pathogen is favored by organic substrate with high content of peat, and its high moisture and temperature. Due to this, the disease manifestations occur with high intensity in greenhouse cultivations. Here, *P. cryptogea* spreads with water during watering, or through capillary ascent in containers, and through infested substrate and seedlings [Orlikowski and Ptaszek 2007]. Phytophtorosis in greenhouse objects spreads already within several weeks from planting. In favorable conditions, development of the disease can lead to complete destruction of plantings. Therefore, measures minimizing losses due to the occurrence of *P. cryptogea* are extremely important. Besides maintaining phytosanitary conditions in a greenhouse and production of plants from healthy seedlings, the basis of protection should consist of integrated protection of plants including selection of appropriate plant protection products.

The objective of the study was the development of effective chemical methods of protection of *Gerbera jamesonii* against *Phytophthora cryptogea* with consideration of the way of their application.

## MATERIAL AND METHODS

The study evaluated the efficiency of 7 chemical plant protection products, including fungicides used for preventive and curative potato protection (Pyton Consento 450 SC) and vegetable plants (Acrobat MZ 69 WG, Infinito 687.5 SC) and in the protection of strawberries (Luna Sensation 500 SC) against fungal diseases (Tab. 1). Only three fungicides were registered during tests for the protection of ornamental plants, including:

– Ridomil Gold MZ Pepite 67.8 WG is a fungicide in the form of granules for aqueous suspension with systemic and contact mode of action, for preventive use during intensive plant growth;

– Mildex 711.9 WG is a fungicide with systemic mode of action for preventive and curative use in the protection of ornamental plants (phytophthora, downy mildew) and vegetable (powdery mildew, potato blight, alternariosis);

**Table 1.** List of tested plant protection products

| No. | Product trade name | Active substances and their content | Registered for ornamental plant protection/ application method |
|---|---|---|---|
| 1. | Acrobat MZ 69 WG | dimetomorf (90 g $kg^{-1}$),<br>mancozeb (600 g kg) | no |
| 2. | Infinito 687.5 SC | propamocarb hydrochloride (625 g $l^{-1}$),<br>fluopicolide (62.5 g $l^{-1}$) | no |
| 3. | Luna Sensation 500 S.C. | fluopyram (250 g $l^{-1}$),<br>trifloxystrobin (250 g $l^{-1}$) | no |
| 4. | Pyton Consento 450 S.C. | propamocarb hydrochloride (375 g $l^{-1}$),<br>fenamidone (75 g $l^{-1}$) | no |
| 5. | Ridomil Gold MZ Pepite 67.8 WG | metalaxyl-M (38.8 g $kg^{-1}$),<br>mancozeb (640 g $kg^{-1}$) | yes / watering |
| 6. | Mildex 711.9 WG | fosetyl-aluminium (667 g $kg^{-1}$)<br>fenamidone (44 g $kg^{-1}$) | yes / spraying |
| 7. | Previcur Energy 840 SL | propamocarb hydrochloride (530 g $l^{-1}$),<br>fosetyl-aluminium (310 g $l^{-1}$) | yes / watering |

– Previcur Energy 840 SL is a fungicide in the form of a concentrate for making a systemic aqueous solution for preventive and curative use in the protection of vegetable and ornamental plants (indoor uses) and in forest nurseries against soil fungi complex.

The experiments applied one representative isolate of *P. cryptogea* Pethybr. & Laff. Tested isolate was selected randomly from the pool of isolates obtained from diseased *Gerbera jamesonii* (var. Eryka) plants. The isolate was identified on the basis of morphological features, using available monographs [Erwin and Ribeiro 1996] and keys [Gallegly and Hong 2008]. Identification was confirmed in PCR (Polymerase Chain Reaction) with species-specific primers CRYF2/CRYR2 [Boersma et al. 2000] and Cryp1/Cryp2 [Minerdi et al. 2008].

The assessment of the efficiency of selected products for the protection of gerbera against *P. cryptogea* was performed in laboratory and greenhouse research.

**Assessment of the activity of the analyzed products in laboratory conditions**

In *in vitro* conditions, the effect of the aforementioned products on linear growth of *P. cryptogea* was analyzed. The research applied 7-day cultures of the species growing on Potato Dextrose Agar (PDA) at a temperature of 25°C in the dark. The analyzed chemical agents were added to flasks with sterilized PDA cooled to 50°C, so that the concentration of the active substance amounted to 1, 10, and 100 ppm, respectively. The control consisted of agar with no addition of the fungicide. Agar with the product was mixed, and then transferred to Petri dishes with a diameter of 90 mm, 25 ml each. The Petri dishes were placed in a laminar flow cabinet for 24 hours until complete solidification of the substrate. Next, agar discs with a diameter of 5 mm overgrown by *P. cryptogea* hyphae were placed in the middle of each Petri dish. On the bottom side of Petri dishes, two perpendicular lines were drawn, crossed at the right angle in the center of the inoculum. The Petri dishes were placed in an incubator, and incubated at 25°C. Observations of the growth of the thallus were performed after four and eight days of incubation. The diameter of colonies was measured along the drawn lines. For each combination, the experiment was conducted in four repetitions, one Petri dish each. The experiment was conducted twice at a two-week interval.

The second stage of the laboratory research involved the assessment of the effectiveness of tested products in limiting the colonization of gerbera leaves inoculated with *P. cryptogea*, in accordance with the methodology described by Orlikowski and Szkuta [2002]. The inoculation involved the application of 7-day cultures of *P. cryptogea* growing on V8 vegetable juice agar at a temperature of 25°C. Solutions of the tested plant protection products at concentrations of 0.1%, 0.15%, and 0.2% were prepared in glass beakers. Young gerbera leaves were soaked in the analyzed solutions for two minutes, and then placed in trays (with dimensions of 32.5 × 25.5 × 5.5 cm) lined with moist, sterile, synthetic mat covered with plastic mesh so that they do not come in direct contact with the moist substrate.

Gerbera leaf blades were inoculated with studied pathogen in three places through a puncture by means of a dissecting needle. The trays were covered with thin foil, and incubated on laboratory tables at a temperature of 22–24°C. Each time, the tests included a non-infested control, in which organs of plants were inoculated with clean agar discs, and an infected control, where fragments of plants were soaked in only distilled water, and then infected with *P. cryptogea* hyphae. The tests were reviewed every day, and depending on the rate of decay development, readings of the necrosis size were performed. The measure of the effects of the analyzed products on the pathogen was the diameter of necrotic spots on leaves.

The experimental design was completely randomized with 4 replications and 5 leaf blades each. The experiments were conducted twice in a two-week interval.

**Assessment of the activity of the analyzed products in greenhouse conditions**

The pathogen inoculum was prepared with the application of a method described by Orlikowski [1999] on oat medium (OM). In the central part of 90 mm Petri dishes, an agar disc overgrown with *P. cryptogea* was placed. The Petri dishes were incubated for 10 days in 25°C. Then, the substrate was homogenized with an addition of distilled water (150 ml per 1 Petri dish). The resulting uniform suspension was mixed with peat substrate in the following proportion: content of 1 Petri dish per 1 liter of peat substrate. The substrate was placed in bags and incubated in a greenhouse for 14 days. Then, rooted gerbera cuttings (with 5–6 leaves) were planted in 1 liter pots filled with infected peat substrate, and placed on sills in the greenhouse. Next, the plants were treated with tested plant protection products (Tab. 1). Two ways of application of the products were used: in the first one, the cuttings were watered with 25 ml of working liquid, and in the second one, they were thoroughly sprayed.

Each time, the research included a non-infected control (without the pathogen) and an infested control (infested substrate with no application of a product). In control samples, plants were sprayed/watered only with clean water.

The plants were cultivated for 5 weeks at a temperature of 17–26°C and relative air moisture of 54–75%. During the experiment, after two, three, and five weeks from planting, the number of leaves with manifestations of rotting of the base of petioles was determined, as well as the number of affected plants in the same terms.

The experiments were established in a system of random blocks in four repetitions with 10 plants each, and repeated twice in a two-week interval.

After the completion of the laboratory and greenhouse experiments, the causal agent of the disease was isolated from necrotic plant tissues and from substrate sampled from under the plants. The isolate was re-identified to the species.

### Statistical analysis

Obtained results were subject to statistical processing by means of variance analysis. The significance of differences between mean values was determined applying Duncan test at the significance level $\alpha = 0.05$.

## RESULTS

### Assessment of the activity of the analyzed products in laboratory conditions

The assessment of the biological activity of products in Petri dish tests showed that only in the case of products Infinito 687.5 SC, Luna Sensation 500 SC, Pyton Consento 450 SC, and Ridomil Gold MZ Pepite 67.8 WG, the introduction of only 1 μg/ml of active substances to the agar slightly limited the growth of *P. cryptogea* (Fig. 1). Complete inhibition

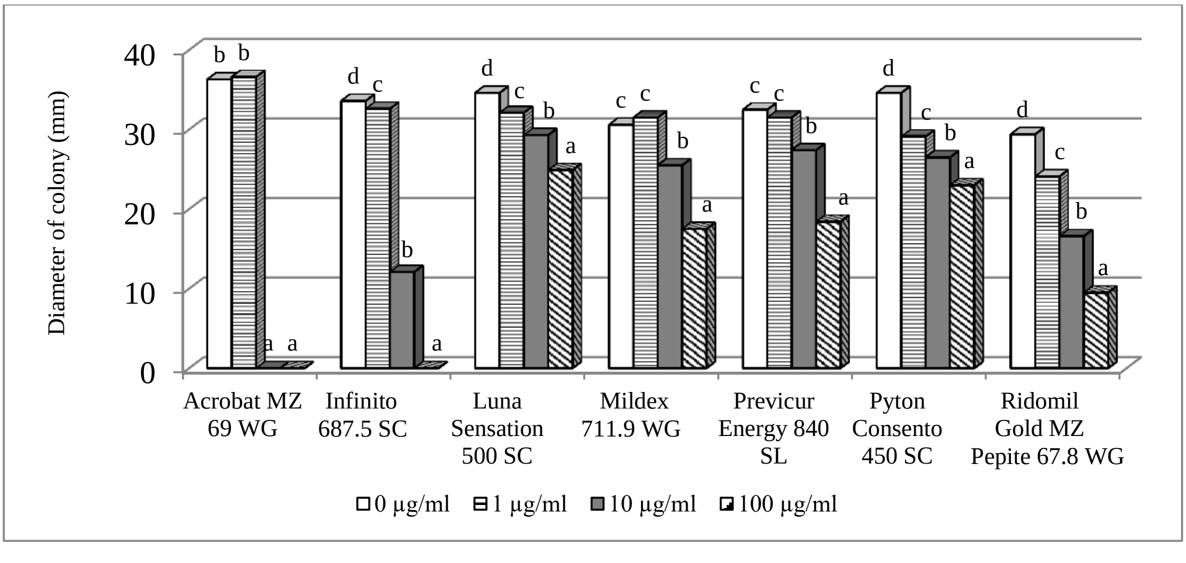


**Fig. 1.** Influence of the concentration of tested products on the growth of *P. cryptogea* after 4 days of incubation

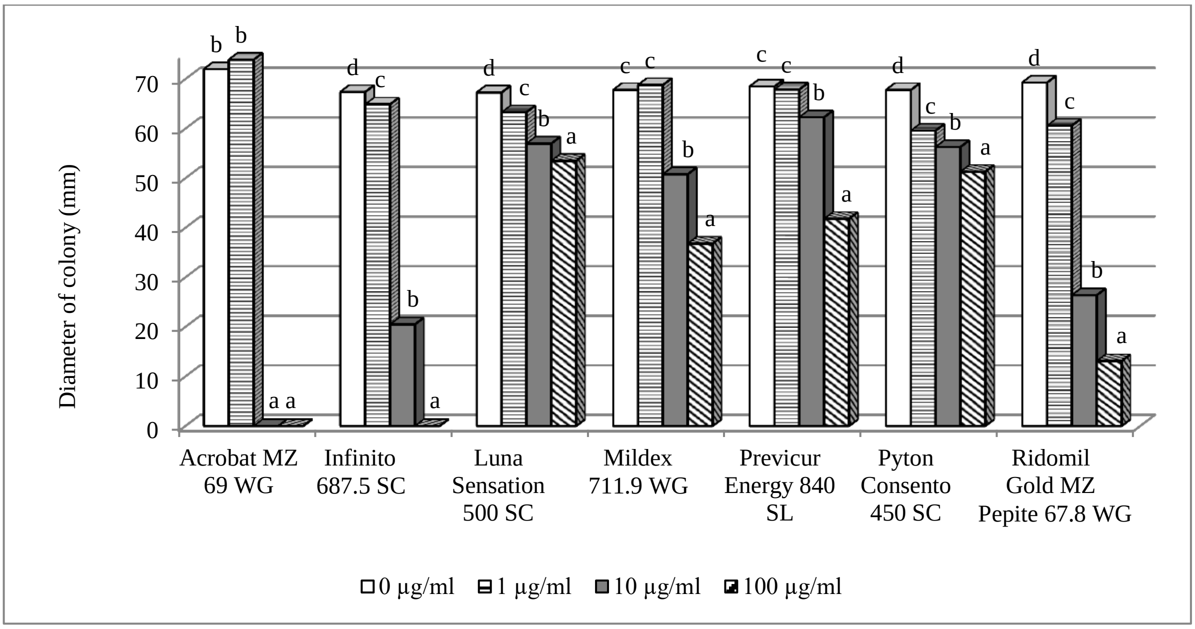


**Fig. 2.** Influence of the concentration of tested products on the growth of *P. cryptogea* after 8 days of incubation

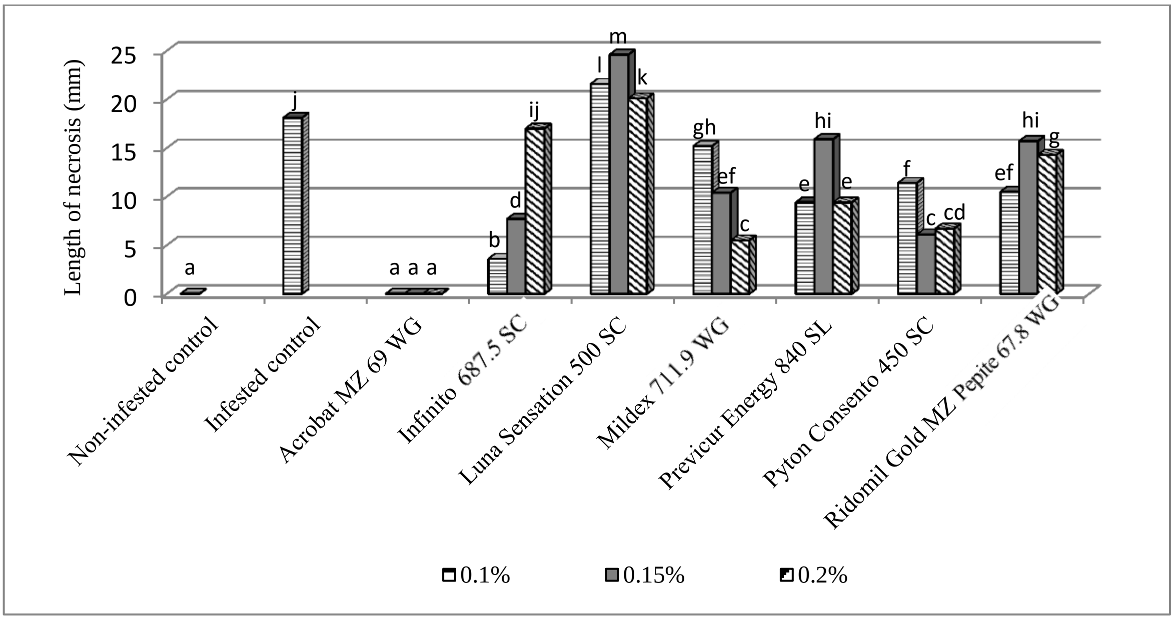


**Fig. 3.** Influence of the concentration of tested products on the colonization of leaved by *P. cryptogea* after, 4 days of incubation

**Table 2.** The effectiveness of compounds in the protection against *P. cryptogea*

| Protection products | Concentration (%) | Application method | Numbers of leaves with decay rot symptoms at the bottom after weeks of planting (No./plant) | | |
|---|---|---|---|---|---|
| | | | 2 | 3 | 5 |
| Non-infested control | – | – | 0 a | 0 a | 0 a |
| Infested control | – | – | 2.7 ef | 3.5 h | 4.95 e–g |
| Acrobat MZ 69 WG | 0.2 | spraying | 2.0 cd | 2.9 de | 4.98 fg |
| | | watering | 1.8 cd | 2.7 cd | 4.9 d–g |
| Infinito 687.5 SC | 0.2 | spraying | 1.6 c | 2.4 c | 4.85 c–g |
| | | watering | 1.9 cd | 2.55 c | 4.93 e–g |
| Luna Sensation 500 SC | 0.2 | spraying | 1.8 cd | 2.55 c | 4.9 e–g |
| | | watering | 1.8 cd | 2.6 cd | 4.7 c–f |
| Mildex 711.9 WG | 0.2 | spraying | 1.6 c | 2.5 c | 4.93 e–g |
| | | watering | 1.9 cd | 2.7 cd | 4.68 c–e |
| Previcur Energy 840 SL | 0.2 | spraying | 2.3 de | 3.2 fg | 5.0 g |
| | | watering | 2.0 cd | 2.5 c | 4.6 cd |
| Pyton Consento 450 SC | 0.2 | spraying | 2.4 de | 3.2 fg | 4.98 fg |
| | | watering | 2.4 de | 3.0 ef | 4.9 d–g |
| Ridomil Gold 68 WG | 0.2 | spraying | 0.6 b | 1.3 b | 4.6 c |
| | | watering | 0 a | 0.1 a | 1.4 b |

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

of *P. cryptogea* de-velopment was only observed after the application of Acrobat MZ 69 WG and Infinito 687.5 SC, whereas in the latter case, only at a concentration of 100 µg of the active substance/ml. Such an effect was maintained during consecutive four days of incubation (Fig. 2).

In laboratory tray tests, only in combination with Acrobat MZ 69 WG, irrespective of the applied concentration, complete inhibition of the pathogen development was observed (Fig. 3). Development of brown decay on gerbera leaves after four days of incubation was largely limited by Mildex 711.9 WG and Pyton Consento 450 SC (Fig. 3). In the case of Previcur Energy 840 SL, the inhibiting effect was obtained at concentrations of 0.1% and 0.2%. Lack of the inhibition effect on *P. cryptogea* development, even at a concentration of 0.2%, was reported in the case of application of Luna Sensation 500 SC.

**Assessment of the activity of the analyzed products in greenhouse conditions**

In the first three weeks of gerbera cultivation, all of the analyzed products limited the development of phytophtorosis (Tab. 2). Irrespective of the way of application, a lower number of infected leaves with rotten petioles was recorded than in control plants. After two weeks of cultivation of gerbera watered with Ridomil Gold 68 WG, no disease symptoms were observed, and in the following weeks, necrotic symptoms were only observed on single leaves. The statistical analysis showed that the soil application of the product was three times more effective than spraying. In remaining combinations, the protective effect was observed only during the first three weeks. In the fifth week after planting, the number of leaves with decay statistically corresponded to that on infected unprotected plants.

Determination of the number of affected plants conducted after three weeks showed that all tested products, irrespective of the way of application, protected gerbera against *P. cryptogea* (Tab. 3). The best effects were recorded in the case of watering and spraying with Ridomil Gold 68 WG. In the second and third week of cultivation, the product protected approximately 9/10 plants. The second group of products, with moderate effectiveness, protecting 8/10 gerbera plants, included Mildex 711.9 WG, Previcur Energy 840 SL, and Pyton Consento 450 SC. The products were applied both into the soil and onto leaves, and they limited the number of affected plants, but only during the first three weeks. Only Ridomil Gold 68 WG, irrespective of the way of application, protected the majority of plants – 7/10 gerberas – against dying.

**Table 3.** The effectiveness of compounds in the protection against *P. cryptogea*

| Protection products | Concentration (%) | Application method | Number of infected leaves after weeks of planting | | |
|---|---|---|---|---|---|
| | | | 2 | 3 | 5 |
| Non-infested control | – | – | 0 a | 0 a | 0 a |
| Infested control | – | – | 2.3 c | 5.0 h | 9.8 e |
| Acrobat MZ 69 WG | 0.2 | spraying | 0.5 ab | 2.3 c–f | 8.5 cd |
| | | watering | 0.5 ab | 3.0 e–g | 8.3 c |
| Infinito 687.5 SC | 0.2 | spraying | 1.3 b | 2.5 d–f | 8.0 c |
| | | watering | 0.8 ab | 2.5 d–f | 9.0 c–e |
| Luna Sensation 500 SC | 0.2 | spraying | 2.5 c | 3.3 fg | 8.3 c |
| | | watering | 2.5 c | 3.8 g | 8.8 c–e |
| Mildex 711.9 WG | 0.2 | spraying | 0.5 ab | 2.0 c–e | 8.3 c |
| | | watering | 0.3 a | 1.8 cd | 8.5 cd |
| Previcur Energy 840 SL | 0.2 | spraying | 0.8 ab | 2.0 c–e | 9.5 de |
| | | watering | 0.5 ab | 2.3 c–f | 8.8 c–e |
| Pyton Consento 450 SC | 0.2 | spraying | 0.3 a | 2.3 c–f | 9.0 c–e |
| | | watering | 0.8 ab | 2.3 c–f | 8.3 c |
| Ridomil Gold 68 WG | 0.2 | spraying | 0.5 ab | 1.3 bc | 2.3 b |
| | | watering | 0 a | 0.3 ab | 2.5 b |

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

## DISCUSSION

The study involved testing of seven two-component plant protection products containing eight different active substances, including procamocarb-HCL, dimethomorph, fluopicolide, fluopyram, fosetyl-aluminium, mankozeb, metalaxyl, and trifloxystrobin. Among products studied in *in vitro* tests, Acrobat MZ 69 WG containing dimethomorph and mancozeb proved to be the most effective. Complete inhibition of *P. cryptogea* development was even observed at small concentrations of active substances. The product also protected gerbera leaves against colonization by *P. cryptogea* in tests on infected plant tissues. In greenhouse research, the effects were considerably weaker. The obtained results show unstable effectiveness of dimethomorph in relation to the pathogen. This is confirmed by results of research by Benson and Parker [2011] evaluating the effectiveness of Orvego (ametoctradin and dimethomorph) in the protection of gerbera against *P. cryptogea*, evidencing its high, although unstable in repetitions, activity.

High effectiveness in *in vitro* tests was also shown by Infinito 687.5 SC, which added to the agar at a concentration of 100 µg/ml, completely limited development of *P. cryptogea*. The results are in accordance with data published by Korzeniowski et al. [2016 a, b] for species *P. cinnamomi* and *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian.

In laboratory studies, positive effect was also observed in the case of Pyton Consento 450 SC, although in greenhouse conditions, its effectiveness was limited. Already in the third week of the evaluation, the product provided no protection.

Considering the number of affected leaves, results similar to those for Acrobat MZ 69 WG were obtained in the case of application of products based on propamocarb-HL and fluopicolide (Infinito 687.5 SC), and products containing fosetyl-aluminium along with fenamidone (Mildex 711.9 WG) and propamocarb-CHL (Previcur Energy 840 SL). In greenhouse studies, their limited protective effect was determined.

Still in the 1980's, Orlikowski [1982] evidenced positive effect of fosetyl-aluminium (Aliette 80 WG) in protection against *Phytophthora* spp., but the substance was not effective in limiting gerbera phytophtorosis.

Own research also involved testing Luna Sensation 500 SC containing 2 active substances from different chemical groups and with different effects, i.e. fluopyram from the group of SDHI and trifloxystrobin from the group of strobilurins. Obtained results showed that foliar application of the product protected gerbera against *P. cryptogea* better than its introduction to the soil, but its effectiveness was limited. Available literature provides data on the effectiveness of products based on strobilurins, applied in protection against phytophtora. Lindermann and Davies [2008] conducted research on the effectiveness of fungicides containing active substances from the group of strobilurins, i.e. azoxystrobin 50% (Heritage) and pyraclostrobin 20% (Insignia). Neither of the products showed inhibiting effect on the development of *P. citricola* and *P. citrophthora* in the case of soil or foliar application. Results obtained by Benson and Parker [2011] also confirmed limited effectiveness of strobilurins in the protection of gerbera against *P. cryptogea*.

In the conducted research, the best solution for protecting gerbera cultivations against *P. cryptogea*, particularly in the case of soil application, proved to be Ridomil Gold MZ Pepite 68 WG, which along with mancozeb also contains metalaxyl. Similar effects were obtained in research on houseleeks [Ptaszek and Orlikowski 2008]. Among tested products, the best effects limiting spreading of *P. cryptogea* were observed for spraying plants with Acrobat MZ 69 WP and Ridomil Gold MZ 68 WP.

## CONCLUSIONS

1. Obtained data show a variable response of *Phytophthora cryptogea* to tested plant protection products.

2. In the *in vitro* tests, the highest effectiveness in the limitation of *P. cryptogea* growth was recorded for Acrobat MZ 69 WG, Infinito 687.5 SC, and then for Ridomil Gold MZ Pepite 68 WG.

3. The best solution protecting gerbera cultivations against *P. cryptogea* proved to be Ridomil Gold MZ Pepite 68 WG. In programs of gerbera protection

against phytophtora, the product should be introduced by means of watering.

4. Acrobat MZ 69 WG, Infinito 687.5 SC, and Mildex 711.9 WG can be considered an element of integrated protection of gerbera.

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