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Full Length Research Paper

Toxicological Assessment of Cucumber Fruits Safety Infected with White Mold Disease in Rat Animal Feeding Model

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Abstract

The blood contents of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) increased obviously in the case of rat fed with cucumber fruits biologically controlled with two bioagents namely *Bacillus subtilis* and *Trichoderma deliquescen.* In contrast, Alkaline phosphatase (ALP), Blood Albumin and Total protein contents decreases as a result of rat fed on meal containing cucumber fruits treated with any of the two bioagents *B. subtilis* or *T. deliquescens*. This decrease was more obvious in the case of *B. subtilis*. Fruits treated with *T. deliquescens*, resulted in lower cholesterol level in the rats blood, this decrease was proportional to the concentration of treated fruits in a meal. For red blood cells (RBC's) and total platelet count (PLT), hemoglobin (HGB), Hematocrit (HCT) and total platelet count (PLT) there was decreased in the blood of the rats fed on meal containing fruits treated with *T. deliquescens* or *B. subtilis*. However, the white blood cell count (WBCs), increases gradually as the concentration of fruits treated with *T. deliquescens* increases but decreases in the case of *B. subtilis*.

Keywords: Biological control, *Sclerotinia sclerotiorum*, *Bacillus subtilis, Trichoderma deliquescen,* Liver enzymes.

INTRODUCTION

White mold of cucumber caused by *Sclerotinia sclerotiorum (S.sclerotiorum*) is a destructive disease causing great economic losses in greenhouses as well as in the field (Hanafy 2011), causing significant yield loss up to 80% in some countries (Tuncer and Damdere, 1997, Yanar and Onaran 2011). Many fungicides were successfully used to control White Rot diseases on cucumber (Leammlen 2001, Bradley et al. 2006).

During the last three decades, environmental pollution has attracted attention and became the main problem facing the world, especially food fungicide residues (Brooks and Roberts 1999, Veneziano et al. 2004). Substitution of the pesticides with other natural compounds has been considered as safe elements to plant insects and disease control and is of great demand. Many bioagents are isolated and tested for their plant diseases control efficiency with a high percentage of success (Pal and McSpadden, 2006, Moorman 2015). *Bacillus subtilis, Trichoderma deliquescens*, were tested against the white mold of cucumber caused by *Sclerotinia sclerotiorum* and gave satisfactory results (Hanafy 2011). Chang et al. (2002) found that 36 isolates of Trichoderma showed a strong inhibitory effect on *S. sclerotiorun* in vitro. Duncan et al. (2002) found that Pseudomonos spp. reduced disease severity of Sclerotinia head rot in sunflower. Savchuk and Fernando (2002) tested 4 antagonistic bacterial strains against *S.sclerotiorum* on canola and found that the treatments gave complete disease suppression.

Janik et al. (2019) mentioned that biological toxins are a heterogeneous group formed by living organisms. They defined these compounds as chemicals produced by living organisms and have toxic properties for another

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organism. Since one of the main modes of actions in biological control is the secretion of metabolites by bioagents such as antibiotics or toxins, thus, this work aims to study the possible toxicological side effect of bioagents on human health.

MATERIALS AND METHODS

Bioagent preparation

Healthy cucumber fruits cv. (Beta alfa) were collected from cucumber plants grown in a plastic house located in El-Garbeia, Governorate, Egypt. After one week of treating with *Bacillus subtilis* or *Trichoderma deliquescens* to control white mold of cucumber (Figure 1) caused by *Sclerotinia sclerotiorum* (Hanafy, 2011), five healthy cucumber fruits from each treatment weighted (about 200 g each). Also, five healthy fruits collected from untreated plants (to serve as control treatment) were labeled and preserved in a clean new polyethylene bags, then kept in a deep freezer.

Twenty-five white albino healthy male rats (a pure strain) of 3-4 months old with the average weight of 180-200 gm were purchased from the Faculty of Medicine, Tanta Univ., Egypt. The rats were reared under the laboratory conditions (25 ± 5 ^{o}C and 65 ± 5% RH) in metallic cages, fed daily with a balanced diet consisted of bread and untreated cucumber fruits, then the rats were made to fast for 4 days.

The treated and untreated cucumber fruit were macerated and homogenized. The rats were randomly divided into 5 groups (5 rats per group), then they were fed on the homogenized cucumber fruits mixed with a balanced diet of bread at the rate of 1: 2 and 1: 1 (each rats group received one concentration of the two treated fruits) and the fifth rats group received the homogenized untreated cucumber fruits mixed with the balanced diet of bread at the rate of 1:1. After 21 days, the rats were sacrificed, the blood from each group was collected in two tubes containing the coagulate substance (EDTA), one was centrifuged at 4500 rpm for 15 min to separate the serum from the plasma. The serum samples were kept at -4^{o}C for the measurement of different parameters.

Effect on liver functions

Transaminases determination including both Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activity were determined using diamond-diagnostics kits (Egypt) based on the method of Reitman and Frankel (1957) at 550 nm. However, Alkaline phosphatase (ALP) determinations was carried out according to Kind and King (1954) at 500 nm. Albumin (Alb) was determined according to Doumas and watstan (1971) method, at 623-630 nm. On the other hand, the total protein amount in the blood was determined using a diamond diagnostic kit according to the method of (Gornall et al. (1949) at 550 nm. Also, Cholesterol was determined using a diamond diagnostic kit according to Watson (1960), at 578 nm. Finally, Haematoxicity of certain treatments was done on albino rats**:**

The blood was collected in small glass vials containing EDTA as an anticoagulant for hematological studies, and then the measurement of blood constituents was done using Hematology analyzer system. The parameters were assessed in respect of complete blood count (CBC), comprised red blood cell (RBC’s), Hemoglobin concentration (HGB), Hematocrit (HCT), white blood cell count (WBC’s), total platelet count (PLT).

RESULTS AND DISCUSSION

Effect on liver functions

Aspartate Aminotransferase (AST)

Feeding the rats on meal containing different concentrations of fruits treated with *T. deliquescens* or *B. subtilis resulted* in a notable elevation of AST in the blood compared with the rats fed on meal containing 1: 2 (treated fruits/bread) and untreated fruits (the control treatment). This elevation was increased as the concentration of the treated fruits in the meal increased. *B. subtilis* resulted in the highest elevation of AST recording 50 and 67 IU/l. When the rats fed on the low (1:2) and high concentration (1:1) of fruits treated with this bioagent recorded an elevation of 67 and 123.3% respectively, compared with 30 IU/l in the control treatment. *T. deliquescens* resulting in moderate elevation (`40 and 46 IU/l) recording 33 and 53.3% respectively (Table 1).

Alanine Aminotransferase (ALT)

Similar data were recorded in the case of ALT, since *B. subtilis* resulted in the highest elevation of ALT when the rats fed on meal containing high (1:1) and low (1:2) treated fruits concentration recording 86 and 98 IU/l respectively compared with 39 IU/l of control, recording with 120.5 and 151.3% elevation; meanwhile, *T. deliquescens* resulted in moderate elevation (`48 and 51 IU/l) with 23.1 and 30.8% respectively (Table 1)**.**

Alkaline Phosphates (ALP)

The two treatments resulted in lowering ALP in the blood of both concentrations of the treated fruits in the meal and this lowering was proportional to the concentration of treated fruits in the meal (low 1:2 and high 1:1). The

Figure 1. Stem white rot of cucumber fruits infected with *Sclerotinia* sclerotiorum (Circle indicates the stem rot)

Table 1. Effect of feeding rats on meal mixed with cucumber fruits treated with the two bioagents *T. deliquescens and B. subtilis* at two concentrations i.e. 1:2 and 1:1 (fruits: bread) on the liver function of these rats.

liver functions	*T. deliquescens*				*B. subtilis*				Untreated (Control) 1:1
	Amount		Disruption %		Amount		Disruption %		
	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	
AST (IU/l)	40	46	33	53.3	50	67	67	123.3	**30**
ALT (IU/l)	48	51	23.1	30.8	86	98	120.5	151.3	**39**
ALP (IU/l)	125	118	-21.9	-26.3	115	111	-28.1	-30.6	**160**
Albumin (g/dl)	3.8	3.5	-13.6	-20.5	3.3	2.4	-25.0	-45.5	**4.4**
T. Protein (g/dl)	67	6.0	-1.5	-11.8	4.0	3.0	-41.2	-55.9	**6.8**
Cholesterol (g/dl)	160	144	-34.4	-41.0	205	300	-16.0	23.0	**244**

*= One portion of fruits collected from untreated plants to one portion of bread

least LPT was found in the blood of rats fed on meal containing fruits treated with *B. subtilis* recording 115 and 111 IU/l compared with 160 IU/l in control representing -28.1 and -30.6% decrease respectively followed by *T. deliquescens,* which recorded 125 and 118 IU/l representing -21.9 and -26.3% respectively (Table 1).

Albumin

Feeding the rats on meal containing fruits treated with *T. deliquescens* or *B. subtilis* resulted in lowering Albumin content in the blood. The highest reduction was found in the blood of rats fed on meal containing 1:1 fruits treated with *B. subtilis,* recording 2.4 g/dl (-45.5%) followed by 3.3 g/dl (-25.0%) in the blood of rats fed on meal containing 1:2 fruits treated with the same bioagent compared with 4.4 g/dl in control. *T. deliquescens* at both concentrations showed a moderate effect on ALP, recording 3.8 and 3.5 g/dl representing -13.6 and -20.5% respectively (Table 1).

Total Protein

Feeding rats on meal containing (1:2) fruits treated with *T. deliquescens,* showed no remarkable effect on total protein contents (6.7 g/dl), and a higher concentration of the treated fruits in a meal (1:1) resulted in lowering of the amount of protein (6.0 g/dl) compared with 6.8 g/dl in the control treatment, *B. subtilis* in a concentration of 1:2 and 1:1 resulted in a remarkable reduction of total protein (4.0 and 3.0g/dl) representing -41.2 and -55.9% respectively (Table 1).

Cholesterol

However, feeding the rats on meal containing fruits treated with *T. deliquescens,* resulted in lowering cholesterol level in the rats blood especially when the rats fed on meal containing high concentration (1:1) of the treated fruits recorded 144 g/dl compared with 244 g/dl in control treatment representing -41.05% reduction. When the rats fed on meal containing low concentration of fruits (1:2), moderate lowering of cholesterol content in the blood of these rats was found (160 g/dl representing -34.4% reduction). Feeding rats on meal with low concentration of fruits treated with *B. subtilis* (1:2) resulted in slight decrease of cholesterol, recording 205 g/dl representing -16%. In contrast, feeding rats on high concentration of fruits treated with *B. subtilis*, a remarkable elevation of cholesterol was noticed recording 300 g/dl (+23%) compared with 244 g/dl in control treatment (Table 1).

Haematotoxicity

Red blood cell (RBC's)

From table (2), red blood cell (RBC's) in blood of the rats fed on meal containing strawberries treated with *T. deliquescens* decreased; especially, when the concentration of the fruits in the meal increased from 1:2 to 1:1, recording 5.0, and 4.8 10^6 c/ccm respectively with decreasing percentage of -5.7 and -9.4% respectively. On the other hand, the rats fed on meal treated with *B. subtilis* at the rate of 1:2 recorded no effect, while increasing the rate of the treated fruit in the meal to 1:1 resulted in lower number of RBC in the blood recording 4.1 x 10^6 c/ccm compared with 5.3 in control treatment with decreasing percentage of -22.6%.

Hemoglobin Concentration (HGB)

Hemoglobin concentration was affected at the same trend as well as red blood cells. Feeding the rats on meal containing any of the two bioagents in both concentration resulted in lowering HGB, this decrease was proportional with the amount of the treated fruits in the rats meal, since, hemoglobin concentrations in blood of rats fed on meal containing low concentration (1:2) of fruits treated with one of *T. deliquescens,* or *B. subtilis* were found to be 12.9, 12.2 gm/dl respectively compared with 14.0 gm/dl in control treatment recording -7.9 and -12.9% respectively. These concentration of Hb decreased to 12.4 and 10.3 respectively while the concentration of the treated fruits in the meal increased (1:1) with decreased percentage of -11.4 and -26.4%.

Hematocrit (HCT)

Feeding the rats on meal containing fruits treated with one of *T. deliquescens* and *B. subtilis* resulted in lowering the percentage of Hematocrit (HCT), this lowering was proportional to the increased amount of the treated fruits in the rats meal, showing 31.2 and 29.6 % respectively at the low concentration (1:2) with disruption of -27.4 and -31.2% respectively and 28.5 and 25.2 % respectively at higher concentration (1:1) compared with 43.0 % in control treatment with disruption of -33.6 and -41.4% respectively .

White Blood Cell Count (WBC's)

Feeding the rats on meal fruits treated with one of the two tested bio agents resulted in different action on the white blood cell count, this effect differed according to the bioagent and concentration of the treated fruits in the meal. Meal containing fruits treated with *T. deliquescens* at low concentrations (1:2 and 1:1) resulted in increased number of the white blood cell, recording 4.90 and 7.00 x10^3 c/cmm respectively compared with 4.5 x10^3 c/cmm in control treatment with increasing percentage of 8.9 and 55.6%; while the meal with low concentration of fruits treated with *B. subtilis* (1:2), white blood cell number showed no clear effect 4.4 x10^3 c/cmm compared with 4.5 x10^3 c/cmm in control treatment. Increasing the concentration of fruits treated with the same bioagent resulted in increased number of the white blood cell to 6.8 x10^3 c/cmm compared with 4.5 x10^3 c/cmm in control treatment with increasing percentage of .51.1%.

Total platelet count (PLT*)*

The two tested bioagents, *T. deliquescens* and *B. subtilis,* in the two concentrations in the rat meal affected negatively the total platelet count (PLT) in blood of the rats fed on meal containing fruits treated with any one of these bioagents; this effect differed according to the concentration of the fruits in the meal. When the fruits treated with *T. deliquescens* was added to the meal at low concentration (1:2), slight decreased number of total platelet was recorded 300 x10^3 c./ccm compared with 354 x10^3 c/ccm in control treatment. Higher concentration of treated fruit with the same bioagent resulted in more decreased number of total platelet recording 219 x10^3 c/ccm with decreasing percentage of -38.1%. The most affected rats were those with low or higher concentration of fruits treated with *B. subtilis* recording 200 and 197 x10^3 c/ccm respectively compared with 354 x10^3 c/ccm in control treatment with decreasing percentage of -43.5and -44.4%.

Substitution of the pesticides with other natural compounds safe elements to control plant pests and diseases became a great demand to save environment and human health. Nowadays biological control is one of the promising approaches to the control of plant diseases. In this toxicological study, the obtained data indicated that feeding rats on meal containing with either low or high concentration of fruits treated with one of the two bioagents, *G. deliquescens* or *B. subtilis,* resulted in disturbances of amount of all the tested blood parameters. These disturbances were in proportion to the concentration of the treated fruit in meals. One of the

Table 2. Effect of feeding rats on meal mixed with cucumber fruits treated with the two bioagents *T. deliquescens and B. subtilis* at two concentrations i.e.1:2 and 1:1 (fruits: bread) on the blood picture of these rats.

Haemato-toxicity	*T. deliquescens*				*B. subtilis*				Untreated (Control) 1:1*
	Concentration		Disruption%		Concentration		Disruption %		
	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	
RBC's (c. x10^6/cmm)	5.0±0.3	4.8±0.23	-5.7	-9.4	5.3±0.3	4.1±0.44	0.0	-22.6	5.3±0.2
Hb. Conc. (Gm/dl)	12.9±0.2	12.4±0.36	-7.9	-11.4	12.2±1.9	10.3±0.57	-12.9	-26.4	14.0±0.6
HCT (%)	31.2±02	28.5±0.1	-27.4	-33.7	29.6±1.4	25.2±1.5	-31.2	-41.4	43.0±0.7
WBCs. Count (x 10^3/cmm)	4.9±0.20	7.0±0.57	8.9	55.6	4.40±0.2	6.80±0.4	-2.2	51.1	4.50±0.1
Platelets Count (x 10^3/cmm)	300±4.7	219±5.53	-15.3	-38.1	200±0.0	197±1.4	-43.5	-44.4	354±1.0

*= One portion of fruits collected from untreated plants to one portion of bread

main modes of action of the most bioagents is the production of some metabolites or mycotoxins. *Gliocladium deliquescens*, produces the metabolites lanosterol, dehydroxy-demethoxy viridin, demethoxy viridin and viridiol (Hanson et al. 1988). Demethoxy viridin and wortmannin inhibit superoxide production and block phospholipase C and D activation in the human neutrophil (Bonser et al. 1991). Since phospholipase D1 plays an important role in the development and progression of rat liver fibrosis (Zhu et al. 2014), it can be suggested that rats fed on meal containing fruits treated with *Trichoderma deliquescens* may suffered liver fibrosis, and in turn all the liver functions and blood parameters were disrupted. Also, Gliotoxin that produced by different spices of *Gliocladium and Trichoderma* i.e. *G. deliquescens, G. fimbriatum* and *G. virens* in addition to other fungi such as *Fusarium equiseti, Talaromyces flavus and Trichoderma viride* (Dolan et al. 2017, Hussain 1994, Anthony et al. 2009; Anonymous 2013). Gliotoxin is belonging to a class of fungal metabolites called epipolythiodioxopiperazines, those are characterized by a disulfide group that connects across the top of the molecule, this mycotoxin is an unusual, highly immunosuppressive mycotoxin (Dolan et al. 2017, Hussain 1994, Anthony et al. 2009, Anonymous 2013), and exposure to this mycotoxin (Gliotoxin) resulted in significant alteration of the expression of 27 proteins including de novo expression of Cu, Zn superoxide dismutase, up-regulated allergen Asp f3 expression and down-regulated catalase and a peroxiredoxin levels and significantly elevated glutathione GSH levels (Carberry et al. (2012), which explain the complete disruption of the liver function and blood picture.

Bacillus subtilis can produce more than two dozen antibiotics with an amazing variety of structures. The produced anti-microbial active compounds include predominantly peptides that are either ribosomal synthesized and post-translationally modified (L-antibiotic-like peptides or non-ribosomal generated), as well as a couple of non-peptidic compounds such as polyketides, an amino sugar, and a phospholipid (Stein 2005). Iturins are a family of lipopeptides produced by various strains of *Bacillus subtilis*. The antifungal activity of iturin is related to the interaction of the iturin lipopeptides with the cytoplasmic membrane of target cells, the K+ permeability of which is greatly increased. It has also been shown that active, iturins interact strongly with sterols, forming lipopeptide/cholesterol complexes (Maget-Dana and Peypoux 1994). On the other hand, researchers found that *B. subtilis* bacterium to be relatively benign in terms of human health, since is not known as a human pathogen or disease-causing agent (Caldwell et. al. 2004). Our data showed considerable disturbance of the blood tests parameters which may metabolites due to this bioagent. Qin Han *et al*. (2015) reported that, treatment with the iturins induced reactive oxygen species bursts, Hog1 mitogen-activated protein kinase (MAPK) activation and defects in cell wall integrity. Later, Torres et al. (2017) studied the potential toxic effects of cell-free supernatant (CFS), and purified lipopeptides fractions (LF) of *B. subtilis*, they found that values of GOT and GPT enzymes did not reveal significant changes; however, the histological analysis showed some signs of liver injury when CFS was administered; but, the liver injury in CFS-treated mice was significantly milder than those observed in the LF group. The two findings may explain the high amount of AST and ALT of cholesterol in the blood of the rats fed on meal containing with a high percentage of fruits treated with *B. subtilis* in our study. From the aforementioned mode of action of both bioagents metabolites, either the antibiotic or the toxin, before the introduction of new bioagent strains in plant protection; especially on crops that are periodically harvested and intensively studied toxicologically.

CONCLUSION

Substitution of pesticides with other safe natural products to control plant diseases has become of great demand to a safe environment and human health. However, this biological agent forming metabolites might accumulate inside the plants and lead to adverse effects on human health. It can be concluded from this study that Rat blood contents of liver enzymes AST and ALT increases as a result of feeding rats on meal containing cucumber fruits treated with one of the two bioagents (*B. subtilis* or *T. deliquescens*), however, ALP decreases. Blood Albumin and Total protein contents decreases as a result of feeding on meal containing cucumber fruits treated with any of *B. subtilis* or *T. deliquescens.* Feeding rats on fruits treated with *T. deliquescens*, results in lowering cholesterol levels in the rat's blood. Red blood cells (RBC's) and total platelet count (PLT), hemoglobin (HGB), Hematocrit (HCT) and total platelet count (PLT) decreases in the blood of the rats fed on meal containing fruits treated with *T. deliquescens* or *B. subtilis*.

The white blood cell count (WBCs), increases gradually as the concentration of fruits treated with *T. deliquescens* increases, but decreases in the case of *B. subtilis*. Based on these results, we recommend the complete avoidance of infected fruits from human consumption.

CONFLICT OF INTEREST

The authors declare no conflict of interests for this study

REFERENCES

Anonymous (2013). Gliotoxin, Trilogy Analytical Laboratory, Washington www.trilogylab.com.

Anthony MH, Ayinla GT, Helmina AO, Ezekiel SO,HarunaOG (2009). Health implications of toxigenic fungi found in two Nigerian staples: guinea corn and rice. African Journal of Food Science. 3, 250-256.

Bonser NTT, Randall RW, Tateson JE, Spacey GD, Hodson HF, Garland LG (1991). Demethoxyviridin and wortmannin block phospholipase C and D activation in the human neutrophil. Br. J. Pharmacol. 103,1237-1241

Bradley CA, Lamey HA, Endres GJ, Henson RA, Hanson BK, McKay KR, Halvorson M, LeGare DG, Porter PM. (2006). Efficacy of fungicides for control of Sclerotinia stem rot of canola. Plant Dis. 90,1129-1134.

Brooks GT, Roberts TR (1999). Pesticide Chemistry and Bioscience: The Food-Environment Challenge. The Royal Society of Chemistry, Cambridge (e-book).

Caldwell B, Rosen EB, Sideman E, Shelton AM, Smart DC (2004). Material fact sheet *Bacillus subtilis*, Organic Resource Guide pp 104 - 108 Cornell Cooperative Extension Publication, USA.

Carberry S, Molloy E, Hammel S, O'Keeffe G, Jones GW, Kavanagh K, Sean Doyle S (2012). Gliotoxin effects on fungal growth: Mechanisms and exploitation. Fungal Genet. Biol. 49, 302–312

Chang KF, Yang Y, Hwang SF, Howard RJ (2002). Biological control of S. sclerotiorum on Echinacea angustifolia. Can . J. Plant Pathol., Vol.24;215-220

Dolan SK, Bock T, Hering V, Owens RA, Jones GW, Blankenfeldt W, Doyle S (2017). Structural, mechanistic and functional insight into gliotoxin bis-thiomethylation in *Aspergillus fumigatus*. Open Biology 7,160292

Doumas B, Watstan W (1971). Colorimetric methods for determination of albumin in serum. Clin. Cheim. Acta. 31, 87 (Germany).

Duncan RW, Fernando WGD, Rasie KY (2002). Biological control for sclerotinia head rot (S. sclerotiorum) in sunflower. Can . J. Plant Pathol.,24:92-94.

Gornall AC, Barswaill CJ,David MM (1949). Colorimetric method for the determination of protein. J. Biol. Chem. 177, 751-766.

Hanafy MF. (2011). Induction of disease resistance of stem rot of some cucumber by some chemical agents and biological agents. Faculty of science, Tanta University. Tanta, Egypt, pp. 263.

Hanson JR, Margaret AO, Wadsworth JH, Boon LY (1988). The biosynthesis of the steroid, viridiol, by (*Gliocladium) deliquescens.* Phytochem., 27, 387–389.

Hussain H (1994). Effect of Soil Water Pressures on Population Dynamics of *Fusarium equiseti*, *Gliocladium virens*, *Talaromyces flavus* and *Trichoderma viride*, Biocontrol Agents of *Verticillium dahliae* in Potatoes, M.Sc. Thesis. Oregon State University, USA.

Janik E, Ceremuga M, Saluk-Bijak J, Bijak M (2019). Biological Toxins as the Potential Tools for Bioterrorism. Int J Mol Sci. 20(5), 1181

Kind BRN, King EG (1954). Determination of alkyl phosphatase activity in serum with 4-amino-antiberine. J. Clin. Pathology. 7, 332.

Leammlen F (2001). Sclerotinia Diseases. Publication 8042, University of California, Agriculture and Natural Resources. Cooperative Extinction Farm Advisor.

Maget-Dana R, Peypoux F (1994). Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties. Toxicol. 87,151–174

Moorman G (2015). Biological Control of Plant Diseases (Greenhouse). Penn State College of Agricultural Sciences research and extension programs are funded in part by Pennsylvania.

Pal KK, McSpadden GB (2006). Biological Control of Plant Pathogens. The Plant Health Instructor DOI: 10.1094/PHI-A-2006-1117-02.

Qin H, Fengli W, Xiaonan W, Hong Q, Liang S, Ang R, Qinghai L, Mingwen Z, Canming T (2015). The bacterial lipopeptide iturins induce *Verticillium dahliae* cell death by affecting fungal signaling pathways and mediate plant defense responses involved in pathogen-associated molecular pattern-triggered immunity. Environ. Microbiol. 17 (4), 1166–1188.

Reitman SMD, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28, 56-63.

Savchuk S, Fernando GD (2002). Microscopic and greenhouse evaluation of biological control of S. sclerotiorum on canola . Can . J . Plant . Pathol . Vol . 24 149-156.

Stein T (2005). Bacillus subtilis antibiotics: structures, syntheses and specific functions: Bacillus subtilis antibiotics. Literature Review *in* Mol Microbiol., 56(4), 845-57.

Torres MJ, Villena J, Paola BP, Fanciotti MN, Audisio MC (2017). Safety Assessment of the Antimicrobial Lipopeptides Synthesized by *Bacillus subtilis* Subsp. *subtilis* CBMDC3f. EC Microbiology 8 (3), 113-122.

Tuncer FE, Damdere H (1997). Biological control of white mold (*Sclerotinia sclerotiorum* (Lib.) De Bary) diseases of vegetables grown in greenhouses of Antalya-Turkey. http://www.tagem.gov.tr/projeler/97/bsag/bsag l8.html.

Veneziano A, Arana GVS, De Simone F, Rastrelli L (2004). Determination of carbendazim, thiabendazole and thiophanate-methyl in banana (Musa acuminata) samples imported to Italy. Food Chem.87, 383–386.

Watson D (1960). A simple method for the determination of serum cholesterol. Clin. Chim. Acta. 5, 637: 643

Yanar Y, Onaran A (2011). Mycelial compatibility groups and pathogenicity of *Sclerotinia sclerotiorum* (Lib.) De Bary causal agent of white mold disease in greenhouse grown cucumber in Antalya-Turkey. Afr Biotech. 10, 3739-3746.

Zhu X, Liu R, Kuang D, Liu J, Shi X, Zhang T, Zeng Y, Sun X, Zhang Y, Yang W (2014). The role of phospholipase D1 in liver fibrosis induced by dimethylnitrosamine *in vivo*. Dig Dis Sci. 59, 1779-1788.

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