SECTION 10: PLANT PROTECTION - PHYTOMEDICINE

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Original scientific paper

ISOLATE MX-95 OF APHANOCLADIUM ALBUM IN THE CONTROL OF COMBINED ATTACKS OF SOIL BORNE PLANT PATHOGENS AND ROOT-KNOT NEMATODES

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Abstract
Two experiments were carried out to verify the efficacy of *Aphanocladium album,* isolate MX-95, against *Pyrenochaeta lycopersici* and *Verticillium dahliae* in combination, each of them, with *Meloidogyne incognita*. Treatments were applied in each trial by the sub-irrigation system. In the first trial, on tomato, treatments were *A.album* isolate MX-95 applied before and after transplant at 5 l/plot ($1x10^7$ CFU/ml) and metham-Na (1,000 l c.p./ha). The second trial was carried out on eggplant and treatments were *A. album* isolate MX-95 at 7.2 l/plot (2×10^7 CFU/ml) applied 7 days before transplant and 15 days after transplant, azoxystrobin (20 l/ha) and fosthiazate (30 Kg/ha). In both trials an untreated control was considered. *A.album* isolate MX-95 reduced severity of symptoms of corky root on tomato roots and the indexes of wilting and vascular discoloration, caused by *V. dahliae* on eggplants in comparison to the untreated control. Also root gall index and soil nematode population density were significantly reduced in both trials.
Keywords: Biological control agent, corky root, Verticillium-wilt.

Introduction

Several studies on the interaction between soilborne plant pathogens and plant parasitic nematodes have been conducted (Ciccarese *et al*., 2001a; Lamberti *et al*., 2001; Gallo *et al*., 2011; Ciccarese *et al*., 2012). During the last decade, severe corky root and *Verticillium*-wilt symptoms, caused by *Pyrenochaeta lycopersici* Schneider *et* Gerlach and *Verticillium dahliae* Kleb., respectively, have frequently been found in association with *Meloidogyne* spp. attacks (Polizzi *et al*., 2004; Ciccarese *et al*., 2012). Generally, control of these pests was based on chemical treatments. The use of pesticides on agricultural crops recently has been deeply restricted and revised by the European Legislation that has focused the attention on human and animal health and enviromental safety. Plant protection from insects, fungi, bacteria, nematodes and other pests should rely on alternative control methods that are enviromentally sound and economically convenient at the same time. Antagonistic microrganisms may represent a potential alternative to the use of pesticides in agriculture as biological control of plant disease and plant parasitic nematodes. Recently an isolate of the fungus *Aphanocladium album* (Preuss) W. Gams, signed as MX-95, has shown appreciable effects in the biological control of powdery mildew, on tomato, squash and cucumber caused by

Oidium lycopersici L. Kiss and *Sphaerotheca fusca* Blumer, respectively (Ciccarese *et al*., 2001b). *A. album* is able to produce hydrolytic enzymes especially chitinase (Kunze *et al*., 1992) responsible of a total or partial degradation of cell walls of numerous plant pests (Ciccarese *et al*., 2003).
Therefore, to verify the possibility of the use of the chitinolytic activity of *A. album* isolate MX-95 also in the soil-borne plant pathogens and plant parasitic nematode biological control, two experiments were carried out on tomato, in a plastic house infested by *P. lycopersici* and *M. incognita*, and on eggplant in an open field infested by the same root-knot nematode and by *V. dahliae*.

Material and methods

First trial

A plastic house at Leverano (province of Lecce, Apulia Region, southern Italy) with a soil heavily infested by *P. lycopersici* and *M. incognita* was selected. The soil was deeply ploughed, rotaveted and subdivided in 96 m^2 plots, spaced 1 m apart, and distributed according to a randomized block design with four replications per treatment. To allow the different treatments a sub-irrigation system (depth 0.2 m) was performed in each plot by PVC drip lines (Ø 1.6 cm) equipped with water emitters (flow rate 4 l/h) every 0.3 m.
Treatments were: a) isolate MX-95 of *A. album* (5 l/plot as a conidial suspension at 1 x 10^7 CFU/ml) applied two times; b) metham-Na (1,000 l p.c./ha) and c) an untreated control. In each plot, one-month old tomato seedlings (cv. Luisa) were transplanted in 3 coupled rows spaced 1 m each other and with 0.8 m between the rows of the same coupled row. Plants were spaced 0.3 m along the row. *A. album* and metham-Na were applied one month before transplant.
A. album treatment, at the same concentration, was repeated one month after transplant. The fungus was reared *in vitro* on PDA Petri dishes incubated in dark conditions at 24 °C for 1 week. To allow the dispersion of hygroscopic conidia, the mycelium was homogenized in sterile water with a tensioactive. The concentration of the inoculum was determined and diluted to obtain 1 x 10^7 CFU/ml standard conidial suspension. The conidial suspension was applied at the rate of 5 l/plot. To avaid the block of the irrigation system a filter was set at the beginning of the sub irrigation system. During the growing season plants received the usual cultural practices. Tomatoes were harvested six times and the total marketable yield calculated. In each plot, plants from the central coupled row were uprooted to estimate the severity of corky root on main and secondary roots and root gall index (RGI). Severity of corky root on main and secondary roots was estimated according to a 0-5 scale (0 = root healthy; 1 = 1-10% affected root surface (a.r.s.); 2 = 11-25% a.r.s.; 3 = 26-50% a.r.s.; 4 = 51-75% a.r.s. and 5 = >76 % a.r.s.). RGI was estimated also according to a 0-5 scale (0 = no galls and 5 root system completely deformed by large and numerous galls) (Lamberti, 1971). Nematodes were extracted from soil samples of each plot processing 500 ml soil sub-sample with the Coolen's method (Coolen, 1979).

Second trial

The second trial was carried out at Valenzano (province of Bari, Apulia region, southern Italy) in open field condition on eggplant (cv. Dalia). The soil was naturally infested by the soil-borne plant pathogen *V. dahliae* and the root-knot nematode *M. incognita*. Treatments were: a) *A. album* isolate MX-95; b) the nematicide fosthiazate (30 Kg/ha) and the fungicide Azoxystrobin (20l/ha) both applied at transplant and c) an untreated control. *A. album* was applied 1 week before transplant and

15 after transplant at the rate of 0.4 l/plant (2 x 10^7 CFU/ml). At the end of the crop cycle Verticillium-wilt index, vascular discolaration and root gall index (RGI) caused by the nematode attack were recorded. Wilting index was assessed according to a 0 – 5 scale (0 = health plant; 1 = yellowing of basal leaves; 2 = diffuse yellowing; 3 = wilting; 4 = diffuse wilting and 5 = dead plant. Stem of each plant was transversely cut 3 cm above the soil level and the severity of vascular discoloration (% area affected) was recorded according to a 0-4 scale (0 = no vascular discoloration; 1 = 1-10% vascular discoloration (vd); 2 = 11-50% vd; 3 = 51-75% vd; 4 = >75% vd). Nematode attack on the eggplant roots was evaluated according to a 0-5 scale (0= no galls and 5 = root completely deformed by the presence of numerous large galls. Nematodes were extracted from soil samples of each plot processing 500 ml soil sub-sample with the Coolen's method (Coolen, 1979).
Data from both trials were subjected to analysis of variance and means compared Duncan's Multiple Range Test using the software Plot It vers. 3.02.

Results and discussion

First trial

Tomato marketable yield was significantly increased by Methan-Na treatment in comparison to untreated control. No statistical difference was observed between *A. album* isolate MX-95 treatment and the other thesis (metham-Na and untreated control) (Fig. 1). In addition, methan-Na treatment significantly decreased severity of corky root either on main and secondary roots, RGI and final nematode population density because of the development of methylisothiocyanate (Figs. 2, 3 and 4). The nematode soil population density and root gall index were also significantly suppressed by *A. album* isolate MX-95 (P=0,05) (Fig. 4).
Number and application time of the chitinolytic fungus can be the cause of the reduction of RGI. Infact, *A.album* isolate MX-95 was applied two times: 1 month before transplanting and 1 month later. So in this way the fungus had the possibility to develop its maximum enzymatic chitinolytic activity protecting the seedlings from nematode attack at transplant. The second application, during the crop cycle, served as post transplant protection. The severity of corky root on secondary roots was not reduced by *A. album* treatment in comparison to untreated control, whereas it was effective on main root with a significant reduction of symptoms (Fig. 2).
The experiments revealed significant and positive correlations between root gall index and the severity of corky root on main ($y = 0.42 + 0.34\ x$; $r^2 = 0.975$) or secondary root ($y = 3.15 - 0.36/x^2$; $r^2 = 0.893$). Therefore, it seems that an increase of the nematode attack increases also the severity of the corky root. Probably the trofic activity of the second juvenile stage of *Meloidogyne* on tomato roots through the mechanical action of the stylet favours *P. lycopersici* infection and penetration.
Metham-Na should be recommended for the control of simultaneous attaks of *P. lycopersici* and *M. incognita*. For this chemical and also for biological control agent application through the sub irrigation technique would results more beneficial than with any other methods of distribution. Infact, in this way it is possible to reduce production costs because of the repeated use of the irrigation system in several cultural practices (irrigation, fertilization and crop protection).

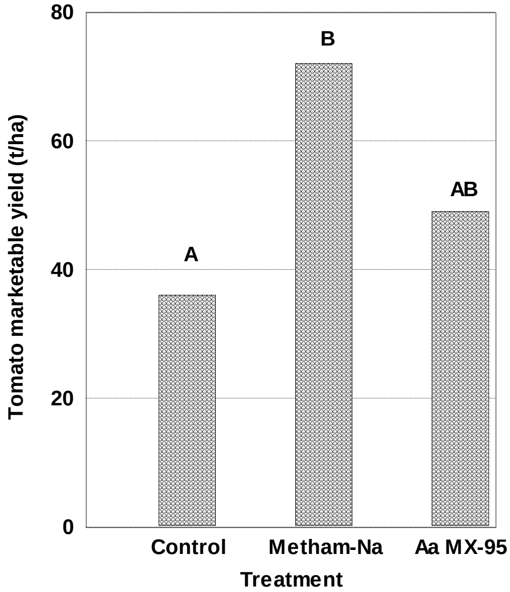

Figure 1. Effect of the chitinolytic fungus *A. album* isolate MX-95 and methan-Na treatments on tomato marketable yield (cv. Luisa) in a plastic house infested by *Pyrenochaeta lycopersici* and *Meloidogyne incognita*.
Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.01)

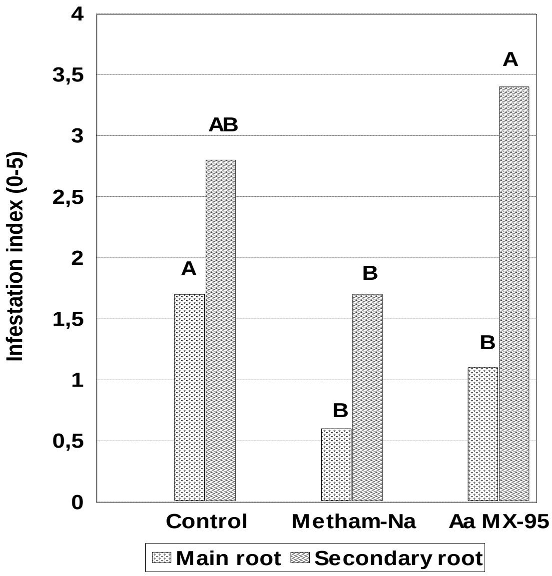

Figure 2. Effect of the chitinolytic fungus *A. album* isolate MX-95 and methan-Na treatments on symptoms of *Pyrenochaeta lycopersici* attack on tomato roots (cv. Luisa) in a plastic-house. For each parameter (main or secondary root) data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (P=0.01)

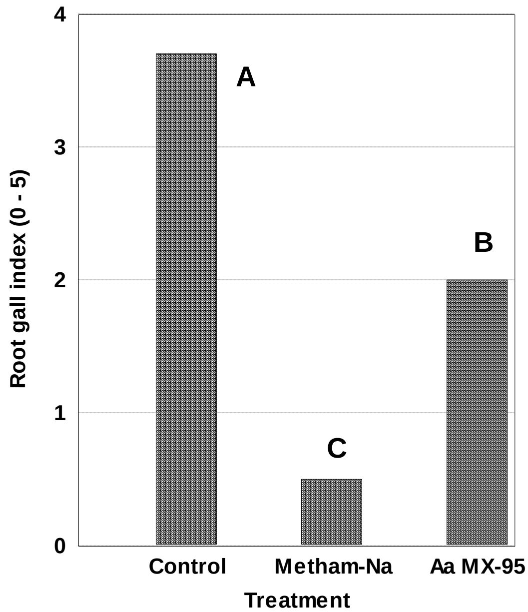

Figure 3. Effect of the chitinolytic fungus *A. album* isolate MX-95 and methan-Na treatments on root gall index caused by *Meloidogyne incognita* on tomato roots (cv. Luisa) in a plastic-house.
Data flanked in each column by the same letter are not statistically different according to Duncan's Multiple Range Test (P=0.01)

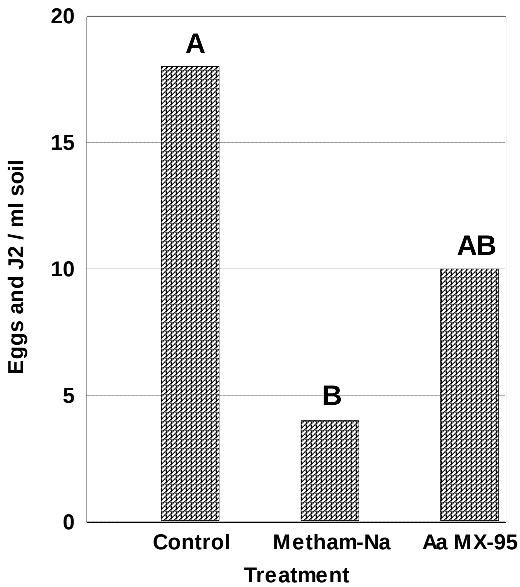

Figure 4. Effect of the chitinolytic fungus *A. album* isolate MX-95 and methan-Na treatments on final *Meloidogyne incognita* population density (*Pf*). Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.01)

Second trial

Treatments with the fungicide (azoxystrobin) and nematicide (fosthiazate) and with the isolate MX-95 of *A. album* significantly increased eggplant yield in comparison to the untreated control. No statistical difference was observed between the chemical treatment and the use of the biological control agent (Fig. 5).

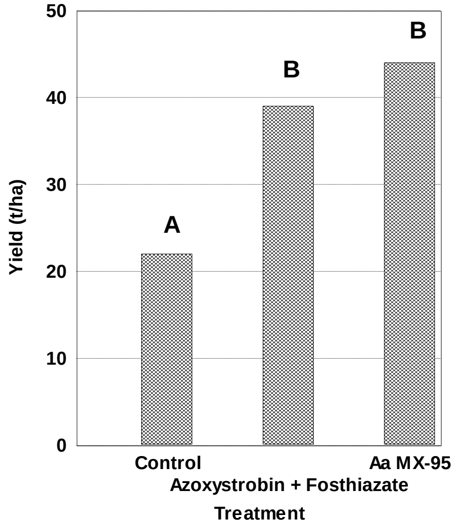

Figure 5. Effect of *A. album* isolate MX-95 and azoxystrobin and fosthiazate treatments on yield of eggplant (cv. Dalia) affected by *Verticillium dahliae* and *Meloidogyne incognita*.

Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.01)

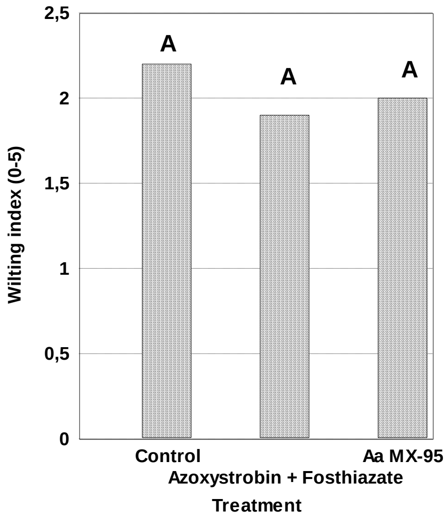

Figure 6. Effect of *A. album* isolate MX-95 and azoxystrobin and fosthiazate treatments on wilting index of eggplant (cv. Dalia) affected by a combined attack of *Verticillium dahliae* and *Meloidogyne incognita*. Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.01)

At the end of the crop cycle no statistical differences were observed in the wilting index between the untreated and treated plants (Fig. 6). The index of vascular discoloration was significantly reduced in plant treated with chemicals and with isolate MX-95 of *A. album* in comparison to untreated control (Fig. 7).

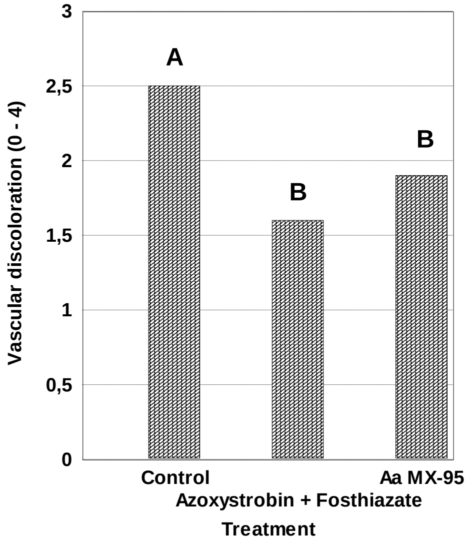

Figure 7. Effect of *A. album* isolate MX-95 and azoxystrobin and fosthiazate treatments on vascular discoloration of eggplant (cv. Dalia) affected by a combined attack of *Verticillium dahliae* and *Meloidogyne incognita*. Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.05)

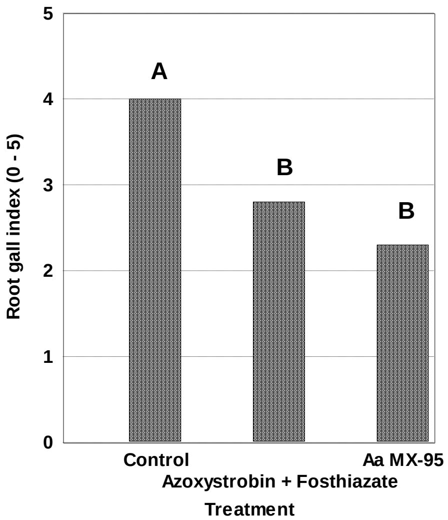

Figure 8. Effect of *A. album* isolate MX-95 and azoxystrobin and fosthiazate treatments on root gall index of eggplant (cv. Dalia) affected by a combined attack of *Verticillium dahliae* and *Meloidogyne incognita*.

Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.05)

RGI observed on the roots of untreated plants was significantly higher than that observed on *A. album* MX-95 and Fosthiazate and Azoxystrobin treated plants (Fig. 8).

Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (P=0.05)

From results of the two experiments the repeated use of the chitinolytic fungus *A. album*, isolate MX-95, seems to be reasonably possible for soil borne plant pathogens and nematode biological control.

The main aim of *A. album* isolate MX-95 use should be a progressive reduction of infestation level under the tolerance limit of the target nematode species also reducing soil borne pathogens attacks (Sasanelli, 1994).

Acknowledgement

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КОНТРОЛА НА ПОЧВЕНИТЕ ПАРАЗИТИ И КОРЕНОВИТЕ НЕМАТОДИ СО ПРИМЕНА НА ИЗОЛАТОТ MX-95 ОД *APHANOCLADIUM ALBUM*

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Апстракт

Двата експеримента се извршени да се потврди ефикасноста на *Aphanocladium album,* изолиран MX-95, против *Pyrenochaeta lycopersici* и *Verticillium dahlia* во комбинација, секој од нив со *Meloidogyne incognita*. Третманите беа применувани во секој опит со под-системот за наводнување. Во првиот експеримент на домати, третирани беа *A.album* изолиран MX-95 аплициран пред и после расадување на 5 l/ површина (1x10^7 CFU/ml) и metham-Na (1,000 l c.p./ha). Вториот експеримент беше спроведен на модриот патлиџан и третманите беа *A. album* изолиран MX-95 на 7.2 l/површина (2 x 10^7 CFU/ml) аплициран 7 дена пред расадување и 15 дена после расадувањето, azoxystrobin (20 l/ha) и fosthiazate (30 Kg/ha). Во двете испитувања нетретирана котрола се разгледуваа. *A.album* изолиран MX-95 ја намалува сериозноста на симптомите на испакнат корен на доматот и индексот на овенување и васкуларната промена на бојата, предизвикана од *V. dahliae* на модриот патлиџан и во споредба со нетретираната контрола. Исто така индексот на галите и густината на почвените нематоди беа значително намалени во двете испитувања.

Клучни зборови: биолошки контролен агенс, плутест корен, Verticillium-wilt.

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Original scientific paper

INTENSITY OF M. GRAMINICOLA ATTACK ON VARIOUS WHEAT CULTIVARS GROWN AT PSEUDOGLEY

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Abstract
This paper gives the results concerning resistance of fifteen winter wheat cultivars to attack of *Mycosphaerella graminicola*, the causal agent of septoria leaf blotch. Climate of Serbia is very favorable for appearing and development of this parasite, so it is considered as one of very harmful pathogens. The investigation has been carried out during 2011/12, in the conditions of natural infection, at the experimental field of Agricultural School in Kraljevo. The trial was set at pseudogley soil type. Experimental plots were 50 m long and 2 m wide. Attack intensity was assessed during the first decade of June, according to the scale of Geshele (1978), by determining infection severity from 0-100%. Majority of the studied cultivars (Ljiljana, Rusija, Biljana, Etida, Gordana, Zvezdana and Arija) showed attack intensity of 30%, which was 46.6% of the total cultivars number. Two of the observed cultivars (13.3%) had 10% of leaf area under septoria blotches. Rapsodija was the most susceptible cultivar with infection intensity of 50%.
Key words: *M. graminicola*, wheat, cultivars, susceptibility, pseudogley.

Introduction
Wheat (*Triticum aestivum* L) is the most important field crop, grown on a large part of arable land throughout the world. Grain yield of wheat is affected by many factors, and among them are parasites, causal agents of various plant diseases, so it is necessary to observe their presence and intensity every year. Septoria leaf blotch (*Mycosphaerella graminicola*) is a disease which can appear during whole vegetation. Under suitable conditions, primary infection happens early, and symptoms of the disease can be seen after emergence of second wheat leaf. The attack causes necrosis and wilting of affected plant tissue, which is often attached to soil surface (Telečki and Jevtić, 2009). This parasite is nowadays regarded as the most influential one in global wheat production, causing main wheat disease in many regions throughout the world (Eyal et al., 1985). Greater damage appears in favorable weather conditions, when the disease can be spread from lower to upper leaves. Wilting of heavily attacked leaves reduces assimilation surface, decreasing grain yield and quality. Wiese (1987) stated that the disease is registered in about 50 countries. Leroux et al. (2007) and Stammler et al. (2008) pointed out to *M. graminicola* as one of the most dangerous parasites in Northern Europe, and Garcia et al. (1992) stated the same for cereal belt of USA. Grain yield loss caused by *M. graminicola* in Western Europe usually is between 30 and 50% (Royle et al., 1986).

Climate of Serbia is very favorable for appearing and development of this parasite, so it is considered as one of very harmful pathogens. Presence of this disease in many locations of Serbia has been reported by Arsenijević (1965), Kostić and Smiljaković (1966), Ivanović et al. (2001). Gudžić et al. (2012) observed that in 2011, on pseudogley soil type, the average infection intensity in ten cultivars amounted 27%. Kalentić et al. (2006) found that in 2006 intensity of septoria leaf blotch attack was higher in winter genotypes, in regard to the spring ones. Proper choice of suitable cultivars plays an important role within integrated plant protection measures. Consequently, this study has been aimed to establish intensity of the pathogen's attack on some winter wheat cultivars grown on pseudogley soil type.

Material and methods

The investigation has been carried out during 2011/12, in the conditions of natural infection, at the experimental field of Agricultural School in Kraljevo. Local coordinates of the location were the following: latitude 43º 42', longitude 20° 42', altitude 215 m. The trial was set at pseudogley soil type. Dugalić (1998) reported this soil as having very bad physical properties and extremely acid soil reaction (pH<4.5). It had low content of humus (about 2.18%) and available phosphorus (7.0-8.0 mg 100^{-1} g of soil). Supplies of available potassium were moderate (13.0-18.0 mg 100^{-1} g of soil), and the amount of total nitrogen was satisfying. The following cultivars were studied: Renesansa, NSR-5, Natalija, Ljiljana, Simonida, Rapsodija, Dragana, Rusija, Biljana, Pesma, Etida, Gordana, Pobeda, Zvezdana and Arija. Sowing was done by seeding machine. Experimental plots were 50 m long and 2 m wide. Fertilization was done by NPK fertilizer 15:15:15 in the dose of 281 kg ha^{-1}. Additional nitrogen fertilization was applied in the stage of tillering with 242 kg ha^{-1} of CAN. The rest of applied production technology was standard.

Intensity of attack by the fungus *Mycosphaerella graminicola* was observed during the first decade of June 2012. Attack intensity was assessed according to the scale of Geshele (1978), by determining infection severity from 0-100%.

Results and discussion

Results of the study have shown the average attack intensity in 2012 was 30.6 % (table 1). Cultivars Natalija and Simonida had 10% of leaf area covered by blotches of the parasite *M. graminicola*. Attack intensity in the cultivar Renesansa was 20%. The most of cultivars (Ljiljana, Rusija, Biljana, Etida, Gordana, Zvezdana and Arija) suffered attack intensity of 30%, and 46.6% of the all studied cultivars belonged to this group. Attack intensity in the cultivars NS rana-5, Dragana, Pesma and Pobeda was 40%, with 18.3% of the cultivars in this group. Rapsodija was the most susceptible cultivar with infection intensity of 50%.

M. graminicola is a pathogen which represents a problem for wheat production. In addition to favorable weather conditions, growing susceptible cultivars and large amount of inoculum from previous vegetations also have a positive effect on this pathogen's development (Kalentić et al., 2006). Breeding and growing resistant cultivars is very important for wheat protection from the causal agent of septoria leaf blotch. Palmer and Skinner (2002) stated that commercial cultivars did not express adequate resistance to this parasite. Jerković et al. (2005) found differences among cultivars regarding resistance to the most dangerous causal agents.

Analysis of the obtained results revealed the highest resistance shown by cultivars Natalija and Simonida, which also had a series of desirable traits. During the observed year, cultivar Renesansa expressed a satisfying resistance to *M. graminicola*. Such behavior of the cultivar has been reported by Gudžić et al. (2011), which is in accordance with the results of our investigation.

Table 1. Intensity of *Mycosphaerella graminicola* attack to wheat cultivars

No	Cultivar	Intensity
1.	Renesansa	20
2.	NS rana 5	40
3.	Natalija	10
4.	Ljiljana	30
5.	Simonida	10
6.	Rapsodija	50
7.	Dragana	40
8.	Rusija	30
9.	Biljana	30
10.	Pesma	40
11.	Etida	30
12.	Gordana	30
13.	Pobeda	40
14.	Zvezdana	30
15.	Aria	30
Average		30.6

Conclusions

On the basis of previously published studies and the presented results, the following conclusion can be drawn out:

Mycosphaerella graminicola is one of common and harmful parasites of wheat in Serbia.

This investigation results showed that the average infection intensity was 30.6%.

Good resistance to *M. graminicola* was shown by the cultivars Natalija and Simonida. Rapsodija was the most susceptible cultivar, with infection intensity of 50%. Breeding and growing resistant cultivars is very important for wheat protection from the causal agent of septoria leaf blotch. In addition to growing resistant cultivars, a special attention ought to be paid to agrotechnical and chemical measures.

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ИНТЕНЗИТЕТ НА НАПАД ОД *M. GRAMINICOLA* КАЈ РАЗЛИЧНИ СОРТИ ПЧЕНИЦА ОДГЛЕДУВАНА НА ПСЕВДОГЛЕЈ

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Апстракт

Овој труд ги дава резултатите во врска со отпорноста на петнаесет зимски сорти на пченица нападнати од *Mycosphaerella graminicola*, како последица од септориозна лисна дамкавост. Климата во Србија е многу поволна за појавување и развој на овој паразит, така што се сметаат за едни од многу штетни патогени. Испитувањата се спроведуваа во текот на 2011/12, во услови на природна инфекција, на експерименталната област на Земјоделското училиште во Краљево. Експериментот беше поставен на псеудоглејна тип на почва. Експерименталните парцели беа 50 метри во должина и 2 метри широчина. Интензитетот на нападот беше оценет во првата декада на Јуни, според скалата на Geshele (1978), детерминирање на инфекција со интензитед од 0 – 100%. Мнозинството од студираните култури (Ljiljana, Rusija, Biljana, Etida, Gordana, Zvezdana и Arija) покажаа надад со интензитет од 30%, кој беше 46,6% од вкупниот број на сорти. Две од набљудуваните сорти (13.3%) имаа 10% на лисната површина од септориозна лисна дамкавост. Rapsodija беше многу подложна сорта на инфекција со интензитет од 50%.

Клучни зборови: *M. graminicola*, пченица, сорти, осетливост, псевдоглеј.

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ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS TO PLANT PATHOGENIC FUNGI *ALTERNARIA SOLANI* SORAUER AND *FUSARIUM GRAMINEARUM* SCHWABE

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Abstract
Eleven essential oils were assayed for inhibitory and lethal activity against *Alternaria solani* Sorauer and *Fusarium graminearum* Schwabe. Fungal pathogens were isolated from diseased plants in Serbia. Early blight of tomato, caused by *A. solani* is an important and widely distributed disease, and *F. graminearum* is the causal agent of Fusarium head blight of small grain cereals in fields worldwide. Essential oils were provided by the „Dr Josif Pančić" Institute for Medical Plant Research in Belgrade, Serbia. Isolates of pathogenic fungi were exposed to the volatile phase of the oils, and then ventilated in order to determine if the effect of the oils was lethal to the pathogens. Five essential oils inhibited growth of *A. solani*: anise (*Illicum verum* Hooker fil.), peppermint (*Mentha piperita* L.), wintergreen (*Gaultheria procumbens* L.), lemongrass (*Cymbopogon flexuosus* L. Stapf) and origanum (*Origanum vulgare* L.). These oils were also lethal to the tomato pathogen, except the wintergreen having only suppresive effect. Pathogen *F. graminearum* was susceptible to four oils: anise, wintergreen, lemongrass and origanum, and they all exhibited fungicidal effect to the fungus. The strongest antifungal activity of natural compounds tested was shown by origanum, followed by lemongrass oil. Origanum oil was lethal to both pathogens at concentrations of 0.02 µl ml^{-1} of air. Lemongrass oil showed fungicidal effect to *A. solani* at 0.02 µl ml^{-1} and to *F. graminearum* at 0.04 µl ml^{-1} of air. Essential oils of rosemary (*Rosemarinus officinalis* L.), eucaliptus (*Eucalyptus globulus* Labill.), pineneedle (*Pinus syilvestris* L.), turpentine (*Pinus pinaster* Ait.) and lavander (*Lavendula angustifolia* Mill.) did not show significant antifungal effects to investigated pathogens.
Key words: essential oils, antifungal activity, *Alternaria solani*, *Fusarium graminearum*.

Introduction
Alternaria solani Sorauer causes two types of disease on tomato plants: early blight and collar rot. Early blight, which defoliates mature plants and contributers to major economic losses for growers, is considered a more serious disease than collar rot. Collar rot is primarily a seedborn disease carried to the field on tomato transplants. Collar rot symptoms are dark, sunken stem lesions near the soil line (Moore and Thomas, 1943). Early blight lesions on tomato leaves are typically surrounded by chlorotic regions that are less extensive in nonvascular tissue than in vascular tissue, indicating that *A. solani* toxins are translocated systemically and may be responsible for the lesions observed (Pound and Stahmann, 1951). Stepanović *et al*. (2007) tested sensitivity of *A. solani*

isolated from Serbia to fungicide formulations, finding that pyraclostrobin, prochloraz, difenoconalzol, iprodione and famoxadone exhibited the highest toxicity, mancozeb and chlorothalonil lower and copper oxychloride the poorest.

Fusarium head blight, or scab, can be a devastating disease on all classes of wheat and barley. Among the species of Fusarium causing scab, *Fusarium graminearum* Schwabe is the predomenimant pathogen. Wheat and barley are highly susceptible to infection when the crop is in flowering to soft dough stages and when weather includes frequent precipitation and high humidity. Multiple symptoms occur: reduced yields discolored and shriveled kernels, depressed seed weights, and reduced seed quality and vigor. The mycotoxin deoxynivelanol (DON, commonly known as vomitoxin) also can be produced by *F. graminearum* on infected grain. Grain contaminated with DON is often unsuitable for flour, cereals, or malt and is also toxic as feed for nonruminant animals (McMullen *et al*., 1997). In recent years, Fusarium head blight has reemerged worldwide as a disease of economic importance. In Serbia, fusarium head blight has been a growing problem over the past three decades. The highest frequency and intensity of up to 67.2% was detected in 2005 for *F. graminearum*, followed by *F. poae* (up to 20.4%) (Lević *et al*., 2008). The fungicides widely used in Serbia for controlling fusarium head blight, leaf rust, septoria leaf blotch, and powdery mildew are: mancozeb, carbendazim, carbendazim + cyproconazole, captan, chlorothalonil, epoxyconazole + thiophanate-methyl, carbendazim + flusilazole, trifloxystrobin + cyproconazole, tebuconazole + triadimenol + spiroxamine, prochloraz + tebuconazole, prochloraz + propiconazole (Savčić-Petrić and Sekulić, 2007; Balaž *et al*., 2008). Various plant essential oils and their components have been tested against pathogenic fungy on cultivated plants. They have demonstrated strong antifungal effects. It is apparent that essential oils should be considered as alternative preharvest natural fungicides. Natural plant-derived fungicides should provide a wide variety of compounds as alternatives to synthetic fungicides, safe for both human health and the environment (Daferera *et al*., 2003). There are few reports about strong activity of essential oils to *A. solani* (Zygadlo *et al*. 1994), Zygadlo and Grow (1995), Özcan *et al*. (2000), Abou-Jawdan *et al*. (2002) Cakir *et al*. (2004) (Mishra *et al*., 2009) and to *F. graminearum* (Agnioni *et al*., 2004; Velluti *et al*. 2004a; 2004b; Singh *et al*., 2005).

Material and methods

Test organisms

Isolates of *Fusarium graminearum* ZE and *Alternaria solani* SV-2 representing isolates identified 2005 and 2006, respectively, during the survey in farms in Serbia, were chosen for this study.

Test substances

Twenty two essential oils of plants were provided by the Dr. Josif Pančić Institute for Medical Plant Research in Belgrade, Serbia. including: *Rosemarinus officinalis* PO124476 (Rosemary Oil Ph. Eur. 50); *Illicum verum* S0100154 (Anise Oil Ph. Eur. 50); *Mentha piperita* P0123884 (Peppermint Oil Ph. Eur. 50); *Eucalyptus globulus* S0100321 (80/85% Eucalyptus Oil Ph. Eur. 50); *Gaultheria procumbens* P0117394 (HAB 2005 Winergreen Oil Ph. Eur. 50); *Pinus silvestris* P0124319 (Pineneedle Oil Ph. Eur. 50); *Citrus limon* P0119551 (Lemon Oil Ph. Eur. 50); *Cymbopogon flexuosus* P0120241 Lemongrass Oil Ph. Eur. 50); *Origanum vulgare* P0125062 (Origanum Oil Ph. Eur. 50); *Pinus pinaster* P0125332 (Turpentine Oil Ph. Eur. 50); *Lavendula angustifolia* P0123527 (Lavander Oil Ph. Eur. 50); *Origanum vulgare* P0125412 (Origanum Oil nat. id.); *Rosmarinus* *officinalis*, Xherdo Co., Albania; *Lavandula angustifolia*, Agroherbal, Albania; *Rosmarinus officinallis* 1, Agroherbal, Ablania; *Rosmarinus officinalis* No-41, Agroherbal, Albania; Pinus pinaster 2, Agroherbal, Albania; *Pinus nigra*, Agroherbal, Albania; *Mentha piperita* No 41/A, Agroherbal, Albania; *Salvia officinalis* Sage, Xherdo Co., Albania; *Abies alba* (Aetheroleoum abietis albie), Xherdo Co., Albania; *Juniperus oxycedrus* (Aethoeroleoum juniperi oxycedri), Xherdo Co., Albania.

Inoculum preparation

The fungal pathogens *F. graminearum* and *A. solani* were prepared as a conidial suspension (approximately 10^6 conidia ml^{-1}). The isolates were initially grown for 14 days on PDA plates. Conidia were harvested by flooding the plates with 10 ml of sterile distilled water and Tween 20 (v/v 0.01%) followed by filtration through double layer of cheesecloth.

Toxicity of the essential oils to fungal pathogens in vitro

Antifungal activity was tested on PDA medium in glass Petri plates (R=90 mm) inoculated with the investigated strains by pipetting 20 µl of the conidial suspension into the well cut at the centre of the plate (R=10 mm). The isolate was exposed to the volatile phase of essential oils for seven days at 20^{o}C. The oils were applied as a drop onto the inner side of the plate covers at concentrations of 0.02, 0.04, 0.08, 0.16 and 0.32 µl m^{-1} of the air inside the Petri plates by using a micropipette. Bottom of the plates were immediately placed on the covers. The plates were left up-side-down and sealed by parafilm to prevent gas exchange with the outside environment. Inhibition of the mycelial growth was estimated four days after the treatment by measuring the radial growth of the isolate treated with different concentrations of the oils and compared to the control. Seven days after the treatment, the plates were observed for the initial mycelial growth without measuring. Concentrations of an oil which completely inhibited the mycelial growth after seven-day-exposure at 20ºC were considered to be fungistatic and the lowest of these concentrations was determined as Minimum Inhibitory Concentration (MIC). Afterwards, the plates were opened and ventilated in a laminar flow hood for 30 min in order to remove volatiles and determine fungicidal effect. The concentrations of the oil were considered as fungicidal if the microbial growth had not been observed seven days after the ventilation. The lowest concentration with fungicidal effect was defined as Minimum Fungicidal Concentration (MFC). Four replicates per treatment were used and the experiment was repeated twice.

Results and discussion

The growth of the isolates was surveyed using 22 essential oils applied in the range of 0.02-0.32 µl ml^{-1}. Thirteen oils did not show either inhibitory or lethal effect to two fungal isolates at tested concentrations (Table 1).

A growth inhibition of tested pathogens after two days was achieved by five oils: *Illicum verum* S0100154, *Gaultheria procumbens* P0117394, *Cymbopogon flexuosus* P0120241, *Origanum vulgare* P0125062 and *O. vulgare* P0125412. Species *A. solani* was more sensitive than *F. graminearum*. Its growth was fully inhibited by nine essential oils, two oils at 0.02, one at 0.08, three at 0.16 and three at 0.32 µl ml^{-1}. Five oils completely inhibited growth of *F. graminearum*: two oils at 0.02, one at 0.08, two at 0.16 and none at 0.32 µl ml^{-1} air after two-day exposure. Essential oils *Cymbopogon flexuosus* P0120241 and *Origanum vulgare* P0125062 exhibited the highest inhibitory effect to both isolates tested, having MIC values of 0.02 µl ml^{-1}. High toxicity was also shown by *Illicum verum* S0100154, *Gaultheria procumbens* P0117394 and *Origanum vulgare* P0125412 with MIC of 0.08 or 0.16 µl ml^{-1} varied depending on the pathogen. Oils *Rosmarinus officinallis* 1, Agroherbal, Ablania; *Rosmarinus officinalis* No-41, Agroherbal, Albania; and *Salvia officinalis*, Xherdo Co., Albania, inhibited *A. solani* at 0.32 µl ml^{-1} air, and without any effect on *F. graminearum* below 0.32 µg ml^{-1}. The most toxic was oil *Origanum vulgare* P0125062 with MBC value of 0.02 to both pathogens, and *Cymbopogon flexuosus* P0120241 with 0.02 to *A. solani* and 0.04 µg ml^{-1} to *F. graminearum*. High toxicity was also obtained by *Origanum vulgare* P0125412, having MBC of 0.08 to *F. graminearum* and 0.16 µg ml^{-1} to *A. solani*. Following toxicity was recorded by *Mentha piperita* P0123884 with MBC of 0.16 µg ml^{-1} to *A. solani*, *Gaultheria procumbens* P0117394 with MBC of 0.32 µg ml^{-1} to *F. graminearum*. The oil *Illicum verum* S0100154 also was highly toxic with MBC of 0.32 µg ml^{-1} to both pathogens.

Results obtained four days after oil application confirmed that the oils varied in their fungicidal effect to *A. solani* and *F. graminearum*. The highest lethal effect was shown by *Cymbopogon flexuosus* P0120241 and *Origanum vulgare* P0125062 to *A. solani*, having MBC value of 0.02 µg ml^{-1}, followed by *Mentha piperita* P0123884 and *Origanum vulgare* P0125412 with MBC of 0.16 µg ml^{-1} and *Illicum verum* S0100154 with MBC of 0.32 µg ml^{-1}. Lethal oils to *F. graminearum* were *Origanum vulgare* P0125062 (MBC=0.02 µg ml^{-1}), *Cymbopogon flexuosus* P0120241 (MBC=0.04 µg ml^{-1}), and *Illicum verum* S0100154 and *Gautheria procumbens* P0117394 (MBC=0.32 µg ml^{-1}). The results indicated that nine oils showed inhibitory effect to *A. solani* and five to *F. graminearum*. Different five oils were lethal to the both pathogens, two of them at 0.02 µg ml^{-1} to *A. solani* and one to *F. graminearum*. *F. graminearum* was less sensitive than *A. solani*. Seventeen oils did not exhibit neither inhibition nor fungicidal activity to *F. graminearum*, and 13 oils were without activity against the *A. solani*.

Among the 22 essential oils analyzed, those of *Illicum verum* S0100154, *Mentha piperita* P0123884, *Gaultheria procumbens* P0117394, *Cymbopogon flexuosus* P0120241, *Origanum vulgare* P0125062 and *O. vulgare* P0125412 expressed the strongest antifungal activity to both of the investigated pathogenic fungi. Only four oils, *Illicum verum* S0100154, *Gaultheria procumbens* P0117394, *Cymbopogon flexuosus* P0120241, *Origanum vulgare* P0125062 and *O. vulgare* P0125412 exhibited fungicidal effect to both isolates tested. The strongest inhibitory and lethal effect to *A. solani* and *F. graminearum* was displayed by *Cymbopogon flexuosus* P0120241 and *Origanum vulgare* P0125062. Oils *Origanum vulgare* P0125062 and *Cymbopogon flexuosus* P0120241 were the most toxic to both isolates.

Antifungal effect of oregano, peppermint and sage oils, from other plant species than used in this study were confirmed in reports of Abou-Jawdan *et al*. (2002), with wild marjoram (*Origanum syriacum*), peppermint (*Mentha longifolia*) and sage (*Salvia fruticosa*), having high activity against *A. solani*. Özcan *et al*. (2000) showed that sage had weak activity, whilst wild thyme and oregano were active against *A. solani*. Zygadlo and Grow (1995) confirmed that essential oils of *Salvia gilliess*, inhibited mycelial growth of *A. solani*.

Contrary to ours findings, inductive effect of Sardinian *Rosmarinus officinalis* L. on the growth of *F. graminearum* was observed (Agnioni *et al*., 2004). Results of our tests about inhibitory effects of oregano and lemongrass to *F. graminearum* were consistent with reports of Velluti *et al*. (2004a; 2004b). Although the cost-effectiveness of oil application must be taken into account, this study justifies further research on practical use of essential oils for the control of plant pathogenic fungi.

Table 1. Toxicity of the essential oils to tested pathogenic fungi.

Essential oils	Effective concentrations of the essential oils (µl/ml of air)			
	Alternaria solani		*Fusarium graminearum*	
	MIC[1]	MFC[2]	MIC[1]	MFC[2]
Rosemary Oil Ph. Eur. 50 (*Rosemarinus officinalis* PO124476)	>0.32	>0.32	>0.32	>0.32
Anise Oil Ph. Eur. 50 (*Illicum verum* S0100154)	0.08	0.32	0.16	0.32
Peppermint Oil Ph. Eur. 50 (*Mentha piperita* P0123884)	0.16	0.16	>0.32	>0.32
Eucalyptus Oil Ph. Eur. 50 (*Eucalyptus globulus* S0100321)	>0.32	>0.32	>0.32	>0.32
Wintergreen Oil Ph. Eur. 50 (*Gaultheria procumbens* P0117394)	0.16	>0.32	0.16	0.32
Pineneedle Oil Ph. Eur. 50 (*Pinus silvestris* P0124319)	>0.32	>0.32	>0.32	>0.32
Lemon Oil Ph. Eur. 50 (*Citrus limon* P0119551)	>0.32	>0.32	>0.32	>0.32
Lemongrass Oil Ph. Eur. 50 (*Cymbopogon flexuosus* P0120241)	0.02	0.02	0.02	0.04
Origanum Oil Ph. Eur. 50 (*Origanum vulgare* P0125062)	0.02	0.02	0.02	0.02
Turpentine Oil Ph. Eur. 50 (*Pinus pinaster* P0125332)	>0.32	>0.32	>0.32	>0.32
Lavender Oil Ph. Eur. 50 (*Lavendula angustifolia* P0123527)	>0.32	>0.32	>0.32	>0.32
Origanim Oil nat. id. (*Origanum vulgare* P0125412)	0.16	0.16	0.08	0.08
Rosemary Oil (*Rosmarinus officinalis;* Xherdo Co., Albania)	>0.32	>0.32	>0.32	>0.32
Lavender Oil (*Lavandula angustifolia*; Agroherbal, Albania)	>0.32	>0.32	>0.32	>0.32
Rosemary Oil (*Rosmarinus officinallis* 1; Agroherbal, Ablania)	0.32	>0.32	>0.32	>0.32
Rosemary Oil (*Rosmarinus officinalis* No-41; Agroherbal, Albania,)	0.32	>0.32	>0.32	>0.32
Turpentine Oil (*Pinus pinaster* 2 Agroherbal, Albania)	>0.32	>0.32	>0.32	>0.32
Black pine Oil (*Pinus nigra*; Agroherbal, Albania)	>0.32	>0.32	>0.32	>0.32
Peppermint Oil (*Mentha piperita* No 41/A; Agroherbal, Albania,)	>0.32	>0.32	>0.32	>0.32
Sage Oil (*Salvia officinalis*; Xherdo Co., Albania)	0.32	>0.32	>0.32	>0.32
Silver fir Oil (*Abies alba*; Xherdo Co., Albania)	>0.32	>0.32	>0.32	>0.32
Cade juniper Oil (*Juniperus oxycedrus*; Xherdo Co., Albania)	>0.32	>0.32	>0.32	>0.32

[1]Minimum Inhibitory Concentration
[2]Minimum Lethal Concentration

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ФУНГИЦИДНА АКТИВНОСТ НА ЕСЕНЦИЈАЛНИ МАСЛА ВРЗ ФИТОПАТОГЕНИТЕ ГАБИ *ALTERNARIA SOLANI* SORAUER И *FUSARIUM GRAMINEARUM* SCHWABE

Емил Рекановиќ, Милош Степановиќ, Биљана Тодоровиќ, Ивана Поточник, Светлана Милијашевиќ-Марчиќ, Мирослав Костиќ

Апстракт

Единаесет есенцијални масла беа испитувани за инхибиторна и смртиносна активност врз *Alternaria solani* Sorauer и *Fusarium graminearum* Schwabe. Габните патогени беа изолирани од заболените растенија во Србија. Рано гниење на доматот, предизвикан од *A. Solani* е важна и широко распостранета болест, и *F. Graminearum* е причински агент на фузариозно гниење на житните култури низ целиот свет. Есенцијалните масла беа обезбедени од „Dr Josif Pančić“ Институт за Истражување на Медицински Растенија во Белград, Србија. Изолатите од патогените габи беа изложени на испарливата фаза на масла, и потоа се проветрени со цел да се потврди дали ефектот на маслата е смртоносен за патогенот. Пете есенцијални масла се инхибитори на порастот на *A. solani*: anise (*Illicum verum* Hooker fil.), peppermint (*Mentha piperita* L.), wintergreen (*Gaultheria procumbens* L.), lemongrass (*Cymbopogon flexuosus* L. Stapf) и origanum (*Origanum vulgare* L.). Овие масла исто така покажаа смртност на патогенот на доматот, освен wintergreen има само спречувачки ефект. Патогенот *F. Graminearum* беше подложен на четири масла: anise, wintergreen, lemongrass и origanum, сите тие се изложени на фунгицидниот ефект на габата. Најсилните антигабни активности од природните состојки што се тестираа беше прикажан од страна на origanum, проследен со lemongrass масло. Origanum маслото беше смртоносен за двата патогена во концентрација од 0.02 µl ml-1 во воздух. Lemongrass маслото покажа фунгициден ефект на *A. Solani* со 0.02 µl ml^{-1} и на *F. graminearum* со 0.04 µl ml^{-1} во воздух. Есенцијалните масла од рузмарин (*Rosemarinus officinalis* L.), еукалиптус (*Eucalyptus globulus* Labill.), pineneedle (*Pinus syilvestris* L.), turpentine (*Pinus pinaster* Ait.) и lavander (*Lavendula angustifolia* Mill.) не покажуваат значителни антигабни ефекти на испитуваните патогени.

Клучни зборови: есенцијални масла, фунгицидна активност, *Alternaria solani*, *Fusarium graminearum*.

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Original scientific paper

NEMATICIDAL EFFECT OF CHESTNUT TANNIN SOLUTIONS ON POTATO CYST AND ROOT-KNOT NEMATODES

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Abstract
Two glasshouse pot experiments were carried out to evaluate the effect of chestnut tannins in the control of the potato cyst nematode *Globodera rostochiensis* patotype Ro1 and the root-knot nematode *Meloidogyne javanica*. Doses of 100, 250 and 450 g/m^2 of tannins in aqueous solutions were applied to the soil at transplant or at transplant and two weeks later to verify their effect on nematode control. In both experiments tannin treatments were compared to untreated controls. Cysts were extracted from dried soil using the Fenwich can. Root-knot nematodes were extracted from roots by the Hussey and Barker's method using 1% NaOCl aqueous solution and from soil processing 500 ml soil by the Coolen's method. Data were subjected to analysis of variance and means compared by Least Significant Difference's test. Significant reductions of *G. rostochiensis* number of cyst/100 g soil and eggs and juveniles/g soil and eggs and juveniles/g root and total *M. javanica* population density were observed in association with decreased reproduction rates.
Key words: *Globodera rostochiensis*, *Meloidogyne javanica*, nematode control.

Introduction

Plant parasitic nematodes are important pests of many horticultural crops in the world. They can be highly specialized or they can attack a large number of plants belonging to numerous botanical families because of their polyphagy. Among the most dangerous pathogenic plant parasitic nematodes there are the cyst forming nematode *Globodera rostochiensis*, a specific parasite of potatoes and the root-knot nematode *Meloidogyne javanica*, ables to attack more than 770 agricultural plants also in higher altitudes and warms climates. Because of the development of these nematodes is related to the rizosphera and they can grow also inside plant root tissues, their control during growing season is difficult. The large restriction and revision of the use of pesticides on agricultural crops according to the last European Legislation (Reg. CE 299/2008 and 1107/2009) forced to find new environmental, human and animal health safety methods for plant protection against these pests. From many years the research on low environmental impact alternatives to chemicals has received a strong impulse with a wide range of options, including the use of natural plant products (Sasanelli & D'Addabbo, 1992; Hewlett *et al.* 1997; Chen *et al.* 1997; Renčo *et al*. 2011; Caboni *et al*., 2012; Cavoski *et al*., 2012).

Plant derivatives are a rich source of pharmaceuticals, pesticides and immune allergens containing important biomolecules with biological activities such as glycosides, tannins and phytosterols

(Upadhyay, 2011). In particular, tannins, secondary plant polyphenols, were found to have nematicidal properties against gastrointestinal nematodes in ruminants (Hoste *et al*. 2006). Their physical and chemical properties can change according to the plants, parts of the plants and the season in which they are produced (Waterman, 1999; Waghorn & McNabb, 2003). Moreover, tannins protect several plants against herbivores (Feeny, 1976) and they are toxic to a wide range of fungi, bacteria and yeasts (Scalbert 1991). Hovewer, studies on the effect of the tannins on plant parasitic nematodes are few (Mian & Rodríguez-Kábana 1982; Hewlett *et al.* 1997; Chen *et al.* 1997).

Threfore, two pot experiments in glasshouse were carried out to evaluate the potential use of chestnut tannins in the control of the potato cyst nematode *Globodera rostochiensis* and the root-knot nematode *Meloidogyne javanica.*

Material and methods

Trial on Globodera rostochiensis (Ro1)

The tested chesnut tannin were provided by Agrostar s.r.l. (Cavriago, RE, Italy), extracted by vapour from chestnut wood without chemical solvents in powder form after dehydration. The population of *Globodera rostochiensis* (Woll.) Behrens pathotype Ro1, was obtained from an infested soil at Avezzano (province of L'Aquila, Italy).

The cysts were collected by Fenwick can from dried soil. Then the extracted cysts were thoroughly mixed into 2.9 kg steam sterilised sandy soil. Five 10 g soil samples were taken from this inoculum and the cysts were extracted, counted and their egg content estimated. Appropriate amounts (100 g) of the inoculum were then thoroughly mixed with steam sterilised soil (3,900 g) in each clay pot to give an initial nematode population density of 5 eggs and juveniles/g soil. After inoculation, in each clay pot one potato tuber (cv. Désirée) was sown.

Three tannin treatments at different concentrations were considered: 1) 100 g/m^2; 2) 250 g/m^2 and 3) 450 g/m^2, applied at sowing or at sowing and two weeks later, with a total of six tannin treatments. Tannins were applied as aqueous solutions (400 ml/pot), calibrated on the holding capacity of the soil. Nematode infested and untreated soil was used as control. The pots were arranged on beaches in a glasshouse at 20 $\pm$ 2 °C according to a randomized block design with four replicates for each treatment. Three months later, at the end of the experiment, potato plants were uprooted and nematological parameters estimated. Soil from each pot was mixed thoroughly and then a soil sample was collected and air dried. Cysts were extracted by Fenwick can from 200 g soil sub-sample. Cysts separated from debries and soil particles were crushed (Seinhorst & Den Ouden, 1966) and their egg content estimated.

Trial on Meloidogyne javanica

The population of *M. javanica* (Treub) Chitwood was obtained from Torchiarolo (Province of Brindisi, southern Italy) from infested tomato roots (*Solanum lycopersicum* L.). *M. javanica* was reared for two months on tomato cv. Rutgers in a glasshouse at 25 ± 2 °C. When large mature egg masses were formed, tomato roots were finely chopped and eggs and juveniles were quantified by processing 10 root samples of 5 g each with 1 % aqueous solution of NaOCl (Hussey & Barker, 1973). The roots were then thoroughly mixed with 3 kg of steam sterilised sandy soil (pH 7.2; sand > 99 %; silt < 1 %; clay < 1 % and organic matter = 0.75 %) and used as inoculum.

Appropriate amounts of this inoculum were then thoroughly mixed with steam sterilised sandy soil contained in clay pots (1,000 ml) to give an initial nematode population density of 5 eggs and juveniles/ml soil (*Pi*). Tannins were applied as described in the previous trial on *G. rostochiensis*. After treatments were performed, in each pot a one-month-old tomato seedling cv. S. Marzano was transplanted.

Pots were arranged on benches in a glasshouse at 25 ± 2 °C according to a randomized block design with 10 replicates per treatment.

At the end of the experiment, two months later, tomato plants were uprooted to evaluate nematological parameters. Root gall index (RGI) was estimated according to a 0 – 5 scale, where 0 = no galls; 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 galls; 4 = 31 – 100 galls and 5 > 100 galls (Taylor & Sasser, 1978). Soil nematode population density in each pot was determined by processing 500 ml soil by the Coolen's method (Coolen, 1979). Numbers of *M. javanica* eggs and second stage juveniles in roots were assessed by cutting up each root system into small pieces and further comminuting them in a blender, containing 1 % aqueous solution of NaOCl for 20 sec (Marull & Pinochet, 1991; Sasanelli *et al*., 1997). The water suspension was then sieved through a 250 µm pore sieve put over a 5 µm pore sieve. Nematodes and root debris gathered on the 5 µm pore sieve were further processed by centrifuging at 2,000 rpm for five min in 400 ml of a magnesium sulphate solution of 1.16 specific gravity. Then eggs and juveniles in the water suspension were sieved through the 5 µm pore sieve, sprayed with tap water to wash away the magnesium sulphate solution and collected in about 30 – 40 ml water in plastic labelled bottles. Nematodes in water suspension were then counted by a microscope and final nematode population density (*Pf*) in each pot calculated summing nematodes recovered from soil and roots. *M. javanica* reproduction rate *r* was expressed as ratio between final and initial population density (*Pf*/*Pi*). Data from both experiments were subjected to analysis of variance (ANOVA) and means compared by Least Significant Difference's test. All statistical analysis was performed using the PlotIT program.

Results and discussion

In the literature, there are only few studies where the impact of tannins application on plant parasitic nematodes population development were investigated, however the investigations on tannins as suppressant of gastrointestinal nematodes, bacteria and diseases prevailing (O´Donovan & Brooker, 2001; Paolini *et al.*, 2003; Nguyen *et al.*, 2005).

Results showed that number of cysts/100 g soil, number of eggs and second stage juveniles/g soil and reproduction rate (*r* = *Pf*/*Pi*) of the potato cyst nematode *G. rostochiensis* were significantly reduced by all tannin treatments (100, 250 and 450 g/m^2) in comparison to the untreated control (Fig 1). The highest reduction of these parameters was recorded in soil treated with tannin at dose of 450 g/m^2 applied at sowing and at sowing and 2 weeks later, although this treatment was not significantly different from that applied twice at 250 g/m^2. Tannin treatments at different doses did not significantly affect number of eggs/cyst (Fig 1C).

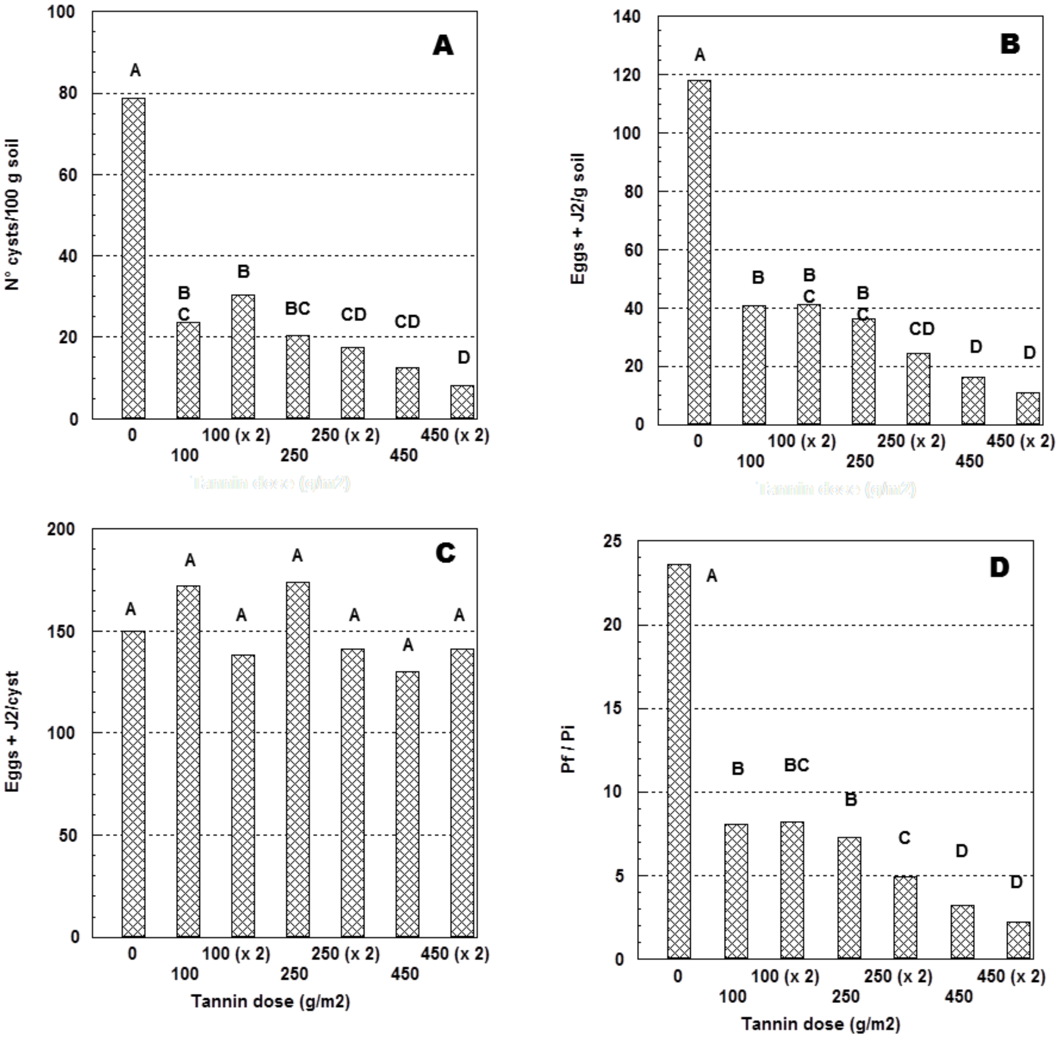

Figure 1. Effect of chesnut tannin at different doses applied at sowing and at sowing and two weeks later on nematological parameters induced by the potato cyst nematode *Globodera rostochiensis* on potato (cv. Désirée). A) N. of cysts/100 g soil; B) eggs and J2/g soil; C) eggs and J2/cyst and D) reproduction ratio *Pf/Pi*. Columns flanked by the same letter are not statistically different according to LSD's test (P=0.01).

No significant differences, in all nematological parameters, were found between the tannin applications at sowing and at sowing and 2 weeks later for all the three tested concentrations (100, 250 and 450 g/m^2) (Fig 1). Similarly to results obtained with the potato cyst nematode, in the pot experiment with *M. javanica* all tannin doses (100, 250 and 450 g/m^2) reduced number of eggs and juveniles/g root, the final population/ml soil and reproduction rate of the root-knot nematode *M. javanica* in comparison to infested and untreated control (Fig 2).

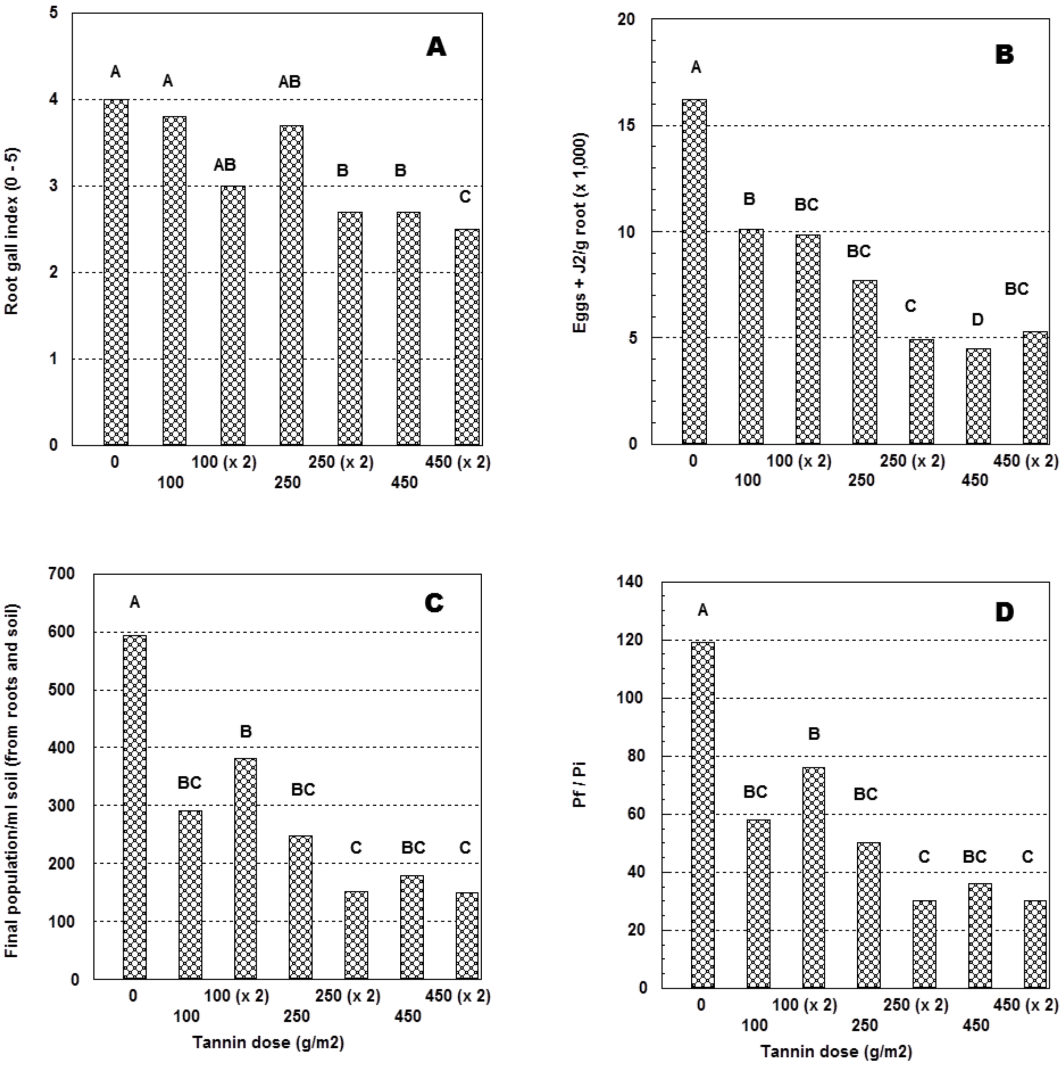

Figure 2. Effect of chesnut tannin at different doses applied at transplant and at transplant and two weeks later on nematological parameters induced by the root-knot nematode *Meloidogyne javanica* on tomato (cv. S. Marzano). A) Root gall index; B) eggs and J2/g root; C) final population/ml soil and D) reproduction ratio *Pf/P*. Columns flanked by the same letter are not statistically different according to LSD's test (P=0.01).

In particular, a significant reduction of RGI was obtained by the use of tannins at 250 g/m^2 applied two times, at transplant and two weeks later, demonstrating the protective and positive effect of repeated treatments (Fig 2, A). However, the lowest dose (100 g/m^2) of tannin was not sufficient to contain the initial nematode attack on the roots.
Results from our pot experiments with the two different type of endoparasitic nematode species, the potato cyst nematode *G. rostochiensis* and the root-knot nematode *M. javanica*, confirm results obtained in previous studies. Mian and Rodríguez-Kábana (1982) found the reduction of

Meloidogyne arenaria (Neal) Chitw. on squash in field after tannic acid treatments and Tylor and Murant (1966) found less number of *Longidorus elongatus* (de Man) Thorne & Swanger in soil treated with two powdered tannin extracts derived from mimosa (*Acacia mollisima* Willd.) and quebracho (*Schinopsis lorentzii* (Griseb.) Engl.). *L. elongatus* is a virus vector of plant and it was found that soil borne viruses were previously suppressed by the addition of tannins to the soil (Cadman & Harrison, 1960). Because of these viruses are not free in the soil but they are transmitted by *L. elongatus*, Tylor and Murant (1966) outlined that plant virus protection was obtained through the control of the virus vector and not because of the effect of the tannin directly on viruses.

On the contrary, Hewlett *et al.* (1999) found that tannic acid extracted from tara (*Caesalpinia spinosa* Mol. Ktz.) was an attractant for the *M. arenaria* and *M. incognita*, whereas it was abhorrent for *Radopholus similis* (Cobb) Thorne and had no effect on *Heterodera glycines* Ichinohe.

On the base of results from these two pot experiments the use of chestnut tannin seems to be a promising method for the control of the potato cyst and root-knot nematodes in sustainable agriculture. Because of the tannins are present in all higher plants, additional studies are suggested to investigate the effect of different tannins on different plant parasitic nematode species as well as on beneficial, soil free living nematodes.

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НЕМАТИЦИДЕН ЕФЕКТ НА ТАНИНИ ОД КОСТЕНОТ ВРЗ КОМПИРОВАТА ЦИСТА И КОРЕНОВИТЕ НЕМАТОДИ

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Апстракт

На два стакленика беа извршени експерименти со цел да се оцени ефектот на танинот од костенот во контрола на компировата цисна нематода *Globodera rostochiensis* патотип Ro1 и корено-јазолски нематоди *Meloidogyne javanica*. Дози од 100, 250 и 450 g/m^2 од танин во водни раствори беа применети на почвата на пресадување или пресадување и две недели подоцна да се потврди ефектот од контрола на нематодата. И во двата експерименти taнинскиот третман беше спореден со нетретирани контроли. Цистите беа естрахирани со користење на Fenwich can. Корено-јазолските нематоди беа естрахирани од коренот со Hussey и Barker’s метод користејќи 1% NaOCl воден раствор и со обработка на почва 500 ml почва од страна на Coolen’s метод. Податоците беја изложени на анализа на варијансата и начин во споредба со најмалку значителна разлика на тест. Значително намалување на *G. rostochiensis* број на цисти/100 g почва и јајца и младите/ g, почва и јајца и младите /g на корен и вкупна популација на *M. javanica* каде се забележани во соработка со намалени репродукциски стапки.

Клучни зборови: *Globodera rostochiensis, Meloidogyne javanica*, контрола на нематоди.

UDC: 632.76-567(497.115)
631.24(497.115)
Original scientific paper

INVESTIGATION OF THE PRESENCE OF THE MOST IMPORTANT WHEAT GRAIN PEST FROM THE ORDER COLEOPTERA IN SOME PROCESSING MILLS IN KOSOVO

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Abstract
The purpose of this study was to identify the most common pest species affecting wheat grain during storage in three electric processing mills in Kosovo. During the year of 2011 and 2012, grain samples were taken from the processing mills located in three different locations of Kosova (Podujevë, Sekiraqë and Bajqinë). The obtained results showed that wheat weevil is the most prevalent pest of the wheat grain in processing mills in Kosovo. Of the total collected pests in the above three processing mills (1522), wheat weevil (*Sitophilus granarius*) takes part with 875 individuals (57%), rice weevil (*Sitophilus oryzae*) 380 (25%), lesser grain borer (*Rhyzopertha dominica*) with 199 individuals (13%) and flour beetle (*Tribolium* spp.) with 68 individuals (5%). The highest number of the grain pests was found in processing mill located in Bajqina (744 individuals), whereas the lowest one in Sekiraqa (328 individuals). According to the analysis of variance (ANOVA) there were found statistically significant differences with regard to pest species found in three electrical mills in different locations.
Key words: Grain pest, Storage, Electric processing mills, Granary weevil, ANOVA.

Introduction

Human beings for centuries has collected, stored and preserved agricultural products. So archaeologists have determined that food storage started back to the Neolithic period (about 8000 years BC), when man began the cultivation of plants and animals.

The storage fauna investigation was subjekt to many authors, thus in Georgia (Voast, 2009), Croatia (Korunić, 1990), etc. Storage pests were investigated also in Kosov by different authors. Thus, according to some authors (Pireva & Musa, 2003) in different grain strage and mills it was identified and investigated in detal lesser grain borer (*Rhizoperta dominica*), while according to Pireva (1990) in different storage and mills in Kosovo were found 32 species from the order Coleoptera and three from the order Lepidoptera. Given that storage conditions are more favorable compared to outdoor condition the multiplication of pests in storage is faster. Thus, as for the pest species *Oryzaephilus surinamensis* and *O. Mercator*, a pair of mature individuals (imago) within 150 days can provide up to 1 million offspring, while the other pest species, *Sitophilus granarius*, some couples formed hundreds of thousands of descendants (Maceljski, 1999). In this manner a very small infestation within a very short period of time will provide a large number of pests which endanger the entire wheat grain stored in storage. In large infestation of some siloses, feed factories

etc. have been found in some cases more than 2816 insects / 1 kg of wheat sample. In 1 kg of cereales grain were found over 29 species of insects, whereas in other cases in 1 kg of waste wheat grain during aspiration found over 100 species of insects (Maceljski, 1999). According to some authors (Korunić, 1990), in wheat grain storages there are shown to be present different types of pests most of whom are from the order Coleoptera (over 100 species), Lepidoptera order (more than 10 species) and mites (33 species).
The goal of our investigation has been the determination of the pests from the order Coleoptera, in some electrical procesing mills in our country.

Material and methods

Our investigations were conducted during the years 2011- 2012 in the Podujevo region. For this purpose, samples were taken at three electricall processing mills (Bajqinë, Sekiraqa and Podujevë). Grain samples were taken according to standard methods for investigation of the storage pests. In each investigated processing mill were taken three samples, each weighing about 1 kg. The samples consisted of wheat grain collected in different places in mills, usually in sievse, corners etc. Samples for analysis were taken once a week from each processing mill. In this case, all the insects collected were taken and placed in plastic bags, were equipped with the all relevant informations (date of sampling, sample number, the name of the mill, etc.). Later on such a samples were brought to the laboratory for plant protection of the Phytosanitary Corporation "Sara & Meti" in Pristina, for identification and description of the different pest species, with the aid of different entomological keys (Lea Schmidt, 1970). The obtained results were processed statistically using MSTAT-C computer program from University of Michigenit.

Results and discussion

During the two-years of the investigations with regard to the presence of different pest species in wheat grain stored in three processing mills in region of Podujeva (Podujevë, Sekiraqa and Bajqinë) there were found these pests: wheat weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhizopertha dominic*a) and flour beetle (*Tribolium* spp.). The level of appearance of differenet grain pests in investigated processing mills was very diferent (Table 1).The obtained results after the two-years of investigations of wheat grain pests in some electric processing mills in Podujevo municipality (Podujevo, Sekiraqa and Bajqinë) showed that wheat weevil (*Sitophillus granarius*) is the most prevalent pest species, while there were also present other pest species, by far with the lowest number (Graph 1). From the total collected pests in the above mentioned mills (1522), wheat weevil takes part with 875 individuals (57%), rice weevil 380 (25%), lesser grain borer with 199 individuals (13%) and flour beetle 68 individuals (5%). Our results are nearly the same as the results reporter by other authors, where it has been shown that the first two pests (*S. granarius* and *S. oryzae*) are the mos present species during storage of wheat grain in siloses and proccesing mills (Pireva, 1990; Korunić, 1990; Musa & Carli, 2004).
The frequency of appearance of different pests of wheat grain stored in different processing mills under ivestigations has been different (Graph 2). Of the total number of pest species found in the wheat grain stored in different processing mills it was shown that there are differences among investigated mills, with regard to the presence of these pests.

Table 1. Frequency of the wheat grain pests in different mills (2011-2012)

Processing Mill	Pest	Sampling										Total
		1	2	3	4	5	6	7	8	9	10	
Podujevë	*S. granarius*	14	9	23	1	52	33	19	83	43	6	283
	S. oryzae	6	7	8	5	20	12	9	23	11	4	105
	Rh. dominica	5	0	8	4	0	1	1	11	8	4	42
	Tribolium spp.	0	3	0	2	5	0	3	4	0	3	20
Sekiraqë	*S. granarius*	1	17	14	12	9	39	34	15	24	5	170
	S. oryzae	0	4	6	12	7	11	13	18	17	3	91
	Rh. dominica	0	0	1	2	1	4	7	11	2	8	36
	Tribolium spp.	1	1	4	4	2	6	9	0	3	1	31
Bajqinë	*S. granarius*	19	22	49	51	31	28	54	21	105	42	422
	S.oryzae	6	11	33	12	15	18	25	19	33	12	184
	Rh. dominica	0	0	15	28	12	9	3	16	5	33	121
	Tribolium spp.	0	5	1	3	0	0	2	5	0	1	17

Legend: the values represents the sum of the imagos collected from three working samples

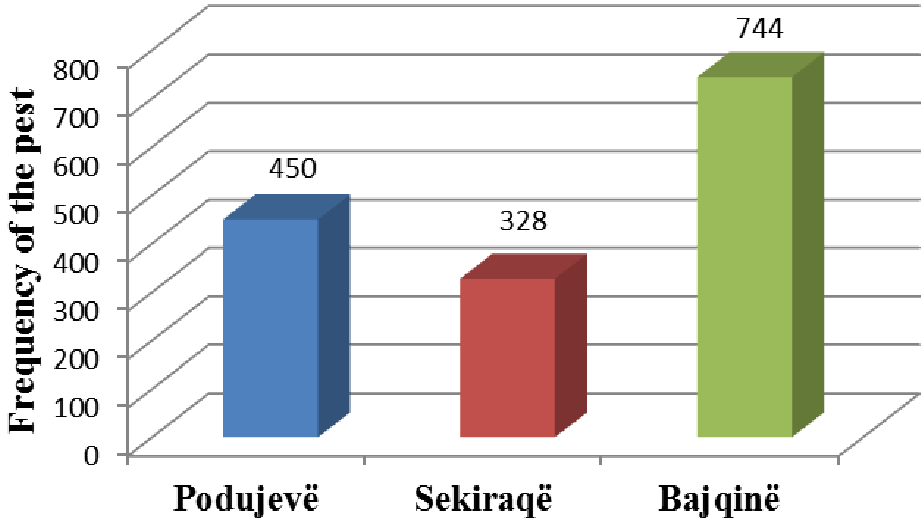

Figure 1. Pest proportion from the total identified in all pocesing mills

With regard to this, the processing mill located in Bajqina has been the most affected, with total of 744 individuals found or 48.88%, mill in Podujevo with 450 or 29.57%, while the least affected was processing mill in Sekiraqa, with 328 individuals or 21.55%, (Graph 3).

The table of analyses of variance and LSD tests shows that different levels of statistical significant differences were found with regard to the number of pests identified in different processing mills, where samples were taken. In this regard, the results of our investigations are nearly the same as those reported by other authors (Kolektiv Autora, 1972).

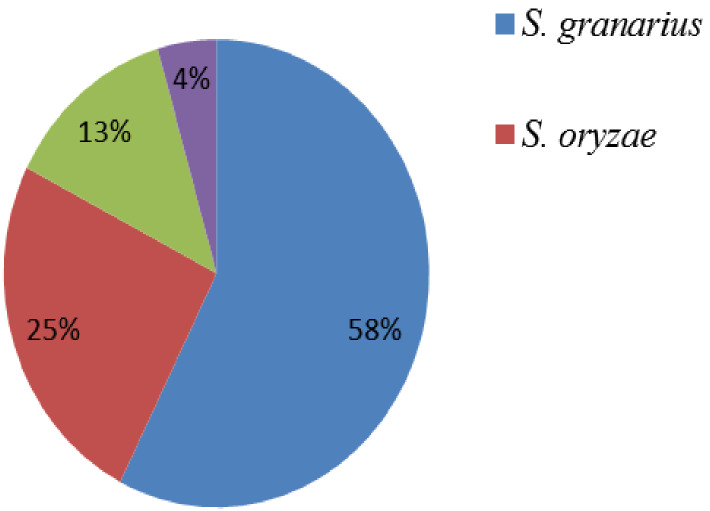

Figure 2. Frequency of the pest by proccesing mills

The highest number of pests, as the average of the total, was found in processing mill located in Bajqina (18.60) and the lowest one in Sekiraqa (8.20). On the other side in processing mill located in Podujevo the average number of pests from the total has been somewhere in between (11.25). In this context we can say that processing mill located in Bajqina has been the most affected by these pests, while the mill located in Sekiraqa has been the least one affected by various grain pests.

Statistically significant differences of different levels were found with regard to pest species as well (Factor B). The highest value (29.17) was found to wheat weevil (*S. granarius*) and lowest one (2.27) to flour beetle (*Tribolium* spp). According to the analyses of variance, it was shown that there are statistically significant differences of different levels among wheat grain pests found (Table 2).

Table 2. Frequency of wheat grain pests, (ANOVA)

Processing mill (A)	Pest (B)				Average (A)
	S.oryzae	*S.granarius*	*Rh.dominica*	*Tribolium* spp.	
Podujevë	28.30	10.50	4.20	2.00	11.25
Sekiraqë	17.00	9.10	3.60	3.10	8.20**
Bajqinë	42.20**	18.40	12.10	1.70**	18.60**
Average (B)	29.17**	12.67	6.63	2.27**	Interaction A x B

Factor		A	B	A x B	B x A
LSD	1%	8.7768	7.3722	14.2483	12.7690
	5 %	6.4072	5.6006	10.6304	9.7005

Regarding to the interactions of the factors, locality and pest species (A x B), also were found different levels of statistical significant differences as it is shown in table 2.
In connection with the appearance of grain pests, various authors have reported results that are approximately in line with the results of our investigations (Maceljski, 1999; Pireva, 1990; Radonjic Jelena 1990).

Conclusions

Based on the two-year investigations, with regard to the spread of different pests species from the order Coleoptera in the wheat grain stored in three electric proccesing mills of Podujevo Municipality (Podujevo, Sekiraqa and Bajqinë), we can conclude that:
Different pest species from the order Coleoptera are widespread in these mills, causing huge losses of wheat grain during it's storage. The obtained results indicates that wheat weevil (*S. granarius*) is the most prevalent pest species from the order *Coleoptera* in wheat grain in storage. From this order there were found also other pest species in wheat samples, rice weevil (S. oryzae), lesser grain borer (*Rh. dominica*) and flour beetle (*Tribolium* spp.) but by far in less number.
The dynamics of the appearance of certain types of grain pests has been various in all investigated processing mils, where the highest number of these pests were found in Bajqinë, then Podujevë and the lowest one in Sekiraqa.
Through the analyses of variante and LSD tests, highly significant statistical differences were found between the pest species found (factor B), locations where processing mills were located (factor A) and interaction between these factors (locality x pest).
Based on the number of the pest found in all investigated processing mills it can be said that there is a neccesserry to undertake protective measures to keep these pests under control starting from preventive to curative ones.

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УТВРДУВАЊЕ НА ПРИСУСТВОТО НА НАЈЗНАЧАЈНИТЕ ШТЕТНИЦИ НА ПЧЕНИЧНОТО ЗРНО ОД РЕДОТ COLEOPTERA ВО НЕКОИ МЛИНОВИ ВО КОСОВО

Гурнер Мурати, Фадил Муса, Скендер Рамадани

Апстракт

Целта на овие истражувања беше да се идентификуваат најчестите штетни инсекти на зрната од пченица при нејзиното складирање во три електрични млинови во Косово. Во текот на 2011 и 2012 година, беа земани примероци од пченични зрна во испитуваните електрични млинови сместени на 3 различни локации во Косово. Добиените резултати покажуваат дека најзастапен штетник е житниот жижак. Од вкупниот број на собрани штетници во трите испитувани млинови (1522), житниот жижак (*Sitophilus granarius*) беше застапен со 875 единки (57%), оризовиот жижак (*Sitophilus oryzae*) со 380 (25%), *Rhyzopertha dominica* со 199 единки (13%) и *Tribolium* spp. со 68 единки (5%). Најголем број на штетници беше утврден во млинот лоциран во Бајкина (744 единки), а најмал во Секирака (328 единки). Со анализа на варијанса (ANOVA) утврдени беа статитистички значајни разлики во однос на штетните видови на инсекти во трите испитувани млинови.

Клучни зборови: штетници на зрна, складирање, електрични млинови, ANOVA.

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THE MOST COMMON HARMFUL AND USEFUL ORGANISMS FOUND IN GRAIN CROPS IN LESKOVAC

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Abstract
The aim of this research is to monitor plant development and register any emergence of harmful and useful organisms in each separate research facility. The research took place in 2011 and 2012 in the research area of Agricultural High School in Leskovac. The following standard entomological methods were used to determine the prevalence and number of insects: visual examination of plants (every 7-10 days during the whole vegetation period) so as to determine the presence of pests; examination of above-ground plant organs (ears and leaves) and determination of the intensity of pest attack; hand reaping as an additional measure for determination of pest presence and density; laboratorical treatment, taxidermy and making a herbarium out of collected materials. The research results show that the following pests are the most common: aphids *(Aphididae)*, cereal leaf beetle (*Lema melanopus*) and scarab beetles (*Anisoplia spp*.). The most common diseases are the following: leaf rust (in 2011) and *Fusarium spp.* (in 2012). Significant presence of some sorts of ladybirds (Coccinellidae) and green lacewings (Chrysopidae) has been registered too.
Key words: grain crops, harmful and useful organisms, monitoring, Leskovac.

Introduction

Crop production is based on modern principles of creating high-quality food sources and providing healthy food products. Crop production can be brought to a higher extent by modernization of the production process. That is achieved by using high-yield crops, adequate fertilization (Tmušić et al., 2012) and pesticides in accordance with the integral (Ančev et Lazarevska, 2000) and organic principles of production . The increase in wheat crop area imposes the need for monoculture which, as a result, leads to specific conditions, high density of cultivated plants and the presence of various harmful organisms. The newly-arizen conditions in agrobiocenosis are suitable for the emergence of various harmful (disease, pests and weeds) and useful organisms (Lazarevska, 1998). Observing the presence, development and ratio of both useful and harmful organisms, taking notice of any faults in normal production process and establishing the extent of harmfulness that certain organisms might have are all part of monitoring system in plant protection. (Nikolić et al., 2012).

Apart from the modernization of production process, the changes in agrobiocenosis are caused by the climate as an environmental factor highly prone to change. Global warming, the phenomenon established by the end of the 20th and the beginning of the 21st century, has a significant influence on normal crop production process and plant protection as it changes the ratio of plants and harmful organisms. As a result, it is necessary to monitor the number of harmful organisms (Čamprag, 2000;

Petrović-Obradović, 2003) and disease-causing agents (Gudžić et al., 2009) in grain crops, as well as all accompanying changes in climatic factors (temperature, precipitation, humidity).
The aim of this research is to monitor plant development, take notice of any emergance of both harmful and useful organisms and the comparison of their emergence in spring and winter crops.

Material and methods

Microexperiment on spring crops (done on 19/04/2011) had 7 genotypes (Vojvoda, Smaragd, Jadran, Slavuj, Rajac, Lovcen and Vranac). Microexperiment on winter crops (done on 18/11/2012) had 8 types (Zemunska Rosa, Aleksandra, Srma, Janja, Zenit, Nirvana, Cipovka, 34/D-durum). The standard entomological methods (Group of authors, 1983) were used to determine the number and the spread of insects. Visual plant examinations were carried out during the whole vegetation period, every 7-10 days, depending on the meteorological conditions and the need for establishing the presence of disease-causing agents and insects (both harmful and useful). This method was also used for establishing the presence of rodents. Examination of aboveground plant parts (ears and leaves) was done to establish the presence of leaf lice. After the ear development stage in plant growth, 100 ears of each type were examined to determine the attack intensity of aphis population on the ears. The attack intensity was graded on the scale from 0 to 4. (Banks): 0 - no pests; 1 - very mild infection (individual plants or small colonies); 2 - mild infection (small number of pest colonies can be found on plants); 3 - infection of medium intensity (aphis can be found widely, several big or incoherent colonies); 4 - infection of high intensity (aphis can be found in large numbers, plant organs are densely covered with coherent colonies).
Aphids infection of medium intensity is calculated when the total sum of graded ears is divided by the number of analysed ears (Group of authors, 1983). Hand reaping was used as an additional measure for establishing the presence of predatory fauna in the period of high plant density. Laboratorical treatment (taxidermy and making a herbarium out of collected materials) was done continuously during the entire research period.

Results and discussion

The research results show that the following pests are the most common: aphids *(Aphididae)*, cereal leaf beetle (*Lema melanopus*) and scarab beetles (*Anisoplia spp*.). The most common diseases are the following: leaf rust (in 2011) and *Fusarium spp.* (in 2012). Significant presence of some sorts of ladybirds (Coccinellidae) and green lacewings (Chrysopidae) has been registered too.
The attack intensity of aphids (Figure 1.) on spring crops was determined three times in 2011 (on June, 19th, July, 5th and July, 18th). The attack intensity of aphids on winter crops was determined three times in 2012 (on May, 28th, June, 11th and June, 29th) (Table 1.). Aphids infection of medium intensity during the mentioned research period is shown in the Table 2. During the research period, it was determined that meteorological conditions such as drought and high temperatures were partucularly suitable for the development of leaf lice. In both research years, there was one treatment done to diminish the aphids population.

Figure 1. Aphids colony on ear and leaf

Table 2. Aphids infection of medium intensity in grain crops

	Visual examination		
2011.	June, 19th	July, 5th	July, 18thv
Spring crops	0,42	1,14	1,85
2012.	May, 28th	June, 11th	June, 29th
Winter crops	0,13	1,00	1,37

The predatory activity of natural enemies from the ladybird Family Coccinellidae and the green lacewing Family Chrysopidae was established during the research period.

- Family Coccinellidae (ladybird):
Coccinella septempunctata L. (Figure 2.)
Adalia bipunctata L.
Hippodamia variegata Goeze
Propylea quatouredecimpunctata L.
Calvia quatourdecimpunctata L.
- Family Chrysopidae (green lacewing):
Chrysopa perla L.
Chrysopa carnea Steph.

All those predatory natural enemies that were taken notice of during the reseach period fed primarily on aphis. They play a significant role in reduction of aphis population and dicrease in the extent of inflicted damage. Therefore, it is of extreme importance to take care and preserve the already-existing predatory species in the agrobiocenosis under research.

Table 1. Aphids infection of genotypes (scale from 0 to 4)

2011.	Visual examination		
Genotypes	June, 19th	July, 5th	July, 18thv
Vojvoda	0	1	1
Smaragd	1	2	2
Jadran	1	2	3
Slavuj	1	1	2
Rajac	0	1	2
Lovcen	0	0	1
Vranac	0	1	2
2012.	Visual examination		
Genotypes	May, 28th	June, 11th	June, 29th
Zemunska Rosa	0	1	1
Aleksandra	0	0	2
Srma	0	1	2
Janja	0	1	1
Zenit	1	2	2
Nirvana	0	1	1
Cipovka	0	1	1
34/D-durum	0	1	1

Cereal leaf beetle (*Lema melanopus* L.) is one of most significant small grain pests. (Čamprag, 2000). Its presence was established in the research area during the research period, but not in numbers large enough to require a special treatment. The first emergence of imaga on the newly-formed leaves of spring crops was established on 10/05/2011. The first emergence in winter crops was establised on 23/04/2012. The first signs of damage caused by larvae of cereal leaf beetles were established on 21/06/2011 and 24/05/2012. Larvae feed on the leaf area causing the leaf to skeletonize, leaving only the lower leaf epidermis (Figure 3.)

The damaged leaves are strikingly yellow and white, whereas the larvae are covered in mucus. The extent of larva damage was higher in spring crops in 2011 in comparison to the larva damage of winter crops in 2012. The preference of this pest for spring crops was pointed out by other authors too. (Group of authors, 1983; Čamprag, 2000).

Sporadic emergence of scarab beetles (*Anisoplia spp*.) was established during the research period. In 2011, larger numbers of *A. austriaca* Hrbst., *A. agricola* Poda and *A. segetum* Hbst .were present in winter crops.

Drought in summer 2011 was suitable for the emergence and spread of rodents, causing minor attacks on winter crops on the outskirts of the research area. (13/07/2011).

In 2011, the emergence of leaf rust (*Puccinia triticina* Ericsson) and stem rust (*Puccinia graminis* Persoon) were established in spring crops, especially in the oats types of Slavuj and Rajac. Orange pustules on leaves were first established on 17/06/2011 and the most intense attack was on 05/07/2011. In 2012, leaf rust has been present in traces in some sorts of winter crops (Figure 4).

Figure 2. *Coccinella septempunctata* L. on ear

Figure 3. Damaged leaves from *Lema melanopus* L.

Figure 4. Leaf rust (*Puccinia sp.*) on leaves

In 2012, fusariosis of wheat ears (*Fusarium graminearum*) was established in the blossoming stage (on 08/06/2012), after a long-lasting period of rain and higher humidity. The characteristic symptoms of this disease are the emergence of "white ears" with poorly developed seed and dying out of the ear above the infected area.

The results are a part of the research project called: "The examination of genetic basis for the improvement of growth and yield of small grains under different environmental conditions" (TP 31092) funded by the Ministry of Science and Technological Development of the Republic of Serbia. The research is going to be continued in the forecoming period.

Conclusions

Modernized production process, monoculture and environmental conditions can result in dense plant growth and emergence of various harmful organisms in grain crops.

The research results show that the most commonly-present pests were: aphids (Aphididae), cereal leaf beetles (*Lema melanopus*), scarab beetles (*Anisoplia spp*.) and rodents. Natural enemies from the Family of ladybirds (Coccinellidae) and the Family of green lacewings (Chrysopidae) play an important role in the reduction of aphis population.

The most commonly present diseases were leaf rust (*Puccinia triticina*) in 2011 and furiosis of wheat ears (*Fusarium graminearum*) in 2012.

The presence of harmful and useful organisms in spring and winter crops is almost identical, with small differences pertaining to the beginning of insect emergence and different attack intensity. The diferences in attack intensity are related to the developmental stages of plants and are under the influence of meteorological conditions.

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НАЈЧЕСТИ ШТЕТНИ И КОРИСНИ ОРГАНИЗМИ КАЈ ЖИТНИТЕ КУЛТУРИ ВО ЛЕСКОВАЦ

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Апстракт

Целта на ова истражување беше преку мониторинг на развојот на растенијата да се детектира појавата на штетни и корисни инсекти во секој испитуван објект. Истражувањата беа извршени во текот на 2011 и 2012 година во истражувачките полиња на Земјоделското Средно Училиште во Лесковац. Во испитувањата беа вклучени стандарни ентомолошки методи за утврдување на застапеноста и бројот на инсекти и тоа: визуелен преглед на растенијата (секои 7-10 дена) за да се утврди присуството на штетните организми; преглед на надземните делови од растенијата и утврдување на интензитет на напад; лабораториски третмани, таксидермија и правење на хербариум од колектираниот материјал. Резултатите покажуваат дека најчести штетни инсекти се следните: растителните вошки *(Aphididae)*, житната пијавица (*Lema melanopus*) и *Anisoplia spp*. Најчести болести кај житариците се: лисната рѓа (во 2011) и *Fusarium spp.* (во 2012). Значајно присуство на некои видови бубамари (Coccinellidae) и златици (Chrysopidae) беа регистрирани исто така.

Клучни зборови: житни култури, штетни и корисни организми, мониторинг, Лесковац.