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THE INFLUENCE OF GLUCOSINOLATES ON THE *IN VITRO* GROWTH OF FUNGI PATHOGENIC TO OILSEED RAPE (*BRASSICA NAPUS* L.)

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Abstract: The influence of glucosinolates isolated from oilseed rape seeds on the growth of pathogenic fungi infecting oilseed rape was studied. The activity of those compounds against 3 fungal species was tested *in vitro*. It was stated that glucosinolates present in the medium did not totally inhibit the growth of the fungi, but considerably confined the area of colonies of 2 out of 3 fungal species studied.

Key words: oilseed rape glucosinolates, pathogenic fungi

INTRODUCTION

Presently the world-wide research is being developed to establish new, proecological solutions in the field of plant protection against pests and pathogens. A special attention is given to the possibilities of application of non-chemical methods of plant protection, and also methods based on utilisation of secondary plant metabolites, specific for a given systematic group (Bettolo 1983; Dawson et al. 1980).

Glucosinolates, compounds containing sulphur, which are secondary metabolites of oil and vegetable type Crucifers, are included to this group (Chew 1988; Krzymańska et al. 1997; Larsen 1981; Schnug 1990). Our interest in glucosinolates occurring in oilseed rape (*Brassica napus* L.) is related to a wide cultivation of this plant species in Poland. The role of glucosinolates in plants is not fully recognized. It was stated that they play certain functions in plant metabolism, as well as in mutual relationship between plants and their pests and pathogens (Birch et al. 1992; Doughty et al. 1991; Giamoustaris and Mithen 1995; Lazzeri et al. 1993; Mithen 1992; Schnug and Ceynowa 1990; Waligóra and Krzymańska 1995). The evidence for enhenced synthesis of glucosinolates in plants at the moment of injury testifies their protective role against pests and pathogens (Birch et al. 1992; Doughty et al. 1980).

As new, double-low varieties of oilseed rape (*Brassica napus* L.) characterized by a very low content of those compounds in seeds have recently been introduced – a question arises – what consequences this will result in, with regard to their resistance against pests and pathogens.

Pathogenic fungi every year cause considerable losses in winter oilseed rape cultures. Some species of those pathogens are especially troublesome: *Botrytis cinerea*, a causal agent of grey mould, *Phoma lingam* the fungus causing dry rot of oilseed rape stems, also *Alternaria brassicicola* and *Alternaria alternata* which are one of causal agents of alternaria blight. They cause infection of stems, petioles, leaves and also buds, flowers and pods.

Reports given by breeders indicate, that oilseed rape varieties with lowered content of glucosinolates (low and double-low) may sometimes be more severely infected by pathogens than old, standard varieties. The objective of presented research work was to elucidate the influence of oilseed rape glucosinolates on the *in vitro* development of some fungi pathogenic to winter oilseed rape.

MATERIALS AND METHODS

The research was performed according to EPPO Bulletin 21, 291–354 (1991), designed for studying the sensitivity of fungi to various chemical compounds. The influence of glucosinolates obtained from oilseed rape seeds on the development of 3 species of pathogens: *B. cinerea, A. brassicicola* and *A. alternata* was studied. Glucosinolates were isolated from oilseed rape seeds according to the method described by Jerzmanowska (Jerzmanowska 1967). Obtained glucosinolates were stored as a powder and then used in experiments. The isolates of fungi were obtained from oilseed rape plants collected on experimental plots. Inoculum was prepared using 21-day old cultures grown on PDA plates at 18–20°C. The tests were performed on PDA medium enriched with glucosinolates, which were isolated from rape seeds, then dissolved in 1ml of water and applied at concentrations of 100 to 800 ppm.

Non-enriched PDA plates and PDA plates to which 1 ml of water was added served as controls (relative and absolute controls). The experiments were performed in 5 replications. Agar plates (diam. 9 cm) were inoculated with small discs (diam. 5 mm) of 3-week old cultures of pathogens studied. The area of colonies was estimated after 6 days of incubation. Two perpendicular diameters of mycelium were measured and the surface area of mycelium was calculated (in mm^2). Results were subjected to statistical analysis of variance, and compared using t-Student's test on the level of significance 0.05.

RESULTS AND DISCUSSION

The results obtained in the experiments on the effect of glucosinolates on the growth of various fungal pathogens infecting oilseed rape are shown in tables 1–3.

In table 1 are given results of tests carried out with the fungus *B. cinerea*. A significant inhibition of the increase of surface area of colony on agar medium containing glucosinolates was observed. This being related to control plates, and the observed effect depended on concentration of those compounds in the medium.

Results of experiments with the fungus *A. brassicicola* are presented in table 2. We did not observe any inhibition of the increase of surface area of mycelium by glucosinolates. In concentrations 100 i 200 ppm we did not state significant differ-

ences, and in concentrations 400 i 800 ppm even some increase of surface area of mycelium was observed, as related to control plates.

In the course of study on the effect of glucosinolates on the growth of the fungi *A. alternata* (Tab. 3) inhibitory action of glucosinolates was also stated. The greatest differences in the mycelial growth were noted between experimental variants with glucosinolates and control without water.

In general, in our experiments total inhibition of mycelial growth was not observed. However, glucosinolates added to the medium, especially in higher concentrations, confined the growth of surface area of colony in the case of 2 out of 3 species of fungi. The fungus *A. brassicicola* was the exception, as glucosinolates did not inhibit its growth, and even in higher concentrations caused a considerable increase of growth rate of its mycelium.

In our previous investigation we have estimated the total amount of glucosinolates in different parts of rape plant, at different stages of plant development. We have stated that the content of glucosinolates in rape plant changes dynamically

Table 1. Mean surface area of *Botrytis cinerea* colonies on media amended with glucosinolates, after 6 days

Treatment	Mean area of fungal colony in mm^2	t-Student's test*
100 ppm of glucosinolates	4 584.16	b
200 ppm of glucosinolates	4 110.61	b
400 ppm of glucosinolates	4 346.00	b
800 ppm of glucosinolates	3 450.46	c
Control + H_2O	5 686.72	a
Control	6 031.27	a

Table 2. Mean surface area of *A. brassicicola* colonies on media amended with glucosinolates, after 6 days

Treatment	Mean area of fungal colony in mm^2	t-Student's test*
100 ppm of glucosinolates	1 450.71	a
200 ppm of glucosinolates	1 250.23	a
400 ppm of glucosinolates	1 633.98	b
800 ppm of glucosinolates	1 584.05	b
Control + H_2O	1 471.91	a
Control	1 288.54	a

Table 3. Mean surface area of *A. alternata* colonies on media amended with glucosinolates, after 6 days

Treatment	Mean area of fungal colony in mm^2	t-Student's test*
100 ppm of glucosinolates	2 118.51	b
200 ppm of glucosinolates	1 721.32	b
400 ppm of glucosinolates	1 372.71	c
800 ppm of glucosinolates	1 339.23	c
Control + H_2O	2 678.42	a
Control	3 048.51	a

* Values followed by different letters are significantly different at level $\alpha=0.05$

during vegetation, and it depends on the variety and also on the stage of plant development (Krzymańska et al. 1997). This was also confirmed by Fieldsend (Fieldsend and Milford 1994) and Giamoustaris (Giamoustaris at al. 1994). In rape varieties investigated in our studies, total content of glucosinolates oscillated from 0.5 to 3.42 μM/g at the stage of 4th leaf; from 0.25 to 2.39 μM/g at the stage of flower buds forming and from 0.16 to 1.92 μM/g at the stage of flowering (Krzymańska et al. 1997).

Many scientists have been working on the problem of antibacterial and antifungal activity of glucosinolates. Results of this investigation proved that glucosinolates indicate strong inhibiting action towards different tribes of pathogenic fungi. Some scientists tested activity of the mixture of glucosinolates against different microorganisms (Makulec et al. 1995). In those experiments however minimal inhibitory concentration of glucosinolates was relatively high and it seemed it might have been caused by using the mixture of glucosinolates. The presence of other compounds in this mixture could dissemble the activity of glucosinolates. In our experiments we also used the extract containing mixture of glucosinolates. As it was stated it showed significant inhibiting properties towards two of tested fungal species and the effectiveness of inhibiting increased as the glucosinolate concentration in the substrate increased.

Some other scientists investigated the influence of particular isolated and purified glucosinolates and their hydrolitic products.There is also a hypothesis that the most active compounds are products of glucosinolates' breakdown. The attack of pest or pathogen causes enzymatic degradation of glucosinolates and releasing of some compounds, mainly isothiocyanates (volatile compounds), which are suspected to be the most toxic. The efficacy of such volatiles was tested by many scientists (Borek et al. 1994; Fenwick et al. 1983; Mayton et al. 1996; Oleszek 1987; Smolińska et al. 1997a; 1997b; Smolińska and Horbowicz 1999).

Mithen (1987) observed that the development of *Leptosphaeria maculans* was inhibited by the degradation products of all glucosinolates tested except progoitrin. It sounds strange that progoitrin which is known as a compound responsible for many antinutritional effects of rapeseed meal has no antifungal properties.

It would appear that accumulation of glucosinolate breakdown products as a result of initial tissue damage restrict the further development of infection, so the low and double-low varieties of rape can be infected in nature more intensively than traditional varieties, this was in some cases observed in practice. It was stated (Mithen et al. 1987) that the ability of the pathogen to spread in tissue varied considerably among different lines of *Brassica*.The extent of colonization was related to the level of glucosinolates: plants showing small localised lesions had high levels of glucosinolates and those with large lesions or systematic infection had low levels of these compounds.

So, from the practical point of view it seems that the reduction of the level of glucosinolates in green parts of oilseed rape plants may not be profitable concerning its susceptibility to the infestation by some species of pathogenic fungi. Reduction of the level of progoitrin in oilseed rape seeds however, would increase the feeding value of the rapeseed meal whilst preserving resistance to disease. Magrath

et al. (1993) suggested that it would be possible within conventional breeding programme to modify the leaf and seed glucosinolate profile of oilseed rape with the use of synthetic *Brassica napus* lines.

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POLISH SUMMARY

WPŁYW GLUKOZYNOLANÓW NA WZROST PATOGENICZNYCH GRZYBÓW PORAŻAJĄCYCH RZEPAK (*BRASSICA NAPUS* L.) W HODOWLACH *IN VITRO*

Wykonano doświadczenia dotyczące wpływu glukozynolanów wyizolowanych z nasion rzepaku (*Brassica napus* L.) na wzrost patogenicznych grzybów porażających tę roślinę. Przetestowano *in vitro* aktywność tych związków w stosunku do trzech gatunków grzybów: *Botrytis cinerea, Alternaria brassicicola* i *A. alternata* – hodując je na pożywce AGZ z dodatkiem glukozynolanów w stężeniach od 100–800 ppm. Nie stwierdzono całkowitego hamowania wzrostu któregokolwiek z badanych grzybów, jednakże glukozynolany dodane do podłoża ograniczały – zwłaszcza w wyższych stężeniach, wzrost pola powierzchni grzybni dwóch spośród trzech badanych gatunków grzybów. W jednym przypadku (*A. brassicicola*) glukozynolany nie tylko nie ograniczały wzrostu grzyba, a powodowały nawet przyspieszenie wzrostu grzybni.