11

Impact of Plant Extracts on Vitality and Root Healthiness of Leguminous Plants Inoculated by *Fusarium oxysporum* (Schl.)

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1. Introduction

Among the factors negatively influencing yielding of leguminous plants are such pests as pathogens, insect pests and weeds. The losses caused by the pathogens and insect pests can reach 30%, a half of which can be ascribed to plant pathogens.

In the condition of light soils, at moisture insufficiency and in periods of high temperature a great harmfulness show root rot diseases caused by such soil pathogens as *Fusarium* sp., *Pythium* sp., *Phoma* sp., as well as *Aphanomyces* sp. The early infection of horse bean *(Vicia faba minor)* by the mentioned pathogens causes a yield loss of about 10%. On lupine plantations the root rot pathogens are more significant than those occurred on overground parts of the plants (Sadowski 1994, Krzysiak 1994).

These pathogens are transmitted most often with a seed material. They can also survive as saprotrophes in soil or parasite on different cultivated and wild plants (Filipowicz 1987, Sadowski and others 1993).

The most effective method of an occurrence limitation of these pathogens and of a restriction of their negative impact on plant sprouts is a seed dressing. But the sowing of healthy or dressed seeds gives not always satisfactory results. It protects the plants only for a short period, but does not secure the sprouts against soil-born pathogens (Sadowski, Piątek 1983). Besides that the intensive chemical treatments lead to an emerging of pesticides resistant pathogen populations (Łacicowa 1989).

One of the principles of integrated pest management is integration of agricultural, biological and chemical method. In the biological methods to the pathogens control may be used chemical substances formed by bacteria and

fungi, and as well as by plants. It is known that most of plants produces compounds with allelochemical, biocidial and biostatical effect (Achremowicz, Cież 1992). The biological activity of plant extracts depends however on several factors, and first of all on content of specific chemical compounds and on their ability to diffuse. Besides that some those compounds may stimulate a pathogen development and increase a degree of contamination and the others can act as inhibition factors (Sas-Piotrowska and others 1995b, 1996). The impact on protected plants is also of importance.

The aim of the investigations carried out was a laboratory evaluation of a fungicidal activity of extracts from polygonaceous plants against Fusarium oxysporum, root rot agents on Papilionaceae plants.

2. Material and methods

The material used in investigations was as follows :

- Polygonaceous plants (*Polygonaceae)* from which the extracts were prepared, and namely *Polygonum bistorta* L., *P. hydropiper* L., *P. convolvulus* L., *P. persicaria* L., *P. aviculare* L and *P. sachalinense* Schmidt. The investigated in vivo plant extracts were prepared in a form of water extracts (maceration and infusion) as well as alcohol and acetone solutions. The way of their preparation was given in earlier studies (Sas-Piotrowska, Piotrowski 1997).
- Seeds of following leguminous plants: horse bean (*Vicia faba L.* ssp. *minor),* white lupine (*Lupinus albus* L.), yellow lupine (*Lupinus luteus* L.)
- Fungus *Fusarium oxysporum* (Schl.) causing the root rot diseases of leguminous plants. Its culture was carried out on Petri dishes with a PDA nutrient medium. From one week old cultures of *F. oxysporum* overgrowed with mycelium, agar discs with a diameter of 5 mm were cut and served as an infection material.

The experimental method was as follows: disinfected and rinsed in a sterile distilled water seeds were dressed in rotary seed dresser with one of the mentioned extracts (7 minutes), and then the seeds were left for 20 hours. The next day the seeds were put in suitably prepared glass tubes, the overgrowed with fungus mycelium agar discs were put on and all was plugged with a cotton wool. After 2 weeks of incubation in conditions of natural lighting and ambient temperature the healthiness and vitality evaluation were made:

- as a criterion of healthiness a degree of root infection was taken, and a 9 - grade scale were applied, where 1^o - lack of infection symptoms , 9^o - dark browned and dead roots. The analysis of variance was carried out on the according to Townsend-Heuberger formula transformed values;
- as a criterion of vitality a root length in cm and their mass in g were taken.

The experiment was conducted in two periods and 4 replications for each of investigated factors performing altogether 14400 evaluations for each criterion. The research results were assessed using a single analysis of variance and a linear correlation.

3. Research results

The extracts made from the polygonaceous plants (regardless of the preparation way), as well as the extracts made according to other procedures (regardless of their origin) significantly differentiated the root infection of *Vicia faba minor*, *L. albus*, *L. luteus* by *F. oxysporum* (Fig. 1) and the vitality of these plants (Fig. 2 and 3).

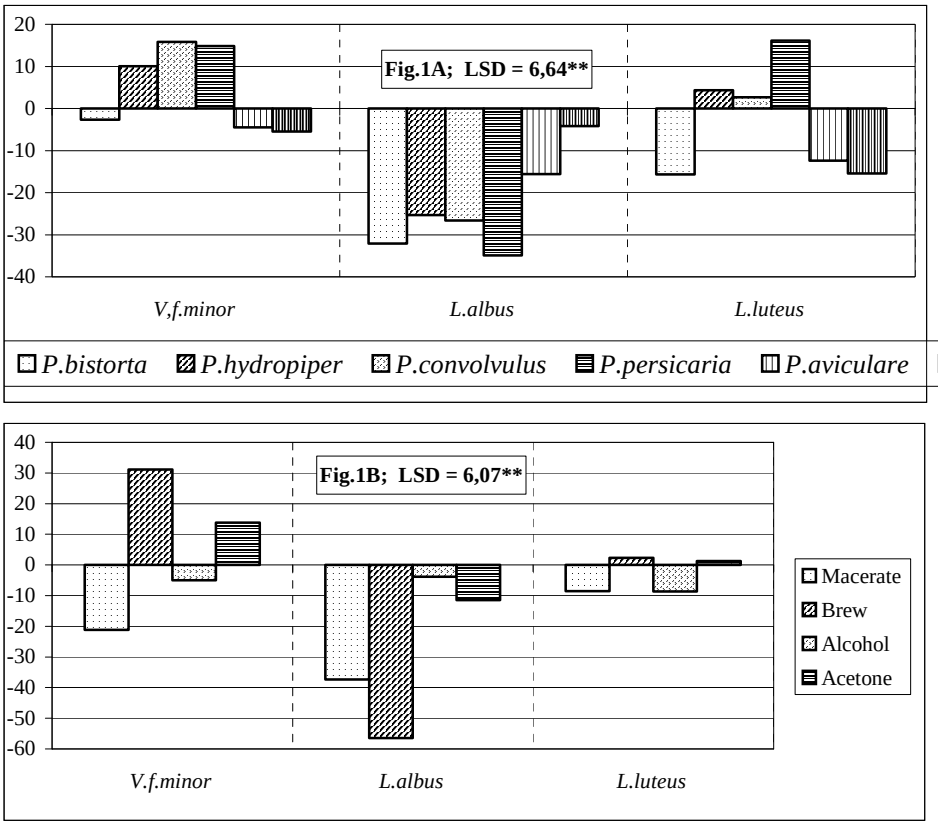


Fig. 1. The root infection by *F. oxysporum* depending on origin (A) and preparation way (B) of an extract (deviation from control, %)

Rys. 1. Porażenie korzeni przez *F.oxysporum* w zależności od pochodzenia (A) i sposobu (B) przygotowania wyciągu (odchylenie od kontroli, %)

The infection of *L. albus* roots by *F. oxysporum* was limited by the extracts made from all investigated *Polygonaceous* plants, and particularly from *P. persicaria* and *P. bistorta* (Fig. 1a). Similar effects were observed in relation to the way of their preparation (Fig. 1b). The most strong action was showed by the water extracts (maceration and infusion). The infection of *V.f. minor* and *L. luteus* roots was limited however by the extracts from *P. bistorta, P. aviculare* and *P. sachalinense*, and especially by maceration and alcohol extracts from *Polygonaceous* plants.

The performed statistical analysis proved furthermore a significant interaction between these factors (extract origin x preparation way)as well as between these ones and investigated leguminous plant species. They differed among themselves with response changeability in relation to the used extracts. It was the highest for *L. albus* (V = 44,0%), and the lowest for *L. luteus* (V = 15,5%). Furthermore, the impact of the extracts on the healthiness on plant species was sometimes different. A significant impact conformity was stated in comparison of the extracts on healthiness of *V.f.minor* and *L. luteus* (r= 0,452*). In remaining cases a tendency to a negative correlation appeared. It found an reflection in final results showed in tables 1, 2 and 3. It results from there that:

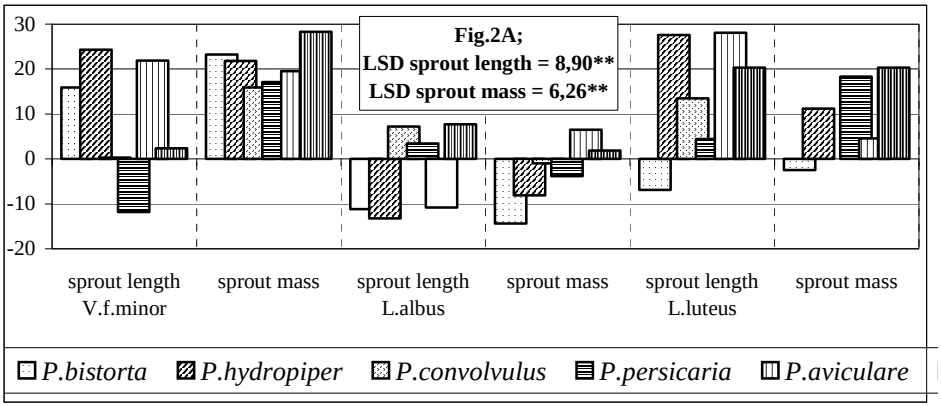
- infection of *V.f. minor* by *F. oxysporum* was limited most strongly by macerated extracts from *P. bistorta* and *P. aviculare* and by alcohol extract from *P. aviculare*;
- in case of *L. albus* there were infusions from *P. persicaria* and *P. hydropiper* as well as acetone extract from *P. convolvulus*;
- in case of *L. luteus* there were alcohol extracts from *P. sachalinense* and *P. aviculare* and macerated extract from *P. sachalinense*.

In addition to root healthiness of leguminous plants those parameters as a root and shoot length and mass were measured. These parameters were considered as an information regarding a possible phytotoxic or stimulating action on plant vitality of compounds contained in the extracts.

It was found, that in most cases, the diminished infection by *F. oxysporum* was accompanied by an increasing of plant vitality. This correlation was however proved only for *V.f.minor* by the impact of the extracts on root infection and their interaction on sprouts length (r=-0,610**) and on roots length (r=-0,733**). When the roots healthiness of *V.f.minor* with a green mass and roots mass, as well as roots healthiness of *L. albus* with a mass and length of green parts were compared a tendency for a reverse phenomenon was observed - the more the extracts from polygonaceous plants limited the root infection by *F. oxysporum* the stronger was also the decreasing of plant vitality.

The results collected on diagrams 2a and 2b show that *L. albus* responded with a clear decrease of length and mass of green parts on extracts

made from *P. bistorta* and *P.hydropiper*. The sprout length was alsow reduced by extracts from *P. aviculare* and its mass by extracts from *P. persicaria*. The impact of the extracts on these features was significantly correlated (r= 0,905**) by *L. albus*, what however was not observed in a case of *V.f.minor*. The sprout length of this plant was limited solely by the extracts from *P. persicaria*. The extracts from *P. bistorta* have also reduced the sprouts length and their mass by *L. luteus* (r = 0,537**). In remaining cases a stimulating action of the extract on sprouts length and mass was observed.



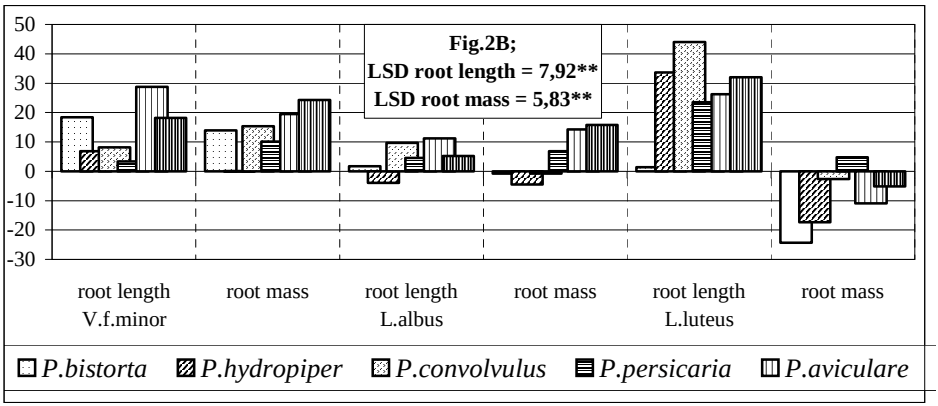


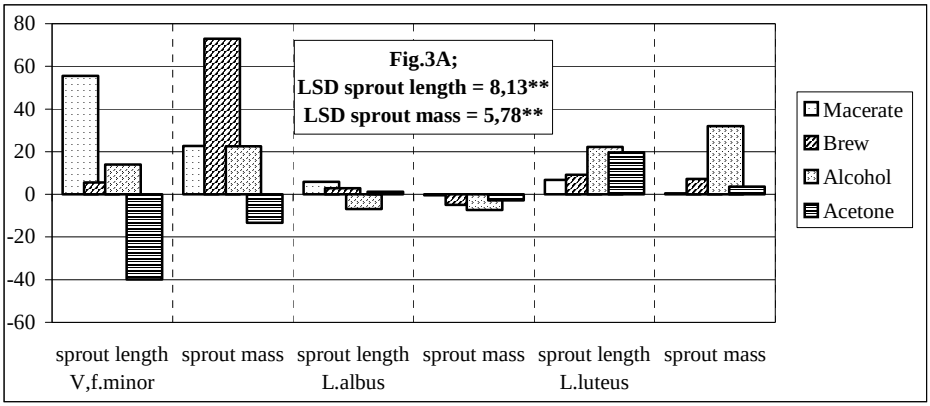
Fig. 2 (A, B). The vitality of leguminous plants depending on an extract origin (deviation from control, %)

Rys. 2 (A, B). Żywotność roślin strączkowych w zależności od pochodzenia wyciągu (odchylenie od kontroli, %)

The action of investigated extracts on green parts (length and mass of the sprouts) and on root system (length and mass of roots) was in a range of *L. albus* and *L. luteus* significantly positively correlated. The values of the correlation coefficients oscillated by *L. albus* from r = 0,560** to r = 0,905**, and by

L. luteus from r = 0,502* to 0,618**. In a case of *V.f.minor* these relationships were proved solely for sprouts and roots length (r = 0,726**) and for sprouts and roots mass (r = 0,912**).

A significant conformity was stated also for the response of a root system of *V.f.minor* (r = 0,407*), *L. albus* (r = 0,709**) and *L. luteus* (r = 0,502*), which was measured by its length and mass. However *L. luteus* has responded on substances contained in most extracts from polygonaceaus plants with a clear decrease of roots mass, by a simultaneous increase of their length (Fig.2a and 2b). A slight reducing of a development of a root system of *L albus* was caused by the extract from *P. hydropiper*. An increasing of a length and mass of *V.f. minor* roots was stimulated by all extracts from those polygonaceous plants investigated.



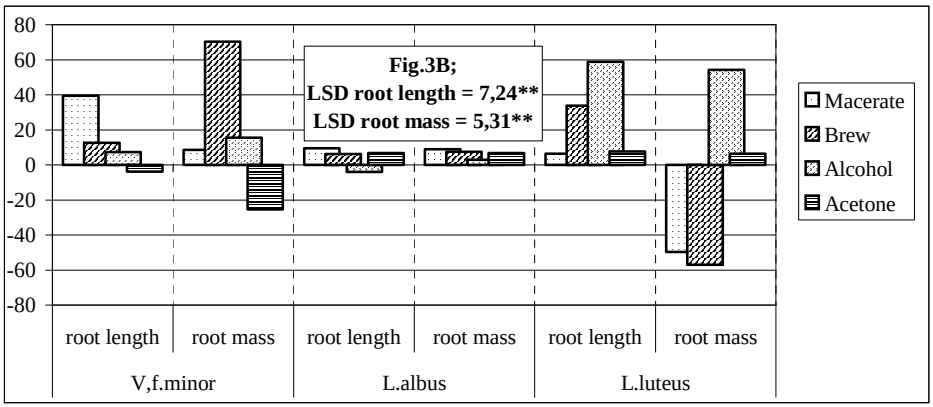


Fig. 3 (A i B). The vitality of leguminous plants depending on a preparation way of an extract (deviation from control, %)

Rys. 3 (A i B). Żywotność roślin strączkowych w zależności od sposobu przygotowania wyciągu (odchylenie od kontroli, %)

A significant influence on plant vitality exerted also the way of extracts preparing (Fig. 3a and 3b). The development of underground and aboveground parts of *V.f.minor* was reduced by acetone extracts, and of *L. albus* – by alcohol extracts. In a case of *L. luteus* a negative impact on root mass exerted maceration and infusion extracts, which however caused the increase of their length.

Furthermore a significant interaction was found between an extract origin and a way of its preparation as well as between these factors and investigated leguminous plant species. They differed between themselves with a reaction variability on used extracts. The variability coefficients (V%) oscillated by *V. faba minor* from 21,02 (root length) to 38,16 (sprout length), by *L. albus* from 10,21 (root mass) to 14,56 (sprout length), by *L. luteur* from 17,35 (sprout length) to 58,84 (root mass).

An extract influence on a vitality of investigated leguminous plant species, which was evaluated with different criteria was not significantly correlated. It indicates that a direction and intensity of *V.f.minor, L. albus* and *L. luteus* reaction on used plant extracts for seed dressing were different (Tables 1,2,3).

Table 1. The healthiness and vitality of *Vicia faba ssp. minor* depending on an extract (deviation from control, %)

Tabela 1. Zdrowotność i żywotność *Vicia faba* ssp. *minor* w zależności od wyciągu (odchylenie od kontroli, %)

Mean	Range	Inhibition	Stimulation
ROOT INFECTION			
3,8	-38,0÷44,9	P.bistorta; macerate (-38,0) P.aviculare; macerate (-37,1) P.aviculare; alcohol (-28,1)	P.convolvulus; acetone (44,9) P.aviculare; brew (42,6) P.hydropiper, brew (36,9)
ROOT LENGTH			
11,1	-40,1÷67,1	P.bistorta; acetone (-40,1) P.convolvulus; acetone (-20,9) P.persicaria; alcohol (-20,5)	P.aviculare; macerate (67,1) P.bistorta; macerate (47,4) P.sachalinense; acetone (42,5)
ROOT MASS			
13,9	-53,0÷97,6	P.bistorta; acetone (-53,0) P.convolvulus; acetone (-51,0) P.aviculare; acetone (-26,6)	P.bistorta; brew (97,6) P.persicaria; brew (91,9) P.convolvulus; brew (83,2)
SPROUT LENGTH			
7,1	-70,6÷78,4	P.convolvulus; acetone (-70,6) P.hydropiper, acetone (-46,9) P.bistorta, acetone (-43,4)	P.convolvulus; macerate (78,4) P.aviculare; macerate (75,0) P.bistorta; macerate (73,6)
SPROUT MASS			
20,9	-43,6÷86,3	P.convolvulus; acetone (-43,6) P.persicaria; acetone (-15,8) P.aviculare; acetone (-15,4)	P.convolvulus; brew (86,3) P.bistorta; brew (83,9) P.hydropiper: brew (71,4)

Table 2. The healthiness and vitality of *Lupinus albus* depending on an extract (deviation from control, %)

Tabela 2. Zdrowotność i żywotność *Lupinus albus* w zależności od wyciągu (odchylenie od kontroli, %)

Mean	Range	Inhibirion	Stimulation
ROOT INFECTION			
-19,7	-68,7÷74,9	P.persicaria; brew (-68,7) P.hydropiper; brew(-57,6) P.convolvulus; acetone (-57,4)	P.sachalinense; acetone (74,9) P.aviculare; acetone (40,1) P.hydropiper; alcohol (-23,2)
ROOT LENGTH			
3,8	-30,8÷38,1	P.hydropiper; alcohol (-30,8) P.persicaria; alcohol (-7,7) P.aviculare; macerate (-5,3)	P.aviculare; acetone (38,1) P.convolvulus; macerate (21,1) P.bistorta; macerate (17,7)
ROOT MASS			
5,3	-17,3÷29,3	P.hydropiper; alcohol (-17,3) P.bistorta; acetone (-12,0) P.hydropiper; acetone (-6,0)	P.aviculare; acetone (29,3) P.persicaria; brew (18,0) P.hydropiper; brew (18,0)
SPROUT LENGTH			
0,6	-36,0÷30,8	P.bistorta; alcohol (-36,0) P.hydropiper; alcohol (-29,9) P.bistorta; acetone (-20,4)	P.aviculare; acetone (30,8) P.bistorta; macerate (16,0) P.convolvulus; alcohol (13,3)
SPROUT MASS			
-3,1	-38,7÷21,3	P.bistorta; alcohol (-38,7) P.bistorta; acetone (-25,9) P.bistorta; brew (-17,0)	P.aviculare; acetone (21,3) P.bistorta; macerate (10,6) P.convolvulus; alcohol (7,3)

Table 3. The healthiness and vitality of *Lupinus luteus* depending on an extract (deviation from control, %)

Tabela 3. Zdrowotność i żywotność *Lupinus luteus* w zależności od wyciągu (odchylenie od kontroli, %)

Mean	Range	Inhibition	Stimulation
ROOT INFECTION			
-2,7	-27,6÷23,0	P.sachalinense; alcohol (-27,6) P.aviculare; alcohol (-21,8) P.sachalinense; macerate (-20,1)	P.hydropiper; acetone (23,0) P.persicaria; brew (22,6) P.persicaria; acetone (20,6)
ROOT LENGTH			
21,4	-42,7÷102,2	P.persicaria; brew (-42,7) P.bistorta; acetone (-18,9) P.persicaria; brew (-18,2)	P.persicaria; alcohol (102,2) P.hydropiper; brew (72,0) P.sachalinense; alcohol (66,7)
ROOT MASS			
-9,1	-73,9÷119,6	P.persicaria; brew (-73,9) P.sachalinense; brew (-62,2) P.bistorta; brew (-58,7)	P.persicaria; alcohol (119,6) P.sachalinense; alcohol (90,0) P.convolvulus; alcohol (80,4)
SPROUT LENGTH			
11,6	-29,0÷48,9	P.persicaria; brew (-29,0) P.aviculare; macerate (-9,1) P.bistorta; acetone (-8,2)	P.aviculare; acetone (48,9) P.aviculare; alcohol (43,4) P.sachalinense; alcohol (42,3)
SPROUT MASS			
8,6	-16,9÷106,8	P.bistorta; alcohol (-16,9) P.persicaria; brew (-9,9) P.aviculare; macerate (-9,9)	P.sachalinense; alcohol (106,8) P.persicaria; alcohol (91,2) P.hydropiper; brew (24,9)

4. Discussion

The occurrence in a soil of pathogenic factors with differeniated susceptibility towards fungicides causes that the chemical control of pathogenic fungi causing root rot is not easy (Jańczak 1992). For seed dressing there are used besides of fungicides also antibiotic substances, and lately a fungicidial compounds produced by plant are searched (Piotrowski, Sas-Piotrowska 1995). With these compounds contained in plant tissue there is connected their resistency against pathogens and pests (Trzebiński 1970). These compounds are known under the name of antibiotics or phytoncides.

Receiving of new plant varieties with high yielding possibilities and good economic properties is negatively connected with a content of these compounds. Decreasing of phenoles in maize was strongly correlated with intensity of attack by *Ostrinia nubilalis* (Dąbrowski 1988).

In investigations of Piotrowski and others (1995) it was proved that among 100 tested maceration extracts only some of these have hampered ger-

mination of spores and caused deformation of hyphae. It was first of all a maceration extract made from *P. bistorta*, which hampered germination of *Ascochyta fabae, Botrytis fabae, Fusarium culmorum* and *Alternaria alternata.* In other papers (Sas-Piotrowska, Piotrowski 1995a, b) where an impact of extracts from polygonaceous plants on pathogenes causing spot diseases by *V.f.minor* was analyzed, a high activity have shown also alcohol and acetone extracts from *P. bistorta*. In present investigations it was found however, that a maceration extract from *P. bistorta* is active also in relation to *F. oxysporum*. An application of this extract for seed dressing have reduced an infection of *V.f.minor* by this pathogene.

A favorable action of an extract from *P. bistorta* on plant healthiness depends on ist chemical composition, and particularly the content of almost 25% of vegetable tannines, as well as gallic and ellagic acids (Ożarowski 1980). Gallic acid inhibited flowering of a plant *Kalanchoe sp.* where it was discovered in bound form. Ellagic acid was however isolated in a free or bound form as tannines from an oak and spruce bark as well as from roots of pomegranate (Kączkowski 1993).

Besides extracts from *P. bistorta* a high activity in relation to *F. oxysporum* have shown in carried out researches following extracts :

- maceration extracts from *P. aviculare* i relation to *V.f.minor* and from *P. persicaria* in relation to *L. albus.* The activity of extracts from these plants is probably connected with a synthesis by *P. aviculare* tannine compounds and chlorogen acid, and by *P. persicaria* with flavonoids and volatile oils;
- a maceration and alcohol extract from green parts of *P. sachalinense* thanks to content of numerous alkaloids, and particularly quercitin (up to 2,5%) has reduced a root infection of *L. luteus.*

It results from investigations carried out by Sas-Piotrowska and Piotrowski (1995 c, 1997) that from among egzamined extracts, which were differentiated in relation to their origin and a way of preparation, the most effective against pathogens of spot diseases, and root rot were alcohol extracts from *P. bistorta* and *P. persicaria*. Present research does not confirm these results fully. As a matter of fact both *P. bistorta* and *P. persicaria* distinguished with high activity, but it took place in case of maceration extracts and not of alcohol extracts.

Some differences in results received in investigation carried out may follow from a different nature of these investigations (in vitro and in vivo), from environment conditions at a given vegetational season, which can modify the development of polygonaceous plants and their content of active substances, as well as from age of these plants in a moment of harvest time (Łakota and others 1993).

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Wpływ wyciągów roślinnych na żywotność i zdrowie korzeni roślin strączkowych zarażonych przez *Fusarium oxysporum* (Schl.)

Streszczenie

Zgodnie ze znowelizowaną Ustawą o Ochronie Roślin, obowiązującą od 2001 roku „środki ochrony roślin można stosować wyłącznie do celów określonych w etykiecie ..... uwzględniając w pierwszej kolejności biologiczne, hodowlane i agrotechniczne metody ochrony roślin, które pozwalają ograniczyć stosowanie chemicznych środków ochrony roślin do niezbędnego minimum".

Jednym z elementów biologicznej metody ochrony roślin może być wykorzystanie zjawiska allelopatii, określanego jako: oddziaływanie jednych organizmów na drugie za pomocą wytwarzanych substancji biologicznie czynnych. Substancje takie, zwane „antybiotykami roślinnymi" wytwarzane są także przez rośliny.

Celem przedstawionej pracy była ocena in vivo oddziaływanie substancji aktywnych zawartych w wyciągach wodnych (macerat, napar) oraz alkoholowych i acetonowych, sporządzonych z *Polygonum bistorta*, *P. hydropiper*, *P. convolvulus*, *P.persicaria*, *P.aviculare*, *P.sachalinense*, na żywotność (długość i masę pędów oraz korzeni) oraz porażenie korzeni trzech gatunków roślin strączkowych (*Vicia faba* ssp. *minor*, *Lupinus albus*, *Lupinus luteus*) przez grzyb *Fusarium oxysporum*. Doświadczenia te przeprowadzono w dwóch terminach i 4 powtórzeniach dla każdego z badanych czynników, dokonując łącznie 14400 obserwacji dla każdego z 5 kryteriów oceny.

W doświadczeniach wykazano, że wyciągi sporządzone z poszczególnych roślin rdestowatych, a także wyciągi przygotowane wg odmiennych procedur istotnie różnicowały porażenie korzeni *V.f.minor*, *L.albus* i *L.luteus* przez *F. oxysporum* oraz żywotność tych roślin. Badane rośliny różniły się między sobą zmiennością reakcji na zastosowane wyciągi (była ona najwyższa u *L.albus*, a najniższa u *L.luteus*) oraz zgodnością reakcji (istotnie zgodnie zaeraowały *V.f. minor* i *L. luteus*. Porażenie korzeni *V.f. minor* przez *F.oxysporum* ograniczały najsilniej maceraty z *P.bistorta* i *P. aviculare* oraz wyciąg alkoholowy z *P. aviculare*; *L.albus* - napary z *P.persicaria* i *P. hydropiper* oraz wyciąg acetonowy z *P.convolvulus*; *L.luteus* - wyciągi alkoholowe z *P. sachalinense* i *P. avivulare* oraz macerat z *P.sachalinense*.

Stwierdzono także, że w większości przypadków zmniejszeniu porażenia roślin przez *F.oxysporum* towarzyszył wzrost ich żywotności. Korelacja ta została jednak uwodniona jedynie w przypadku *V.f.minor* dla porównania wpływu wyciągów na porażenie korzeni z ich oddziaływaniem na długość pędów i długość korzeni. Gdy porównywano zdrowotność korzeni *V.f. minor* z masą części zielonych i masą korzeni, a także zdrowotność korzeni *L.albus* z długością i masą części zielonych obserwowano tendencje do zjawiska odwrotnego.

Oddziaływanie badanych wyciągów na części zielone (długość i masę pędów) oraz na system korzeniowy (długość i masę korzeni) było w obrębie *L. albus* i *L.luteus* istotnie pozytywnie skorelowane. W przypadku *V.f.minor* zależności te udowodniono jedynie dla długości pędów i korzeni oraz masy pędów i korzeni.