24. THE USAGE OF CHITOSAN IN PROTECTION OF SOME PEPPERMINT AND LEMON BALM PATHOGENS

Agnieszka Szczeponek, Stanisław Mazur[1)], Jacek Nawrocki[1)]

Department of Genetics, [1)]Department of Plant Protection,
Plant Breeding and Seed Science Production,
Agricultural University of Kraków,
Al. 29 Listopada 54, 31-425 Kraków, Poland
e-mail: smazur@org.ar.krakow.pl

1. Introduction

Although bacteriostatic and fungistatic properties of biological activity substances gained from herbs, there are observed occurrence of disease symptoms on this kind of plants caused by pathogens. The overgrowth, especially by fungi, tissues of overground parts of herbs, is particularly dangerous, because caused damage of secretory cells, and this fact induces on debasement of contents ethereally oils and on modification of composition aeriform fractions (D'Aulerio et al. 1995; Shukla et al. 1999; Shukla et al. 2000; Kalra et al. 2001; Edwards et al. 1999; Edwards et al. 2000; Margina et al. 1996). Poland is significant producer of herbs in Europe, since area herb cultivation is about 50% of European Union acreage. Because EU countries request for herbs covered mainly by import from South-American countries and from Centrally-East European countries, Poland has great chance of further development of herb production (Wolski 1998; Jambor 2001). Intensification of herb plants production and constantly increasing of interests of herbs stock by modern medicine, cosmetic and food industry, is important to gain of high-quality stock and also with even and repeatable chemical composition (Krawczyk 1995; Lutomski 1997). The herbs used as raw material for medicament production, should be free from any pathogens. Therefore herb plants plantations can not be deprivation of protective interventions. The necessity of greatest limitations of pesticides usage in cultivations of herb plants payed attention to possibility usage of biopreparates and bioactive substances. One of this compound is chitosan, which in some conditions could play role of potential biopreparate. Investigations of some authors pointed, that the main activity of chitosan is mainly to induce natural resistance relation in invaded plants (Benhamou 1992, Cohen 1993). Additionally, chitosan get to inhibit in vitro conditions the development of some fungi species and could play role of mechanical barrier for virus infection (Pospieszny and Struszczyk 1994). The aim of

the experiments conducted from a few years by authors of this paper, was to determine an effect of chitosan and it's derivatives used at different concentrations to control main fungal pathogens on peppermint and lemon balm plants.

2. Material and methods

Spore suspension of tested fungi made drenched surface coat of 14-days mycelium using distilled water and filtrated through sterile gauze. Concentrations of spores were measured by using Thom's chamber (Kiraly et al. 1977). For Sclerotinia sclerotiorum concentration established on the base of fragments of mycellium hyphae.

To the investigations were chosen fungi species, which were the most often isolated from diseased plants of lemon balm and peppermint. For lemon balm were tested the following fungi: *Alternaria alternata*, *Fusarium avenaceum*, *Fusarium culmorum*, *Epicoccum purpurascens*, *Sclerotinia sclerotiorum*.

For peppermint were chosen: *Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Epicoccum purpurascens, Fusarium avenaceum*.

Chitosol (content 2.26% of polymer) was used at the concentrations 0.1%; 0.05% and 0.025%. Comparatively were employed Biosept 33 SL (33% extract from grapefruit) at the concentration of 0.1%, Bioczos BR (garlic pulp) at the concentration of 1.0%, Polyversum (*Pythium oligandrum* – 1×10^6 spores of fungus/1 g of confection) at the concentration of 0.1% and Amistar 250 SC (BAS azoxystrobin 250 g/dcm^3) at the concentration of 0,025%.

Sterilized garden soil was put in multipallets. Prepared suspensions, at the concentration of 1 mln spores or parts of hyphae in l ml water, watered using pipette prepared soil in multipallets (l ml suspension/l g of soil) and were transfered into climatic chamber at the following conditions: constant temperature 25 °C and humidity 90% with 16 hours of daylights. After 24 hours into prepared soil were sowed seeds. Earlier, seeds were drenched in tested preparations: in chitosol during 72 hours, and the rest - during l hour. After sowing, seeds were watered of tested preparation suspensions. In this experiment were used 2 types of control: control with the soil infected by fungi and control with not infected soil, which were watering with tap water. Each combination contained 100 seeds in three replications. Efficacy was estimated on the percentage of germinated health seedlings after 5 weeks. The results of the experiments were subjected to analysis of variance. The statistical significance of differences between mean values was estimated by t-Duncan's test at 5% level of significance.

In the glasshouse experiment the soil-wheaten according method described by Bojarczuk and Bojarczuk (1988) was prepared. The experiment and infection factors were the same as in previous experiment on the estimation of efficiency of tested preparations used to control of herb plants conducted in climatic chamber. For pots, disinfected

with 70% solution of C_2H_5OH and filled sterilized garden soil, 20 ml of prepared soil medium overgrown suitable fungus species was added. Each combination contained 100 seeds in three replications. In this kind of pots filled infected garden soil, were sowed seeds of herb plants and was watered suspensions of suitable preparations (seeds were drenched of chitosol during 72 hours, and the rest - during l hour). In this experiment were used also two types of control: control with the infected soil and control with not infected soil, which were watered with tap water. The number of germinated seedlings was assessed after 6 weeks. The results were subjected to statistical analysis as in the previously described experiment.

3. Results and discussion

The results of experiments conducted in climatic chamber and glasshouse conditions pointed out, that the herb seedlings can be efficiently protected against pathogenic fungi using seed dressing before sowing and watering soil with preparation solution immediately after sowing. Efficiency of used preparations in the protection of herb plant seedlings in climatic chamber was higher than in glasshouse conditions. Probably this fact is connected with constant favourable conditions for growth of herb plants (Tables 1 - 4).

The protective activity of chitosol depended on the used concentration. On the ground received results founded, that the highest seed germination was in climatic chamber in the following combinations: Chitosol used at concentration of 0.025% with *Sclerotinia sclerotiorum* (isolate from lemon balm) and *Cladosporium cladosporioides* (isolate from peppermint) (Tables 1 and 3). However in the experiment conducted in glasshouse conditions we received the highest number of health seedlings also after using the lowest tested concentration of chitosol, which was used in combination with *Fusarium avenaceum* (isolates from mint and lemon balm) (Tables 2 and 4). Chitosol

Table 1. *The effect of used control on the number of seedlings healthiness (lemon balm – climatic chamber).*

Preparation	*Alternaria alternata*	*Fusarium avenaceum*	*Fusarium culmorum*	*Epicoccum purpurascens*	*Sclerotinia sclerotiorum*
Amistar 0,025 %	72 f	65 e	71 f	80 g	79 f
Biosept 0,1 %	56 d	56 c	68 e	68 e	69 e
Polyversum 0,1 %	21 b	28 b	89 h	43 b	92 g
Bioczos 1 %	45 c	29 b	38 b	61 c	56 b
Chitosol 0,025 %	85 h	73 f	81 g	87 h	91 g
Chitosol 0,05 %	79 g	62 d	60 d	72 f	64 d
Chitosol 0,1 %	64 e	56 c	49 c	65 d	59 c
Control with inoculation	4 a	3 a	6 a	9 a	5 a
Control without inoculation	100 i	100 g	100 i	100 i	100 h

Note: *means followed by the same letter do not differ at 5% level of significance (Duncan's multiple range test).*

Table 2. *The effect of used control on the number of seedling healthiness (lemon balm – glasshouse).*

Preparation	*Alternaria alternata*	*Fusarium avenaceum*	*Fusarium culmorum*	*Epicoccum purpurascens*	*Sclerotinia sclerotiorum*
Amistar 0,025 %	60 g	57 f	74 h	48 g	76 g
Biosept 0,1 %	56 f	36 d	57 f	23 e	54 f
Polyversum 0,1 %	12 e	25 c	63 g	42 f	51 e
Bioczos 1 %	10 cd	0 a	9 b	5 b	16 c
Chitosol 0,025 %	11 de	53 e	11 c	16 d	27 d
Chitosol 0,05 %	10 cd	37 d	14 d	9 c	11 b
Chitosol 0,1 %	9 bc	16 b	25 e	5 b	3 a
Control with inoculation	2 a	1 a	4 a	3 a	2 a
Control without inoculation	99 h	94 g	100 i	99 h	100 h

Note: *see table 1.*

Table 3. *The effect of used control on the number of seedling healthiness (peppermint – climatic chamber).*

Preparation	*Alternaria alternata*	*Botrytis cinerea*	*Cladosporium cladosporioides*	*Epicoccum purpurascens*	*Fusarium avenaceum*
Amistar 0,025 %	81 g	21 b	21 c	56 e	39 d
Biosept 0,1 %	50 d	79 g	41 e	54 d	34 c
Polyversum 0,1 %	17 b	42 c	14 b	9 b	24 b
Bioczos 1 %	47 c	45 d	39 d	43 c	39 d
Chitosol 0,025 %	74 f	85 h	92 h	89 h	87 g
Chitosol 0,05 %	58 e	70 f	80 g	70 g	72 f
Chitosol 0,1 %	51 d	62 e	75 f	65 f	60 e
Control with inoculation	1 a	1 a	3 a	0 a	2 a
Control without inoculation	100 h	100 i	100 i	100 i	100 h

Note: *see table 1.*

Table 4. *The effect of used control on the number of seedling healthiness (peppermint – glasshouse).*

Preparation	*Alternaria alternata*	*Botrytis cinerea*	*Cladosporium cladosporioides*	*Epicoccum purpurascens*	*Fusarium avenaceum*
Amistar 0,025 %	51 f	67 e	13 b	53 e	42 f
Biosept 0,1 %	39 e	75 f	39 d	38 d	22 c
Polyversum 0,1 %	13 b	43 d	13 b	7 b	8 b
Bioczos 1 %	35 d	42 d	40 d	35 c	43 f
Chitosol 0,025 %	38 e	43 d	45 e	35 c	52 g
Chitosol 0,05 %	25 c	31 c	22 c	7 b	34 e
Chitosol 0,1 %	12 b	12 b	8 a	1 a	25 d
Control with inoculation	3 a	2 a	9 a	6 b	2 a
Contol without inoculation	100 g	100 n	100 f	100 f	100 h

Note: *see table 1.*

in each combination didn't control absolutely disease development. On the ground realised investigations ascertained, that in relation to some fungi increasing of chitosol concentration caused decreasing its efficiency (Tables 1 - 4). This fact was confirmed by earlier investigation of Wojdyła et al. (1996), who on example *Peronospora sparsa* and *Sphaerotheca pannosa* var. rosae founded, that higher concentration of chitosan used to rose spraying decreased its efficiency. On the other hand different results were obtained by El-Ghaouth et al. (1992), who proved, that increasing concentration of chitosan inhibits growth and formation of spores *Rhizopus stolonifer* and *Botrytis cinerea*. For each scheme chitosan-fungus could exist different biochemic mechanisms and this fact caused need separate qualification of effect of chitosan on growth and development individual pathogens. In investigations conducted by Ohta et al. (2004) relating to qualification the effect of chitosan at concentration 1% mixed with soil on growth and florescence period: *Torenia fournieri, Exacum affine, Begonia hiemalis, Sinningia speciosa,, Lobelia erinus, Mimulus hybridus, Calceolaria herbeohybrida, Campanula fragilis* proved, significant effect on acceleration of seedlings growth and plants florescence, except *Calceolaria herbeohybrida* and *Campanula fragilis*. Authors pay attention (according their initial results of experiments), that chitosan can be active in soil as elicitor or also can be directly absorbed by plant roots and utilized. In experiments conducted by Chang et al. (1998) instead displayed, that tested chitosan was good elicitor of *Mentha piperita*, enlarged production of menthol, which protected plants against infections. Pięta et al. (2003) inform, that dressing bean seeds (cultivar Złota Saxa) chitosan at concentration of 0,1% caused obtainment the highest number of healthy seedlings. Investigations of Beausejour et al. (2003) proved that chitosan used against *Streptomyces scabies* protected potato bulbs in field conditions. Application of chitosan to water peat, significantly inhibited development of Fusarium wilts of pink and propagation of pathogen in containers (Orlikowski and Skrzypczak, 2003). Our earlier experiments conducted on isolates of *Fusarium avenaceum* and *Alternaria alternata* obtained from peppermint and lemon balm plants, confirmed also reduction of their linear growth by chitosol (Mazur et al. 2003). Specific mechanism of activity consists in blocking the first stage of infection and induction of Systemic Acquired Resistance (SAR) in plants. According investigations of Pospieszny (1997) the level of SAR induced by chitosan depends on plant species and kind of chitosan. Chitosan is one of the substations, which was numbered to group stimulators of plant of resistance. According many investigators, chitosan induced plant resistance and protects against viral, bacterial and fungal infections (Pospieszny et al. 1991, Pospieszny and Struszczyk 1994, Pospieszny 1997, Wojdyła and Orlikowski 1997, Pięta et al. 1998). Other authors showed that chitosan can find practical application in control of powdery mildew and downy mildew on rose, Fusarium rot of tulip bulbs and Phytophthora rot of Lawson cypress (Orlikowski et al. 1996, Wojdyła et al. 1996). Further experiments conducted by this authors on chitosan activity on *Fusarium oxysporum* f. sp. *dianthi* on pink and *Myrothecium roridum* on diffenbachia, confirmed its efficiency under glasshouse conditions. In case of grey mould on rose chitosan limited development of disease only in the first 3 days after inoculation (Wojdyła and Orlikowski 1997). Efficiency of chitosan can depend also on manner application. Benhamou et al. (1998) displayed,

that chitosan inhibited development of *Fusarium oxysporum* f. sp. *radicis lycopersici* on tomato and caused changes in host physiology. This kind of activity was observed when tomato was sprayed chitosan solution. However seed dresing delayed appearance of disease symptoms caused by this pathogen, but the control was not so much efficient. The influence of chitosan on the growth and development of *Alternaria alternata* f. sp. *lycopersici seems* to be very interesting. At higher concentration chitosan stimulated germination and growth of fungus, but on the other hand decreased vitality of conidial spores. Moreover, chitosan at lower concentration inhibited of mycotoxin production, and also created changes in structure of mycellium hyphae (Reddy et al. 1998). Similar, inhibitory influence of chitosan at higher concentration was observed in relation to *Aspergillus niger* and *A. parasiticius*. He prevented also aflatoxin production (Fang et al. 1994). Chitosan induced formation of phenolic compounds, which limited development and the growth of *Aspergillus flavus* and production aflatoxin Bl (Fajardo et al. 1995). It has also effect on limitation of ability to colonization of *Pythium aphanidermatum*. Moreover, create morphological changes mycelium hyphae, which don't cause phytotoxic changes of cucumber (El-Ghaouth et al. 1994). In *in vitro* conditions inhibited growth and development of *Botrytis cinerea*, *Sclerotinia sclerotiorum* and fungi from species: *Pythium, Rhizoctonia* and *Fusarium* (Pięta et al. 1998).

Concluding results of our work we can ascertain, that chitosol shows protection activity against pathogens. Advisable should be also continuation more detailed experiments in this domain, especially in field conditions.

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