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Original Research

The Effect of Foliar Fertilizers on Mycelial Growth of Select Pathogenic Fungi under *in vitro* Conditions

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Abstract

Fungal communities colonizing the stems of three potato cultivars fertilized with Basfoliar 12-4-6, ADOB Mn, and Solubor DF were analyzed in the present study. The response of eight fungal potato pathogens (*Alternaria alternata*, *Colletotrichum coccodes*, *Fusarium concolor*, *F. culmorum*, *F. oxysporum*, *F. solani*, *Helminthosporium solani*, and *Rhizoctonia solani*) to the above foliar fertilizers added to the PDA medium at the following concentrations: 10, 100, 500, 1,000, and 10,000 mg·dm^{-3} was determined during *in vitro* tests.

The predominant fungal species isolated from potato stems were *Colletotrichum coccodes* (Basfoliar 12-4-6 treatment and control treatment) and *Alternaria alternata* (ADOB Mn and Solubor applied in combination). Fungi showing antagonistic activity against pathogens had a low share of the fungal community.

Foliar fertilizers added to the PDA medium at the studied concentrations significantly inhibited the mycelial growth of *A. alternata*, *C. coccodes*, *F. solani*, and *R. solani*. *F. concolor*, *F. culmorum*, *H. solani*, and *F. oxysporum* grown on the PDA medium containing ADOB Mn were least sensitive to the applied fertilizers. Solubor DF had the most toxic effect on fungal pathogens – when applied at the highest concentration, it almost completely suppressed the mycelial growth of pathogenic fungi, except for *A. alternata* and *F. concolor.*

Keywords: foliar fertilization, potato stems, pathogenic fungi, mycelial growth

Introduction

Colletotrichum coccodes, *Alternaria alternata*, *Fusarium* species, *Helminthosporium solani*, and *Rhizoctonia solani* are considered major fungal potato pathogens [1-3]. Fungicides are efficient in reducing the severity of fungal diseases [4], but due to their adverse environmental effects, in recent years they have been increasingly replaced with plant extracts [5] and biocontrol agents [6]. Organic and inorganic salts as well as organic and mineral fertilizers, in particular foliar fertilizers, can also be a viable alternative to fungicides. Zinc sulfate and copper sulfate are effective in controlling *P. infestans* infections [7]; salts (chlorides, sorbates, carbonates, and phosphates) containing calcium, potassium, sodium, and aluminum contribute to reducing the severity of infections caused by *Helminthosporium solani* [8]; and aluminum chloride shows potential for biocontrol of *F. solani* var. *coeruleum* and *F. sambucinum*, the causal agents of potato dry rot [9]. Foliar and organic fertilizers supply macronutrients (N, P, K, Mg, Ca, S) and micronutrients (Fe, Mn, Cu, Zn, B, Mo) needed by crops [10]. The inhibitory effect of organic fertilizers on the population size of pathogenic fungi such as *P. infestans*, *R. solani,* and members of the genus *Fusarium* has been widely discussed in literature [11, 12]. Crops supplied with adequate and balanced nutrients

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are more resistant to pathogenic attacks. In a study by Osowski [13], Basfoliar 12-4-6 retarded the development of early blight. The foliar application of phosphorus reduced potato tuber infection by the fungus-like organism *Phytophthora infestans* [14]. The severity of infections caused by *R. solani* was reduced by the foliar application of Mikrosol U (4.0% weight N-NH_4, 2.8% Mg, 2.5% S and B, Cu, Zn, Fe, Mn, Mo) [15], sulfate and elemental sulfur [16].

Metal ions applied at certain concentrations under laboratory conditions may lead to the death of the tested microorganisms, whereas in a natural environment they can even stimulate microbial growth. The effects exerted by metal ions are determined by their chemical form, availability, and environmental factors [17]. Foliar fertilizers provide disease suppression when they contain at least one component at a concentration sufficient to inhibit the growth of pathogens, which is not toxic to the crop. Copper salts, zinc salts, calcium hydroxide, potassium hydroxide and selected nitrogen, sulfur and molybdenum compounds have been found to be highly toxic to pathogenic fungi. Mycelial growth can be stimulated by some heavy metals (Al, Fe, Mo, Pb) and inhibited by others (Cd, Co, Ni, Se) [18].

The objective of this study was to determine the effects of three foliar fertilizers on fungal communities colonizing potato stems and on the growth of pathogenic fungi under *in vitro* conditions.

Experimental Procedures

The experimental materials comprised stems of three potato cultivars – Adam, Rudawa and Skawa – grown in a three-year field trial (with randomly selected sub-blocks and four replications), in Bałcyny (northeastern Poland) on grey-brown podzolic soil developed from light silty clay (complex 4 class III in the Polish soil classification system). Healthy, certified seed tubers were planted. Identical agrotechnical (as recommended by the Institute of Soil Science and Plant Cultivation – National Research Institute, Puławy) and plant protection measures (as recommended by the Institute of Plant Protection – National Research Institute, Poznań) were applied in all plots. The following soil mineral fertilization was applied: 80 kg N·ha^{-1}, 80 kg P·ha^{-1}, 120 K·ha^{-1}. The following foliar fertilizers were applied:

B – Basfoliar 12-4-6 (8 l·ha^{-1})
A – ADOB Mn (4 l·ha^{-1})
S – Solubor DF (2 l·ha^{-1})
A+S – ADOB Mn (2 l·ha^{-1}) + Solubor DF (1 l·ha^{-1})
A+B – ADOB Mn (2 l·ha^{-1}) + Basfoliar 12-4-6 (4 l·ha^{-1})
B+S – Basfoliar 12-4-6 (4 l·ha^{-1}) + Solubor DF (1 l·ha^{-1})
B+A+S – Basfoliar 12-4-6 (2.7 l·ha^{-1})+ADOB Mn (1.3 l·ha^{-1}) + Solubor DF (0.7 l·ha^{-1})

Non-fertilized potato plants served as control – **C**. The fertilizers were applied once, at the beginning of flowering (BBCH 61). The content of particular components in % weight was as follows: Basfoliar 12-4-6 (N – 12, K – 6, P – 4, Mg – 0.2, B – 0.02, Mn – 0.01, Cu – 0.01, Fe – 0.01, Zn – 0.005, Mo – 0.005), ADOB Mn (N – 6.5, Mg – 2, Mn – 10), and Solubor DF (B – 17.5).

Stem segments (samples – 30 stems collected randomly per treatment) collected five weeks after foliar treatment were cut into 0.5 cm pieces, disinfected with 50% ethanol and 0.1% sodium hypochlorite, and rinsed three times with sterile water. Fungi were cultured on the PDA medium, and were identified to the species level by microscopic observations.

In vitro tests were conducted to determine the mycelial growth of eight fungal potato pathogens: *Alternaria alternata* (Fr.) Keissler, *Colletotrichum coccodes* /Wallr./ Hughes, *Fusarium concolor* Reinking, *F. culmorum* (W. G. Sm.) Sacc., *F. oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Helminthosporium solani* Dur. et Mont., and *Rhizoctonia solani* Kühn on the PDA medium containing the studied foliar fertilizers at the following concentrations: 10, 100, 500, 1,000, and 10,000 mg·dm^{-3}, which corresponds to the concentrations of: 0.001, 0.01, 0.05, 0.1, and 1% in foliar application. Sterile aqueous solutions of foliar fertilizers were added to a liquid medium at a temperature of around 50ºC. Five mm agar disks overgrown with four- to five-day-old cultures of fungal species were transferred to the solidified medium, and were incubated at 22ºC. The diameters of fungal colonies were measured from the moment the medium had been thoroughly overgrown with fungal mycelium in the control treatment (plates with fungal inoculum on a medium containing no fertilizers). The experiment was carried out twice, in four replications (four plates). The rate of colony growth and the index of colony growth inhibition were calculated from the following formula:

$$I = [(\varphi_k - \varphi)/\varphi_k] \cdot 100\%$$

...where φ_k – control culture diameter, φ – culture diameter on a medium containing fertilizers.

The results of *in vitro* tests performed in a completely randomized design were verified statistically by an analysis of variance (STATISTICA® 9.0, 2010). Means were compared using Duncan's test, at a significance level of 0.01.

Results and Discussion

A total of 31 species of filamentous fungi, non-sporulating fungi, and yeast-like fungi were isolated from the stems of three potato cultivars (Table 1). Pathogenic fungi accounted for approximately 83% of all isolates. The predominant species were *Alternaria alternata* and *Colletotrichum coccodes*, but differences in the numbers of isolates between treatments were statistically non-significant. Species of the genus *Fusarium*, *Helminthosporium solani*, and *Rhizoctonia solani* were relatively rare, and *Botrytis cinerea* was sporadic (Table 2).

Foliar fertilization may reduce the abundance of potential pathogens (*A. alternata*, *R. solani*, fungi of the genus *Fusarium*, and other) colonizing the aboveground parts of different plant species [19]. In the present study, the causal agents of early blight and dry rot had the highest share (approximately 35% and 12%, respectively) of the fungal

Table 1. Percentage of fungi isolated from potato stems (2007-09).

Fungi	Percentage
Pathogens: *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Pers., *Colletotrichum coccodes* /Wallr./ Hughes,, *Fusarium* spp. (*F. culmorum* (W. G. Sm.) Sacc., *F. equiseti* (Corda) Sacc. *F. oxysporum* Schlecht., *F. solani* (Mart.) Sacc.), *Helminthosporium solani* Dur. et Mont, *Rhizoctonia solani* Kühn)	82.8
Antagonists: *Gliocladium catenulatum* Gilman Abbott, *Paecilomyces roseum* (Thom) Samson, *Trichoderma hamatum* (Bon.) Bain, *T. polysporum* (Link and Pers.) Rifai	2.4
Mucorales: *Mortierella* spp., *Mucor hiemalis* Wehmer	3.9
Other: *Acremonium strictum* W. Gams, *Aureobasidium pullulans* de Bary (Arnaud), *Chaetomium globosum* Hughes, *Cladosporium cladosporioides* Fres. de Vries, *Epicoccum* spp., *Gliomastix murorum* (Corda) Hughes, *Humicola* spp., *Monodictis glauca* (Cooke & Harkn.) Hughes, *Penicillium* spp., yeast-like fungi, nonsporulating fungi	10.9
Total (number)	1188

Table. 2. Some fungi isolated from potato stems (mean number of isolates / % of isolates, 2007-09).

Foliar fertilizers	*Alternaria alternata*	*Colletotrichum coccodes*	*Fusarium* spp.	Other pathogens	Antagonists	*Mucorales*
B*	11.67a** /22.6/	29.33a /56.8/	2.33ab /4.5/	4.00a /7.7/	1.00b /1.9/	0.33a /0.6/
A	12.33a /27./4/	22.67a /50.4/	4.00ab /8.9/	0a /0/	0b /0/	1.00a /2.2/
S	9.33a /19.6/	22.00a /46.2/	2.33ab /4.9/	0.67a /1.4/	7.00a /14.7/	1.00a /2.1/
A+S	19.00a /34.5/	18.33a /33.3/	7.00a /12.7/	1.00a /1.8/	0.33b /0.6/	2.67a /4.8/
A+B	15.67a /29.7/	23.00a /43.7/	3.33ab /6.3/	0.33a /0.6/	0b /0/	3.00a /5.7/
B+S	13.67a /25.8/	26.33a /49.7/	1.33b /2.5/	2.67a /5.0/	0.33b /0.6/	2.67a /5.0/
B+A+S	12.33a /23.9/	23.00a /44.5/	3.67ab /7.1/	1.67a /3.2/	0b /0/	4.33a /8.4/
C	8.67a /22.0/	23.67a /60.2/	2.67ab /6.8/	0a /0/	0.67b /1.7/	0.33a /0.8/

*B – Basfoliar 12-4-6, A – ADOB Mn, S – Solubor DF
**homogeneous groups according to Duncan's test for the comparison of means for species

community isolated from potato plants treated with ADOB Mn and Solubor DF applied in combination. The lowest number of *A. alternata* isolates was obtained from the stems of potato plants fertilized with Basfoliar 12-4-6, Solubor DF and control plants. Other authors [13, 20] observed an inhibitory effect of Basfoliar 12-4-6 on the development of the causal agent of early blight. Kolaei et al. [21] noted an inhibitory effect of calcium sulfate and ammonium sulfate on the development of *Fusarium sambucinum* in potatoes. The species *C. coccodes* dominated in the control treatment and in the Basfoliar 12-4-6 treatment (ca. 60%). In other studies [22], the severity of anthracnose was reduced in nitrogen-rich soils. In the present experiment, the highest number of isolates of *H. solani*, recognized as an important potato pathogen [23], were obtained from potato stems sprayed with Basfoliar 12-4-6. Antagonistic fungi were isolated much less frequently, and they most often colonized the stems of potato plants fertilized with Solubor DF. Species of the order *Mucorales* had a 4% share of saprotrophic fungi.

Regardless of their concentrations, Basfoliar 12-4-6, ADOB Mn and Solubor DF added to the PDA medium significantly inhibited the growth of *A. alternata*, *C. coccodes*, *F. solani*, and *R. solani*. The mycelial growth of the causal agent of early blight was suppressed to the highest degree (60%) in a medium containing Basfoliar 12-4-6 at a rate of 100 $mg \cdot dm^{-3}$ (Table 3). Increasing concentrations of ADOB Mn and Solubor DF in the medium, to 1000 $mg \cdot dm^{-3}$, stimulated mycelial growth. During *in vitro* trials [24], the mycelial growth of *A. alternata* was strongly inhibited by aluminum chloride and copper sulfate. Feng and Zheng [25] demonstrated that potassium chloride and sodium chloride suppressed the growth of *A. alternata*. Other authors [26] found that KNO_3, KCl, K_2SO_4, and KH_2PO_4 had an inhibitory effect on the mycelial growth of *A. solani* and *A. macrospora*, and spore germination of *A. solani*.

Solubor DF applied at the highest concentration had the most toxic effect on *C. coccodes*. In the other treatments, the diameter of fungal colonies ranged from 58.3 mm (31.5% inhibition of mycelial growth – ADOB Mn at the highest concentration) to 78.5 mm (7.7% inhibition of mycelial growth – ADOB Mn at a concentration of 500 $mg \cdot dm^{-3}$ of the medium). Among *Fusarium* species, *F. equiseti*, and *F. culmorum* were resistant to the applied foliar fertilizers Basfoliar 12-4-6 and ADOB Mn. Solubor DF applied at the highest concentration completely suppressed

Table 3. Effect of content of foliar fertilizers in the medium on the linear growth of pathogens in the laboratory (colony diameter mm/% of mycelial growth inhibition).

Foliar fertilizer	Concentration mg·dm^{-3}	*A.alternata*	*C. coccodes*	*F. equiseti*	*F. culmorum*	*F. oxysporum*	*F. solani*	*H. solani*	*R. solani*
		9 days	11 days	7 days	5 days	9 days	9 days	9 days	5 days
Basfoliar 12-4-6	10	56.5e*/33.5/	72.8ef /14.4/	85a /0/	85a /0/	52.8g /37.9/	75.0b /11.8/	85a /0/	75.3e /11.4/
	100	34.0i /60/	75.4b-f /11.4/	85a /0/	85a /0/	60.1f /29.3/	75.1b /11.6/	85a /0/	78.2cde /8/
	500	47.4f /44.3/	71.9f /15.5/	85a /0/	85a /0/	65.5e /23/	69.1c /18.7/	85a /0/	80.1bcd /5.7/
	1,000	62.0d /27.2/	64.3g /24.4/	85a /0/	85a /0/	76.0d /10.6/	69.6c /18.1/	85a /0/	82.2abc /3.3/
	10,000	67.6c /20.5/	74.6c-f /12.2/	85a /0/	85a /0/	81.1bc /4.6/	70.0c /16.2/	71.4c /16.1/	77.6de /8.7/
ADOB Mn	10	39.5gh /53.5/	77.6bcd /8.7/	85a /0/	85a /0/	80.5c /5.3/	75.0b /11.8/	85a /0/	83.0ab /2.4/
	100	42.7g /49.8/	78.3bc /7.9/	85a /0/	85a/0/	82.6ab /2.8/	74.8b /12,1/	85a /0/	79.4b-e /6.6/
	500	68.5c /19.4/	78.5b /7.7/	85a /0/	85a /0/	85a /0/	73.8b /13.2/	85a /0/	80.2bcd /5.6/
	1,000	76.3b /10.3/	72.8ef /14.4/	85a /0/	85a /0/	85a /0/	69.2c /18.6/	48.3d /43.1/	83.2ab /2.1/
	10,000	55.6e /34.6/	58.3h /31.5/	85a /0/	85a /0/	85a /0/	59.6d /29.9/	46.0d /45.9/	47.4h /44.2/
Solubor DF	10	38.7h /54.5/	74.4def /12.5/	85a /0/	80.6b /5.2/	74.6d /11.5/	75.0b /11.8/	85a /0/	70.2f /17.4/
	100	38.2h /55/	77.1bcd /9.3/	85a /0/	85a /0/	76.4d /10.2/	74.6b /12.2/	85a /0/	75.6e /11.1/
	500	55.6e /34.6/	76.4b-e /10.2/	85a /0/	83.8a /1.5/	79.4c /6.6/	68.8c /19.1/	85a /0/	56.8g /33.2/
	1	69.0c /18.8/	73.1ef /14/	85a /0/	81.3b /4.3/	67.3e /20.9/	46.1e /45.7/	81.3b /4.4/	25.7i /69.8/
	10	58.9de /30.8/	5.0i /100/	34.4b /59.6/	9.6c /88.7	5.0h /100/	5.0f /100/	6.0e /92.9/	5.0j /100/
Control		85a	85a	85a	85a	85a	85a	85a	85a

*homogeneous groups according to Duncan's test for the comparison of means for species

the development of the first fungal species, and Solubor DF applied at 10, 1,000, and 10,000 mg·dm^{-3} significantly inhibited the mycelial growth of the other fungal species. ADOB Mn had the weakest inhibitory effect on the mycelial growth of *F. oxysporum* – the fungus did not respond to the addition of the above fertilizer to the medium at a rate of 500 mg·dm^{-3} and higher. Basfoliar 12-4-6 and Solubor DF at the studied concentrations exerted a toxic effect on *F. oxysporum*. It should be noted that increasing concentrations of Basfoliar 12-4-6 and ADOB Mn in the medium stimulated, while Solubor DF inhibited mycelial growth. The growth of *F. oxysporum* was entirely suppressed in a medium with 10,000 mg Solubor DF·per dm^{-3}. Solubor DF exerted extremely strong fungistatic activity also against *F. solani*. The tested fertilizers inhibited the development of fungal colonies, and their inhibitory effect was proportional to the concentration applied. Foliar fertilizers have different fungistatic properties. In a laboratory experiment conducted by Boligłowa and Gleń [27], the foliar fertilizers Insol-7 (15.0% weight N, 0.5% B, 0.5% Cu, 1.1 % Mn, 1.5% Zn), Tytanit (0.8% Ti and N, P, K, Ca, Mg, Na, S, B, Co, Cu, Fe, Mn, Mo, Zn, Si), and Yeald (6% N, 0.25 Cu, 5% Zn, 0.25% Fe, 0.25% Mn) suppressed the growth of *F. culmorum*, *F. solani*, and *F. sulphureum*, but Yeald had a stronger inhibitory effect than Insol-7. The growth rate of pathogenic fungi is also affected by the rates of fertilizers. According to the cited authors, the fertilizers stimulated the growth and spore germination of pathogenic fungi when administered at lower concentrations. In another experiment [28], microelement fertilizers Mikrovit Fe (3.0% Fe) and Mikrovit Mn (6.0% Mn) applied at different concentrations had varied effects on the linear growth and sporulation of *F. culmorum*. During *in vitro* trials [9], aluminum chloride inhibited the growth of *F. sambucinum* and copper sulfate suppressed the development of *F. solani* var. *coeruleum* [24]. Recent research [29] shows that the mycelial growth of *F. solani* var. *coeruleum* can be inhibited by organic and inorganic salts (aluminum chloride, sodium benzoate, potassium sorbate, trisodium phosphate).

In the present study, only the highest concentration of the tested foliar fertilizer Basfoliar 12-4-6, and ADOB Mn and Solubor DF at a rate of 1,000 and 10,000 mg in the medium inhibited the growth of *H. solani* under *in vitro* conditions, and the diameters of fungal colonies were significantly smaller than in the control treatment. The most toxic effect (92.9% of mycelial growth inhibition) was exerted by Solubor DF at the highest concentration. In an experiment conducted by Olivier et al. [30], organic and inorganic salts of sodium, potassium, calcium, copper, and aluminum, applied *in vitro*, suppressed the mycelial growth and spore germination of *H. solani* [30].

All analyzed foliar fertilizers, irrespective of their concentrations, inhibited the growth of *R. solani*. The fungus was most sensitive to Solubor DF added to the PDA medium at 1,000 and 10,000 mg (mycelial growth was inhibited in 69.8% and 100%, respectively) and to the ADOB Mn at the highest concentration (44.2% mycelial growth inhibition). In a different study [31], potassium added to agar inhibited the growth of *R. solani*. Mikrovit Fe at all tested concentrations had the strongest inhibitory effect on the mycelial growth of *R. solani* and on the development of *Sclerotinia sclerotiorum* and *Phoma exiqua* var. *exiqua* [32]. The cited authors reported that the foliar fertilizer Fostar (14.8% P, 34.1% P_2O_5) had a strong fungistatic effect on *Phoma exiqua* and *R. solani*, whereas the growth of those pathogenic fungi was only insignificantly suppressed by Wapnovit (12.2 % Ca, 10.0% N-NO_3, 0.48% MgO, 0.05% B, 0.02% Zn). In another experiment [33], *R. solani* was found to be sensitive to the water extracts of soils fertilized with NPK, NPK + ammonium sulfate, and NPK + Potafoska (13.0% P, 13.0% K-K_2O, 13.0% Ca, 4.0% Mg, S).

Conclusions

1. The effect of foliar fertilizers on the populations of fungal pathogens colonizing potato stems varied.
2. Foliar fertilizers added to the PDA medium at the studied concentrations significantly inhibited the mycelial growth of *A. alternata*, *C. coccodes*, *F. solani*, and *R. solani*.
3. *F. equiseti* and *F. culmorum* were not sensitive to the addition of Basfoliar 12-4-6 and ADOB Mn to the medium at all studied concentrations. The effects of the analyzed fertilizers at the tested concentrations on the linear growth of *H. solani* and *F. oxysporum* were ambiguous.
4. Solubor DF applied at the highest concentration had the most toxic effect on the tested fungal pathogens (except for *A. alternata*) which had the lowest share of the fungal community isolated from potato stems treated with this fertilizer.

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