International Journal of Agricultural Technology 2014 Vol. 10(4): 951-961
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Allelopathic Impact of Some Antioxidants on *Fusarium solani* Causing Root Rot on Faba Bean (*Vicia fabae*)

El Gamal, N. G. [1*] and El Shamy, A. R. [2]

[1]Plant Pathology Department, National Research Centre, El-Behose St. Dokki, 12622, Giza, Egypt, [2]Chemistry of Natural and Microbial Products Dep. National Research Centre, Cairo, Egypt.

El Gamal, N. G. and El Shamy, A. R. (2014). Allelopathic impact of some antioxidants on *Fusarium solani* causing root rot on faba bean (*Vicia fabae*). International Journal of Agricultural Technology 10(4):951-961.

Abstract Some elements mainly allelochemicals, released from living roots or decayed plants might be associated with the diseases. Results showed that the highest reduction of *Fusarium solani* was found by treatment with coumarin at all concentrations (33.3, 55.6, 72.2and 83.3) respectively followed by Argenin, Petrocin and control. These antioxidants at concentrations 5, 10, 15 ppm reduced the enzymatic activity of polyglatronase (PG) and cellulolytic (CX) of *Fusarium solani*. The great decreasing showed at 15 ppm of coumarin after 7 and 14 days. In greenhouse seed treatment with different concentrations of antioxidants can be significantly reduced the disease incidence of pre and post emergency also the combination of coumarin at 15 ppm with *Trichoderma harzianum* was highly effective of reducing pre and post emergency comparing with coumarin or *T. harzianum* alone.

Keywords: allelochemicals, coumarin, Bean, *Fusarium solani*

Introduction

Faba bean (*Vicia fabae*) is one of the most important winter vegetable of the leguminous crops in Egypt its grown mostly to fulfill food and feed requirements of human and protein 26%: 28% and some other compounds Morsy and tarred, 2005), and considered as a meat and skim milk substitute in diet for its high protein and nutritional quality., being cultivated on an area of~160,000 ha and yielding ~640,000 tons (Anonymous, 2006). Dry seeds of high quality are used for meals and in the food industries. Low quality seeds, in addition to plant wastes, are used for animal feed.

Root rot and wilt diseases are still the most important diseases for many crops (cotton, legumes, and some fruit trees) in many countries (Culter and Culter, 1999). Several fungi are recorded as causal pathogens of root rot and wilt diseases such as *Rhizoctonia solani*, *Fusarium solani*, *Sclerotium rolfsii*

[*] **Corresponding author:** El Gamal, N. G.**; E-mail:** nadia_gamil2000@yahoo.com

and *Macrophomina phaseolina* causing root rot diseases. This disease appears during the growing season at the seedling stage of plant growth. Many soilborne fungi attack earlier at the pre-emergence stage, thus forcing the farmer to replant the missed hills or dead plants. Due to the economic importance of faba bean, the farmers plant repeatedly on the same land. This practice leads to a high build-up of pathogens, causing serious losses that could reach up to 12% (Anonymous, 2006).

The management strategy followed by the farmers included an unwise, intensive use of fungicides. This strategy was not a satisfactory solution for controlling root rot disease. An investigation of root rot disease is considered particularly important due to its wide prevalence in Egypt, particularly in sandy soils. Thus far, because of scientific and practical difficulties, there is no economic way to control root rot disease in many crops.

The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries (Sivan, 1987). However, this is not an easy method, and it is costly to apply. It is possible to use biological controls as the best control measure under greenhouse conditions. Abdel-Kader (1997) reported that *Trichoderma harzianum* introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. *Trichoderma spp*. are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan and Chet 1986; Whipps and Lumsden 2001; McLean *et al*. 2004). Hadar *et al*. (1979, 1984) and Elad *et al*. (1980) observed that the application of wheat bran colonized by *T. harzianum* to soil infested with *R. solani* and *S. rolfsii,* reduced the incidence of root diseases caused by these pathogens in beans.

Pathogens frequently display resistance to current drugs, which frequently lack selectivity/efficacy and have detrimental side effects. Thus, there is a constant need fornovel therapeutic agents. Coumarins belong to the family of lactones, having a benzopyrone system that can be isolated from plants as well as total synthesis that can be carried out in the laboratory. To date, many chemical reactions have been established that can be used to synthesize coumarins. The synthesis of coumarins and their derivatives has attracted the attention of organic and medicinal chemists, as these are widely used as fragrances, pharmaceuticals and agrochemicals, Kasumbwe *et al.* (2014).

Coumarin (2H-1-benzopyran-2-one) is a plant-derived natural product known for its pharmacological properties such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective properties. Dietary exposure to benzopyrones is significant as these compounds are found in vegetables, fruits, seeds, nuts, coffee, tea, and wine Venugopala *et al*. (2013). Coumarin exhibited antibacterial activities against gastroenteritis diseases Nitiema *et al.* (2012). Coumarins consist of a large class of phenolic substances found in plants and are made of fused benzene and α-pyrone rings Venugopala *et al*. (2013).

Coumarins are widely distributed in nature and are found in all parts of plants (1). These compounds are especially common in grasses, orchids, citrus fruits, and legumes.(1,2) Being so abundant in nature, coumarins make up an important part of human diet. Based on chemical structure (Fig. 1), they can be broadly classified as (a) simple coumarins (e.g., coumarin, 1), (b) furanocoumarins of the linear (e.g., psoralen, 3) or angular (e.g., angelicine) type, and (c) pyranocoumarins of the linear (e.g., xanthyletin, 6) or angular (e.g., seselin) type.1 Simple coumarins are very widely distributed in the plant kingdom.1 Interestingly, citrus oils, in particular, contain abundant amounts of both simple as well as furanocoumarins.3Humans are also exposed to furanocoumarins (e.g., bergapten andxanthotoxin) in umbelliferous vegetables, such as parsnips, celery,and parsley in substantial amounts.4 A number of furanocoumarins ac t as inhibitors of drug metabolizing enzymes.

The purpose of this work was to evaluate the possible impact of allelochemicals (coumarin , Argenin and Petrocin) released from living roots or decayed plants against *Fusarium solani* the causal agent of root rot of faba bean.

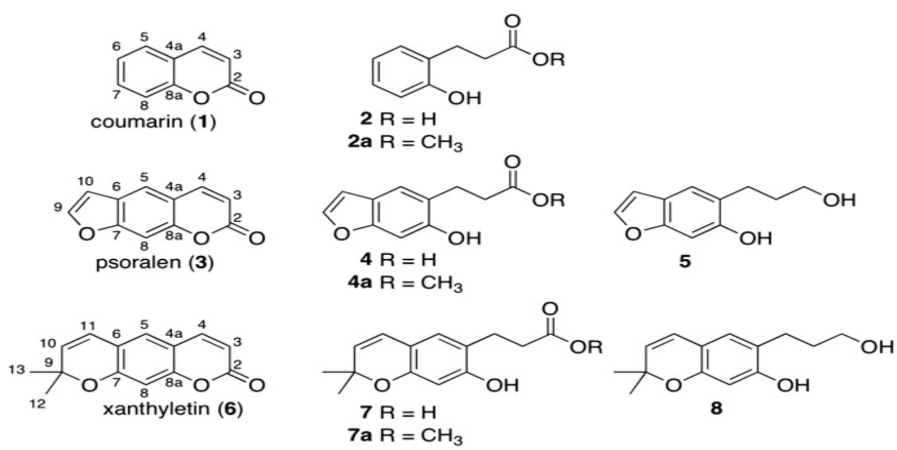

Fig. 1. Chemical structures of coumarin, psoralen, and xanthyletin derivatives

Materials and methods

The present work was developed at the laboratory and greenhouse of Plant Pathology of National Research Centre (NRC), Egypt.

Laboratory tests

Three antioxidants Coumarin, Argenin and Petrocin .at different concentration 5ppm, 10ppm, 15ppm, and 20ppm were evaluated for their inhibitory effect on *Fusarium solani radial* growth through *in vitro* tests. Growth reduction of faba bean root rot in response to different concentrations was recorded.

Emulsified stocks at high concentrations of tested antioxidants were prepared by dissolving in sterilized distilled water. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the antioxidants volumes to obtain an emulsion feature. Different volumes of the antioxidants emulsion were added to conical flasks containing 100 ml of sterilized PDA medium before its solidification, to obtain the proposed concentrations. The supplemented media were poured into Petri-dishes (9 cm) about 20 ml each. The control check treatment was PDA medium which was free of antioxidants. Disks (5 mm -diameter) of *Fusarium solani* taken from seven day-old cultures were placed on the centre of Petri dishes. All plates were incubated at 25±2 ℃ until the tested fungi reached full growth in the check treatment. Reduction in mycelia growth was calculated as the percentage of fungal growth diameter in the treatment, relative to the growth diameter in the control.

Effect of different concentration of antioxidants on polygalacturonase and celluolytic enzyme activity

The efficacy of some antioxidants, namely Coumarin, Argenin and Petrocin,at concentrations of 5ppm, 10ppm, 15ppm in reducing the activity of polygalacturonase (PG) and cellulolytic (Cx) enzymes in vitro tests were determine using the methods described by Khairy *et al.* (1964) and Abdel-Razik (1970).

The production of PG enzyme was carried out using the following medium {4.6 g Citrus pectin, 5.0 g yeast extract, 5.0 g peptone and 5.0 g K2HPO4} (MacMillan and Voughin, 1964). The same medium supplemented with 4.6 g carboxymethyl cellulose (CMC) instead of citrus pectin was used for the production of Cx enzymes. The conical flasks which contained 50 ml of each medium were autoclaved. Each antioxidant was added to the medium flask to obtain the tested concentration. The test flask was inoculated with an equal disc (1 cm diameter) of pathogenic fungus. Three flasks were used as replicates for each treatment as well as the control. The test flasks were incubated at 28C. PG and Cx enzyme activity was determined after 7 and 14 days of incubation. The supernatants were obtained by filtration and centrifugation at 5000 rpm for 15 min and then the supernatants were used for crude enzyme preparation.

Assays of PG and Cx enzymes activity were determined by the viscometer method (Abdel-Razik 1970). The viscometer was employed according to the method described by Khairy *et al*. (1964). PG enzyme activity was assayed by estimating the loss in viscosity of 1.2% citrus pectin solution after an incubation period of 3 h at 30C. Cx enzyme activity was determined by measuring the loss in viscosity of 1% CMC solution after incubation for 3 h at 30C. A boiled crude enzyme served as a blank. The steps applied on the sample were repeated on the blank and distilled water. The enzyme activity was measured in terms of loss of viscosity (%) using the following formula (Tallalys and Buch 1970):

Loss in viscosity% = Tb _ Ts / Tb _ Tw x100
Where Tb = Time of flow of the blank, Ts = Time of flow of the sample, Tw = Time of flow of water.

Greenhouse experiments

Effect of the antioxidants, *i.e.* Coumarin, Argenin, Petrocin on root rot disease incidence of faba bean was studied in a pot experiment. Loamy soil was artificially infested individually (at the rate of 5% w: w and 10x108 cfu/gram medium) with the inoculum of tested fungus which had which previously been growing for two weeks on sand barley medium (1:1, w: w and 40% water) at 25±2 ℃. Faba bean seeds (cv. Giza 3) were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized water, then dried between two sterilized layers of filter paper.

The disinfected faba bean seeds were coated with different antioxidants tested at the different concentration. Seed dressing was carried out by applying the tested antioxidants to the gum moistened seeds in polyethylene bags and shaking well to ensure even distribution of the added materials. The treated seeds were then left on a plastic tray to airdried. In addition, disinfected, untreated faba bean seeds were sown as a control.

The infested soils were filled in plastic pots (40 cm in diameter) and sown with treated faba bean seeds relevant to the specific treatment. Ten faba bean seeds were sown in each pot and ten replicated pots were used for each particular treatment. The percentage of root rot disease incidence was calculated as the pre- and post emergence infection after 15 and 45 days of sowing date, respectively. Recorded data were calculated as the average percentages of pre- and post-emergence root rot incidence.

Combined application of coumarin as effective antioxidant and biocontrol agents *Trichoderma harzianum* for the management of *Fusarium solani* causing root rot in bean plants was done. The fungal inocula of *T. harzianum* was added to the soil drenched at the rate of 5% w.w. seed dressing was carried out by applying coumarin to the gum moistened seed in polyethylene bags and shaking well to insure even distribution of the added materials.

Statistical analysis

Tukey test for multiple comparison among means was utilized (Neler *et al*., 1985).

Results and discussions

In this study the inhibitory effect of antioxidants as Argenin,Petrocin and coumarin against the mycelia growth of pathogenic *F. solani* in table 1. Fungal mycelia growth decreased significantly as the concentrations of antioxidants were increased, to reach the fungal growth's minimum at the highest concentration used. The highly inhibition in fungal growth was observed with 20ppm of coumarin reduced mycelia growth by 83.3. These results are in agreement with (Hong-sheng *et al*., 2008) who found that the hyphal growth of Fusarium oxysporum niveum on plates was stopped at high (>400 mg 1-1) concentrations of Coumarin. In previous investigations, (Blum and Shafer, 1988). Coumarin was considered as a carbon source utilized by microbes in soil, so that it stimulated growth of microorganisms at low concentrations but inhibited growth at high concentrations. In contrast, current results demonstrate that such inhibition or stimulation did not result from its putative role as a carbon source for Fusarium spp. But may be ascribed to hormone – like action of coumarin. Further analysis showed that coumarin added to soil was affected by soil and other microbes that may explain the differences result reported in the literature. Coumarin that is released in root exudates and decayed plant residue is considered to have some specific actions on plants and the surrounding microorganisms. It has been isolated and identified as an allelochemical (Blum and Shafer, 1988). Mostly it has been studied as an inhibitor in the growth of other plants. However, it also appears to affect growth of pathogens (Patrik, 1971).As such, it could be considered as a signaling chemical resulting from the interactive process between the *Vicia faba* and *Fusarium spp*.

Table 1. Effect of different concentrations of Coumarin, Argenin and petrocin on reduction of linear growth of *Fusarium solani* causing root rot on Faba bean

Antioxidants	Concentration	Linear growth (mm)	Reduction (%)
Coumarin	5ppm	60d	33. 3
	10ppm	40f	55.6
	15ppm	25g	72.2
	20ppm	15i	83.3
Argenin	5ppm	85b	5.6
	10ppm	50e	44.4
	15ppm	35f	61.1
	20ppm	20g	66.7
Petrocin	5ppm	70c	77.7
	10ppm	55e	38.9
	15ppm	40f	55.5
	20ppm	25g	72.2
Control		90a	0.0

Figures with the same letters are not significant ($P \leq 0.05$).

Results in Table (2) Showed that different concentrations of the three antioxidants showed sharply decrease in enzymes activity upon increasing their concentrations from (5 to 15ppm.); the grate decreasing has been shown at 15ppm concentration of coumarin after 7 and 14 days (45.5 and 54.1) in comparing to control. Some hydrolytic enzymes secreted in the infecting progression of *Fusarium spp*. are also important pathogenic factors which are associated with pathogensis pectic and cellulolytic enzymes of phytopathogenic fungi are stimulated by the infection process in many plant diseases. They facilitate the penetration of the fungus into the plant by a hydrolysis of polymers (pectic substance, cellulose), which constitute the plant cell walls (Fuches *et al*., 1965). These observation would be the result of different mechanisms for the expression of these two enzymes in presence of coumarin. Different pathogenic enzymes activities would lead to different infection capability of *Fusarium spp*. In which diverse range of *Vicia fabae* resistance to the pathogen could result.

Table 2. Effect of different concentrations of Coumarin, Argenin and petrocin on Pectolytic and Cellulolytic activity of *Fusarium solani* in vitro tests

Antioxidants	Concentration	Cellulolytic			Polygalctronase	
		Days	Activity	Red. %	Activity	Red. %
	5ppm	7	80.9	8.7	70.1	26.2
		14	85.5	12.9	60.0	39.6
	10ppm	7	71.8	19.3	65.5	31.1
Argenin		14	83.5	15.0	68.3	31.2
	15ppm	7	68.8	22.3	58.3	38.6
		14	75.4	23.2	75.6	23.9
	5ppm	7	60.3	31.9	60.0	31.6
Coumarin		14	78.0	20.6	63.5	36.1
	10ppm	7	55.2	37.7	54.0	43.2
		14	65.0	33.8	55.7	43.9
	15ppm	7	48.6	45.1	40.0	57.9
		14	53.5	45.5	45.6	54.1
	5ppm	7	80.3	8.8	85.0	10.5
		14	88.5	9.9	97.2	2.1
Petrocin	10ppm	7	75.5	14.8	63.8	32.8
		14	80.8	17.7	80.3	19.1
	15ppm	7	60.6	31.6	55 .4	41.7
		14	78.3	20.3	72.0	27.5
Control		7	88.6	-----------	95.0	----------
		14	98.2	-----------	99.3	---------

Average of percentages the relative loss in viscosity of 1.2 % pectin solution after 3 hr. incubation with crude enzyme. Average of percentages the relative loss in viscosity of 1.2 % CMC solution after 3 hr. incubation with crude enzymes

Greenhouse experiments

Results of greenhouse experiment in Table (3) indicated that all treatments of antioxidants reduced the damping off disease incidence comparing with control. Results showed that treatment with coumarin was highly effective in reducing the incidence of pre emergence damping off after 15days(10.0,6.7 and 3.3) with increasing the concentration also at post emergence disease after 45 days reduced the DI and increased the reduction of DI (53.5,77.7 and 89.6) respectively on bean plants followed by Argenin , Petrocin and control. On the other hand Argenin showed moderate effect.

Combined application of coumarin as effective antioxidant and biocontrol agents *Trichoderma harzianum* for the management of *Fusarium solani* causing root rot in bean plants.

Table 3. Incidence of faba bean root rot caused by *Fusarium solani* in response to seed dressing with different concentration of antioxidants under greenhouse conditions

Treatment	Conc.	Pre emergency		Post emergency	
		DI	R%	DI	R%
Argenin	5ppm	16.7	37.5	16.0	49.7
	10ppm	13.3c	50.2	11.5d	63.8
	15ppm	6.7d	74.9	10.7d	66.4
Coumarin	5ppm	10.0c	62.5	14.8c	53.5
	10ppm	6.7d	74.9	7.1e	77.7
	15ppm	3.3e	87.6	3.4e	89.6
Petrocin	5ppm	23.3b	12.7	26.0b	18.2
	10ppm	10.0c	62.5	14.8c	753.5
	15ppm	6.7d	74.9	7.1e	77.7
CONTROL	-	26.7a	0.0	31.8a	0.0

Figures with the same letters are not significant ($P \leq 0.05$).
DI – disease incidence R- reduction over control

Data in Table (4) showed that combined treatment with *T.harzianum* with coumarin caused significantly reduction to pre and post emergence compared to control, coumarin or *Trichoderma harzianum* each alone. The respective average for pre emergence damping off was 6.7 and reduction 66.5 and 3.4, 86.4 for post emergence root rot diseases.

We concluded that coumarin is an allelochemical substance strongly inhibited *Fusarium solani.* It highly suppressed hyphal growth, the activities of pathogensis –related enzymes, also coumarin strongly stimulate the growth of plant and reduced the percentage of disease incidence of root rot causing by *Fusarium solani*.It mainly suppresed hyphal growth,and the activities of pathogenesis-related enzymes. While strongly stimulating mycotoxin yield.

Table 4. Incidence of faba bean root rot caused by *Fusarium solani* in response to seed dressings with different concentration of antioxidants and *T . harzianum* under green house conditions

Treatments	Pre emergency		Post emergency	
	DI	R%	DI	R%
Coumarin	6.7c	66.5	10.7c	57.2
Trichoderma harzianum	13.3b	33.5	15.4b	38.4
T.harzianum +Coumarin	6.7c	66.5	3.4d	86.4
CONTROL	20.0a	0.0	25.0a	0.0

Figures with the same letters are not significant ($P \leq 0.05$). DI –disease incidence R – reduction over control

References

Abdel-Kader, M. M. (1997). Field application of *Trichoderma harzianum* as biocide for control of bean root rot disease. Egyptian journal of phytopathology 2.

Abdel-Razik, A. A. (1970). The parasitism of white *Sclerotium cepivorum* Berk., the incitant of white rot of Onion. (Ph.D. Thesis). Faculty of tgricultureAssiut University.

Anonymous. (2006). Yearbook of Statistics of Ministry of Agriculture. Agricultural Economical and Statistical Department, Arab Republic of Egypt, Cairo. 238 pp.

Blum, U. and Shafer, S. R. (1988). Microbial populations and phenolic acids in soil. Soil Biology and Biochemistry 20:793-800.

Culter, H. G. and Culter, S. J. (1999). Biologically active natural productsAgrocemicals. CRC Press.

Elad, T., Chet, J. and Katan, J. (1980). *Trichoderma harzianum*: a biocontrol effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70:119–121.

Fuches, A., Jobsen, J. A. and Wouts, W. M. (1965). Arbanase in phytopathogenic fungi. Nature 206:714-715.

Hadar, Y., Chet, I. and Henis, Y. (1979). Biological control of *R. solani* damping-off with wheat bran culture of *Trichoderma harzianum.* Phytopathology 69:64–68.

Hadar, Y., Harman, G. E. and Taylor, A. G. (1984). Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. Phytopathology 74:106–110.

Hong-Sheng, W. U., Wasseem, R., Dong-Yang Liu, Cheng_Long, W. U., Ze-Shen Mao, Yang-Chun, X. U. and Qi-Rong, S. (2008). Allelopathic impact of artificially applied coumarin on *Fusarium oxysporum* f.sp. *niveum*. World Journal of Microbiology and Biotechnology 24:1297-1304.

Kasumbwe, K., Venugopala, K. N., Mohanlall, V. and Odhav, B. (2014) Antimicrobial and antioxidant activities of substituted halogenated coumarins. Journal of Medicinal Plant Research 8:274-281.

Khairy, E. M., Sammour, H. M., Ragheb, A., Ghandour, M. F. and Aziz, K. (1964). A laboratory manual of practical chemistry. Cairo, Egypt. pp. 1–142.

Nitiema, L. W., Savadogo, A., Simpore, J., Dianou, D. and Traore, A. S. (2012). In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. International Journal of Microbiological Research 3:183-187.

MacMillan, J. D. and Voughin, R. H. (1964). Purification and properties of a polyglacturonic acidtranseliminase produced by Clastridium multiformentans. Biochemistry 3:564–572.

McLean, K. L., Dodd, S. L., Sleight, B. E., Hill, R. A. and Stewart, A. (2004). Comparison of the behavior of a transformed hygromycin resistant strain of Trichoderma atoviride with the wild-type strain. New Zealand Plant Protection 57:72–76.

Morsy, K. M. M. (2005). Induced resistance against damping-off, and wilt diseases of lentil. Egyptian journal of phytopathology 33:53–63.

Patrik, Z. A. (1971). Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Science 111:13-19.

Sivan, A. (1987). Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. Plant Disease Journal 71:587–592.

Sivan, A. and Chet, I. (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum.* Journal of Phytopathology 116:39–47.

Tallalys, P. W. and Buch, L. V. (1970). Pectic enzymes produced by *Verticillium* species. Transactions of the British Mycological Society 55:367.

Venugopala, K. N, Rashmi, V. and Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity BioMed Research International Volume 2013:563-568.

Whipps, J. M. and Lumsden, R. D. (2001). Commercial use of fungi as plant disease biological control agents: status and prospects. Fungi as Biocontrol Agents: Progress, Problems and Potential. Wallingford, UK: CABI Publishing. 9–22 pp.

(Received 27 March 2014; accepted 30 June 2014)