IMPACT OF ANTIOXIDANTS AND MICRONUTRIENTS AS FUNGICIDES ALTERNATIVES IN IMPROVEMENT OF ANTAGONISM OF *TRICHODERMA* spp. AGAINST *SCLEROTINIA SCLEROTIORUM*

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SUMMARY

S. sclerotiorum is a strong and resident soil pathogen with high resistance to synthetic fungicides. The biocontrol agent *Trichoderma* spp. alone provides moderate control of this fungus. In present study, potassium tartrate, ascorbic acid, folic acid, thiamine and micronutrients mixture were used to improve the number of spores, fresh and dry weights, crude extracts, volatile compounds, and secondary metabolites produced by four isolates of *Trichoderma* against pathogen. A onetime amendment of thiamine (0.1%) or micronutrients mixture improve the antagonism efficacy of most *Trichoderma* isolates showing complete prevention of pathogen growth and inhibition of sclerotia formation.

Keywords: sclerotia formation, thiamine, *Trichoderma* species, secondary metabolites.

INTRODUCTION

In Egypt and worldwide, *Sclerotinia sclerotiorum* is capable of causing significant yield losses in numerous crops through infection of flowers, leaves, fruits and stems (Attanayake *et al*., 2013). The host range is rather extending and covering 400 plant species worldwide (Garg *et al*., 2010). The fungus differentiates and propagates by forming sclerotia which are crucial in its survival for long periods of unfavorable conditions (Bolton *et al*., 2006). Chemical management is largely unreliable as fungicide-resistant strains of the pathogen may develop (Li *et al*., 2008). All these reasons are driving the researchers from all over the world to develop alternative methods which are, by definition, safe in the environment and are rapidly biodegradable. One such strategy is use of biocontrol agents, but for example, biological control of soil-borne diseases of bean provides a moderate level of disease suppression in both greenhouse and field (Duffy *et al*., 1997). For improving the efficiency of bioagents, several reports assess the addition of some chemical compounds. In this connection, Alejandro and Blanka (2011) evaluated the effect of $KHCO_3$ in the growth and development of *Sclerotinia cepivorum* and on its interaction with *Trichoderma* strain R39. Charles *et al*. (1997) studied the biocontrol efficacy of *T. virens* in combination with fungicides against cotton seedling disease pathogens.

Humic and folic acids with furfural and *T. harzianum* as soil drench resulted in high reduction in the incidence of root rot of cantaloupe grown under plastic house conditions comparing with fungicides treatment (El-Mougy *et al*., 2014). Sodium bicarbonate and micronutrients mixture formula showed synergistic effect with *T. harzianum* against white mold of bean (Yousef *et al*., 2015). Addition of micronutrients, particularly boron, increased the biocontrol activity of *T. viride* against *F. oxysporum* f. sp *cubense* (Sanjeev, 2008). In our previous work Yousef *et al*. (2013) noticed the direct effect of some micronutrients mixtures and antioxidants which showed good results in inhibiting the growth and sclerotia formation of *Rhizoctonia solani*. In the present study, improving the growth and antagonicity of four *Trichoderma* spp. against *S. sclerotiorum* growth and sclerotia formation by simple addition of antioxidants and micronutrients are discussed.

MATERIALS AND METHODS

Fungal isolates. Isolates of *T. harzianum* (T1, T4 &T8) and *T. koningii* (T6) were isolated from the rhizosphere of healthy bean at Ismailia governorate, Egypt. They were kindly identified by Mycological Research and Plant Disease Survey Department, Plant Pathology Research Institute, A.R.C., Giza, Cairo, Egypt, according to Park *et al*. (2005). The causal organism of bean white mold was isolated from naturally infected plant collected from cultivated fields in Ismailia governorate, Egypt. The isolated

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Table 1. Effect of some antioxidants and micronutrients on linear growth, spore number, fresh and dry weights of *Trichoderma* spp. after 10 days of incubation.

Isolates	Treatments	LG*	Spore N. (x10^{-6})	Fresh w. (gm)	Dry w. (gm)	Increase in dry w. (fold)
	Control**	6.00 e	15.7 op	3.29 B	0.12 F	
	K tartrate	6.68 c	26.3 mn	7.19 x	0.53 s	3.4
T. harzianum	Micronutrients	6.43 d	21.0 nop	6.13 A	0.34 B	1.8
T1	Ascorbic acid	6.60 cd	13.3 p	6.44 z	0.41 x	2.4
	Folic acid	6.85 b	20.7 nop	6.99 y	0.49 v	3.1
	Thiamine	9.00 a	23.0 no	8.62 w	0.56 p	3.7
	Control	4.00 n	47.0 kl	12.00 s	0.35 A	
	K tartrate	9.00 a	68.3 abcd	19.64 c	0.63 l	1.8
T. harzianum	Micronutrients	4.17 mn	66.7 bcde	20.00 a	0.64 k	1.8
T4	Ascorbic acid	9.00 a	41.7 l	18.00 f	0.32 C	0.0
	Folic acid	9.00 a	71.7 abcd	13.47 mn	0.76 d	2.2
	Thiamine	4.50 jkl	70.7 abcde	19.30 d	0.72 e	2.1
	Control	3.50 qr	56.3 ij	18.90 e	0.49 v	
	K tartrate	9.00 a	56.3 ij	13.17 o	0.66 i	1.4
T. koningii	Micronutrients	4.33 lm	68.3 abcd	14.19 k	0.67 h	1.4
T6	Ascorbic acid	4.80 h	68.7 abcd	15.69 i	0.77 c	1.6
	Folic acid	5.43 f	59.7 ghi	20.03 a	0.51 t	1.1
	Thiamine	4.53 ijk	67.3 abcd	12.47 q	0.59 o	1.2
	Control	4.10 n	49.3 jk	13.05 m	0.30 D	
	K tartrate	4.50 jkl	49.7 jk	11.11 t	0.54 r	0.8
T. harzianum	Micronutrients	4.33 lm	65.3 bcd	11.20 t	0.65 j	1.2
T8	Ascorbic acid	4.37 kl	50.0 jk	9.80 v	0.23 E	0.0
	Folic acid	4.73 h	60.7 fghi	14.20 k	0.36 z	0.2
	Thiamine	4.67 hij	64.7 defghi	16.00 h	0.41 x	0.4

*linear growth. **untreated.

fungus was identified based on cultural and microscopic morphological characters (Hanlin and Richard, 1998).

Effect of antioxidants and micronutrients on growth and sporulation of *Trichoderma* spp. The mycelial growth of *Trichcoderma* isolates was studied in gliotoxin medium (GM) ammonium tartrate, 2 g/l; dipotassium hydrogen orthophosphate, 2 g/l; glucose, 25 g/l; ferrous sulfate, 1 mg/l and agar, 20 g/l dissolved in distilled water. Tested compounds are potassium tartrate (A), 300 mg/l, ascorbic acid (A.A) 100 mg/l, folic acid (FA) 20 mg/l, mixture of micronutrients (MN) containing Zn sulfate 500 mg/l, Mn sulfate 500 mg/l, Cu sulfate 100 mg/l, boric acid 100 mg/l and selenium 1.0 ppm, thiamine (TH) 100 ppm and combination of A and MN was added to sterilized GM. The prepared media were poured in Petri dishes. The mycelial disc of each *Trichoderma* isolate was placed in the center of the Petri plate and incubated at 23°C. The growth diameter for each *Trichoderma* spp. was measured. To determine the number of spores, for each isolate, the fungal growth on GM medium was filtered and the filtrate of each treatment was used to prepare serial dilutions up to 10^{-6}, poured in plate and incubated at 23°C. Growing colonies were counted daily and expressed as colony forming units (CFU). Three replicates for each treatment were used.

Effect of antioxidants and micronutrients on antagonistic potential of *Trichoderma* spp. against *S. sclerotiorum*. The antagonistic potential was studied by using the dual culture technique (Cherif and Benhamou, 1990) in Petri dishes with PDA medium. A pathogen disc (5 mm in diam.) was equidistantly placed on the other side of the Petri dish, facing the desired *Trichoderma* disc. The control was a disk from the untreated *Trichoderma*. The plates were incubated at 23°C and the mean colony diameter of the pathogen was measured. The percent of inhibition growth was calculated. The degree of antagonism between each *Trichoderma* isolates and the pathogen was scored on scale of 1-5 as proposed by Bell *et al.* (1982).

Effect of alternative fungicides on the activity of *Trichoderma* spp. crude extracts against *S. sclerotiorum*. *S. sclerotiorum* growth inhibition by substances secreted by *Trichoderma* spp. in liquid medium was determined as follows. In 250 ml Erlenmeyer flasks, 50 ml of GM was amended with each of the tested compounds and was inoculated with two discs of active mycelium of each *Trichoderma* isolate and incubated at 23°C for 14 days. The supernatant was filtered, centrifuged at 10,000 rpm for 20 min and sterilized by Millipore membrane (0.25 µm) filtration. Twenty ml of the filtrate was mixed with 80 ml PDA medium. The PDA medium containing the filtrated *Trichoderma* supernatant poured in Petri dishes was inoculated with active mycelium of *S. sclerotiorum* (5 mm disk) and incubated at 23°C. When the control treatment was completely covered, the growth was measured and the results were expressed as the percentage of mycelial growth inhibition relative to the control (Carisse *et al*., 2001). The

number and weight of sclerotia of the fungus were determined after 10 days.

Effect of alternative fungicides on the activity of *Trichoderma* spp. volatile compounds against *S. sclerotiorum*. The effect of volatile compounds produced by *Trichoderma* isolates, e.g. grown in liquid media supplemented by the antioxidants, was determined using the technique of Boubekeur *et al.* (2012). A 5 mm disc from the pathogen culture and another disc of treated *Trichoderma* were put each in the center of a Petri dish containing PDA medium. The lids were removed aseptically and the bottom of each dish containing the antagonist was placed inverted on that contain the pathogen and was scaled tightly by three layers of parafilm to prevent leakage of volatile substances. Petri dishes containing PDA without antagonist served as control. The average growth diameter of growth in the treatments was measured after 10 days of incubation at 23°C. The effect of volatile material was expressed in terms of mycelial growth inhibition, number and weight of sclerotia.

Effect of alternative fungicides on the activity of *Trichoderma* spp. secondary metabolites against *S. sclerotiorum*. Treated *Trichoderma* isolates were cultured on GM overlaid with a sterilized cellophane membrane (Dennis and Webster, 1971). A mycelial disc (5 mm in diameter) of treated and untreated *Trichoderma* was cut from the margin of an actively growing culture and placed on the center of cellophane overlaid GM. After incubating the plates at 23°C in dark for 2 days, the cellophane membranes were removed and a 5 mm disc of *S. sclerotiorum* culture was placed at the center of each plate. The plates were incubated at 23°C for 7 days and the diameter of the *S. sclerotiorum* colony in each plate was measured. The number and weight of formed sclerotia were also determined.

Statistical analysis. Treatments were arranged in completely randomized blocks design. The means were compared using Duncan multiple range test at probability level ($P) \leq 0.05$, using the statistical analysis software CoStat (version 6.4).

RESULTS AND DISCUSSION

Effect of antioxidants and micronutrients on improving growth and sporulation of *Trichoderma* spp. The data in Table 1 show the significant effect of some antioxidants and micronutrients on linear growth, spore number, fresh and dry weights of four *Trichoderma* spp. The mean colony diameter of *T. harzianum* T1 in the thiamine amended agar medium recorded 9 cm compared to 6 cm in control one (Fig. 1) with 3.7 fold increase in dry weight. Potassium tartrate had a positive effect on growth of the isolates *T. harzianum* T4 and *T. koningii* T6. In addition, folic acid and thiamine lead to 2.2 and 2.1 fold increase in dry weight of *T. harzianum* T4 respectively. On the contrary, *T. harzianum* T8 was slightly affected by the addition of antioxidants.

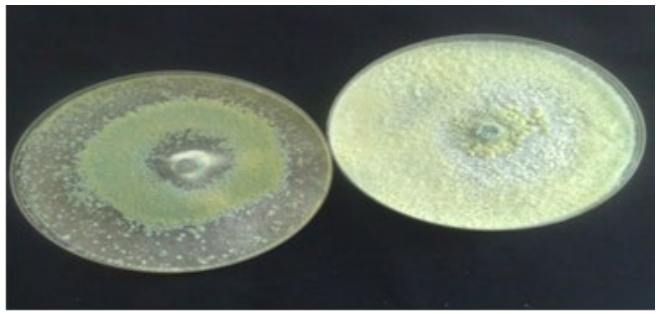

Fig 1. Effect of thiamine in improving *T. harzianum* (T1) growth, the plate in left without thiamine and the right plate treated with thiamine.

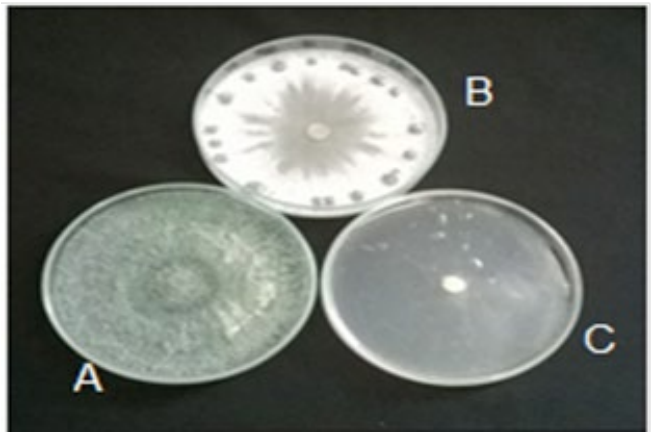

Fig. 2. PDA plates showing the efficiency of volatile compounds of compared with untreated one, A, potassium tartrate treated *T. harzianum* T8; B, control *S. sclerotiorum*; C, complete inhibition of B by the action of volatile compounds of A.

The positive effect of antioxidants and micronutrients was an expected result as most of them improved the metabolic activity and can be used by the fungus as a nutrients or coenzyme. Ivashechkin *et al.* (2015) mentioned that addition of an antioxidant increased the biomass yield of *Lentinus tigrinus*, as well as *Cunninghamella japonica*.

Effect of antioxidants and micronutrients on improving antagonism degree of different *Trichoderma* spp. against *S. sclerotiorum*. Table 2 shows a great variation in the antagonistic effect of treated and untreated *Trichoderma* spp. against *S. sclerotiorum*. Where the antagonism of *T. harzianum* T1 was not affected by treatment, the untreated *T. harzianum* T4, *T. koningii* T6 and *T. harzianum* T8 revealed 59.2%, 41.9% and 44.4% of *S. sclerotiorum* inhibition respectively. Addition of potassium tartrate, micronutrients and ascorbic acid enhanced the antagonism leading to complete inhibition of *S. sclerotiorum* growth.

Table 2. Effect of antioxidants and micronutrients on antagonism degree of different tested *Trichoderma* spp. against *S. sclerotiorum* after 7 days of incubation.

Isolates	Treatments	%inhibition of *S. sclerotiorum*	Bell scale
T. harzianum T1	Control	74.4	2 c
	K tartrate	57.4	3 b
	Micronutrients	75.6	2 c
	Ascorbic acid	81.5	2 c
	Folic acid	72.2	2 c
	Thiamine	72.2	2 c
T. harzianum T4	Control	59.2	3 b
	K tartrate	100.0	1 d
	Micronutrients	100.0	1 d
	Ascorbic acid	100.0	1 d
	Folic acid	83.3	2 c
	Thiamine	83.3	2 c
T. koningii T6	Control	41.9	3 b
	K tartrate	100.0	1 d
	Micronutrients	100.0	1 d
	Ascorbic acid	100.0	1 d
	Folic acid	83.3	2 c
	Thiamine	100.0	1 d
T. harzianum T8	Control	44.4	3 b
	K tartrate	100.0	1 d
	Micronutrients	100.0	1 d
	Ascorbic acid	100.0	1 d
	Folic acid	**100.0**	**1 d**
	Thiamine	**100.0**	**1 d**

Thiamine showed the same effect with *T. koningii* T6 and *T. harzianum* T8, whereas folic acid showed it with *T. harzianum* T8 only. The mechanisms of action of *Trichoderma* against fungal pathogens include secretion of antibiotics, competition for space and nutrients and production of lytic enzymes (Harman *et al*., 2004) and this can be improved the biocontrol potential of *T. viride* against *M. phaseolina* in micronutrients amended medium (Sundaravadana and Alice, 2006) as *Trichoderma* spp. prefer and grow well in acidic pH and in soil or media having high organic matter (Upadhyay and Rai, 1979).

Effect of fungicides alternative on improving crude extract, secondary metabolites and volatile compounds activity of *Trichoderma* spp. against *S. sclerotiorum*. According to Table 3, using *Trichoderma* crude extracts, the pathogen linear growth is completely suppressed with folic acid and thiamine addition in variants with *T. harzianum* T1. By *T. koningii* T6 the 100% efficiency is noted with all additives and by *T. harzianum* T8 as well, except potassium tartrate. The secondary metabolites of *T. harzianum* T4 greatly enhanced by potassium tartrate from no effect to complete linear growth inhibition of the pathogen. Comparing to previous results, volatile compounds of *Trichoderma* species are slightly affected by the additives except *T. harzianum* T8 which showed complete linear growth

Table 3. Effect of antioxidants and micronutrients on the activity of antagonistic crude extract, secondary metabolites and volatile compounds of *Trichoderma* spp. against *S. sclerotiorum* after 10 days of incubation.

Isolates		%inhibition of L.G.		
		C. E	S. M	V. C
T. harzianum T1	Control (T1 untreated)	70	77.8	51.5
	K tartrate	0.0	0	1.9
	Micronutrients	80	0	21.9
	Ascorbic acid	70	77.8	5.6
	Folic acid	100	100	0
	Thiamine	100	100	76.7
T. harzianum T4	Control (T4 untreated)	0.0	0	0
	K tartrate	100	100	0
	Micronutrients	0.0	0	13.9
	Ascorbic acid	0.0	0	70.4
	Folic acid	70	61.0	7.4
	Thiamine	0.0	0	0
T. koningii T6	Control (T6 untreated)	80	77.8	0
	K tartrate	100	100	0
	Micronutrients	100	100	27.8
	Ascorbic acid	100	100	0
	Folic acid	100	100	0
	Thiamine	100	100	0
T. harzianum T8	Control (T8 untreated)	80	77.8	6.5
	K tartrate	80	77.8	100
	Micronutrients	100	94.4	100
	Ascorbic acid	100	100	75.6
	Folic acid	100	100	77.0
	Thiamine	100	94.4	100

C.E = crude extract, S.M = secondary metabolites and V.C = volatile compounds.

inhibition of the pathogen with the addition of potassium tartrate, micronutrients and thiamine.

All *Trichoderma* isolates with all antioxidants (Table 4) showed great effect in the new sclerotia formation by the pathogen. Crude extracts, secondary metabolites and volatile compounds of *T. harzianum* T1 are very effective in inhibition of sclerotia formation of the pathogen. Addition of antioxidants have no remarked effect in its efficiency except thiamine. Addition of all antioxidants to *T. koningii* T8 caused complete inhibition of new sclerotia formation. The activity of secondary metabolites of *T. koningii* T6 is high and further increased to inhibit completely the new sclerotia formation with addition of potassium tartrate and micronutrients mixture where the fungus have no inhibition effect by its volatile compounds in untreated case, the addition of micronutrients steeply increased it to 100% efficiency.

In discussion of these results there are several lines of evidence. Morid and Zafari (2013) found that the highest specific activity of the chitinase enzymes was found in isolates *T. brevicompactum* and *T. koningiopsis* grown in medium containing manganese micronutrient. Peciulyte and Lugauskas (2000) reported that antifungal activity

Table 4. Effect antioxidants and micronutrients on improving crude extract, secondary metabolites and volatile compounds activity of *Trichoderma* spp. against production and weight of *S. sclerotiorum* sclerotia.

Isolates	Treatments	% inhibition of sclerotia production			Wt. of sclerotia (gm)		
		C.E	S.M	V. C	C. E	S. M	V. C
	S. sclerotiorum	0.0	0.0	0.0	0.8 a	0.5 a	0.6 a
T. harzianum T1	Control	78	100	87	0.1 b	0 i	0.1 mn
	K tartrate	63	64	72	0.1 b	0.1 f	0.21 e
	Micronutrients	68	61	72	0.1 b	0.1 d	0.13 hi
	Ascorbic acid	70	89	75	0.1 b	0.005 i	0.15 fg
	Folic acid	65	93	71	0.1 b	0.005 i	0.21 e
	Thiamine	67	100	100	0.09 b	0 i	0 o
T. harzianum T4	Control	34	29	62	0.28 a	0.23 b	0.17 f
	K tartrate	47	100	64	0.18 a	0 i	0.22 de
	Micronutrients	44	72	100	0.23 a	0.12 d	0.1 kl
	Ascorbic acid	49	57	100	0.14 a	0.12 d	0 o
	Folic acid	54	78	87	0.12 a	0.073 h	0.08 lm
	Thiamine	35	56	100	0.27 a	0.203 c	0 o
T. koningii T6	Control	51	92	0	0.13 a	0.003 i	0.2 e
	K tartrate	59	100	67	0.11 a	0 i	0.31 b
	Micronutrients	58	93	100	0.10 a	0.003 i	0 o
	Ascorbic acid	43	100	37	0.17 a	0 i	0.09 jk
	Folic acid	66	89	7	0.11 a	0.002 i	0.23 cd
	Thiamine	56	93	13	0.12 a	0.003 i	0.21 e
T. harzianum T8	Control	56	100	12.7	0.13 a	0 i	0.25 c
	K tartrate	62	96	100	0.10 a	0.001 i	0 o
	Micronutrients	51	93	100	0.15 a	0.001 i	0 o
	Ascorbic acid	25	100	100	0.32 a	0 i	0 o
	Folic acid	54	93	100	0.12 a	0.003 i	0 o
	Thiamine	58	100	100	0.12 a	0 i	0o

against 13 fungal species was dependent on copper sulfate or copper oxychloride concentration in the medium. Metal ions may influence the growth rate, mass, sporulation and enzymatic activity of produced fungal mycelium (Gadd, 1993). In case of potassium tartrate, potassium is the most abundant cation in all living cells and several basic physiological functions such as protein synthesis and enzyme activation require relatively high concentrations. To fulfill this basic physiological function, fungi use a diversity of potassium uptake systems (Rodriguez-Navarro, 2000) and the great variation in response of *T. harzianum* T1 and T4 (Table 3) can be explained by the demonstration of Olivier *et al.* (1998) and Fagundes *et al.* (2013) that microbial strains varies in response to different organic and inorganic salts.

Sclerotia differentiation of the pathogen is triggered by high oxidative stress, Georgiou (1997) found that sclerotial biogenesis in *S. rolfsii* was accompanied by the accumulation of high levels of peroxidized lipids. This theory was supported by other studies showing that vitamin C and carotene as a hydroxyl radical scavengers inhibited sclerotial differentiation of *S. rolfsii*, *S. minor*, *S. sclerotiorum*, and *R. solani* (Georgiou *et al.*, 2006) and by relating the thiolredox state of these fungi to their sclerotial differentiation (Patsoukis and Georgiou, 2008). Cherednichenko *et al.* (2002) found that beta-carotene increased the viability of *T. reesei* 6/16 protoplasts to the greatest extent and the effect of ascorbic acid depended on the presence of Fe ions. Moreover, addition of antioxidants at concentrations less than 5 mM can lead to fungal growth, increase resistance structures, and stimulate secondary metabolite gene expression and accumulation by *Aspergillus flavus* RCP08108 (Nesci *et al.*, 2003).

The advantage of *T. harzianum* isolates can be explained by its production of vast types of secondary metabolites as harzianic acid, dimethyl-harzianic acid, homoharzianic acid, 1-hydroxy-3-methyl-anthaquinone, 1,8-dihydroxy3-methyl-anthraquinone, harzianolide, trichoharzin, harziandione, cyclonerodiol, harzianopyridone, melanoxadin, trichodenones A, B and C, harziphilone, fleephilone, mevalonolactone, harzianolide, MR566B, trichosetin, trichorzianines A, B, trichokindins I-VII, harzianins HC, trichorozins I-IV, isonitrin A and D (Keswani *et al.*, 2014). In support of our results, Reverberi *et al.* (2005) documented a link between increased secondary metabolites production and oxidative stress based on chemical induction.

Our results put the studied antioxidants and micronutrients in enhancers list which play a positive role in production of primary and secondary metabolities of

Trichoderma spp. involved in biocontrol of *S. sclerotiorum* growth and its sclerotia formation and differentiation.

This work will be extended to gene expression and enzymatic analysis studies besides scanning and transmission electron microscope imaging to better understand the enhancing mechanism of this bioagent.

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REFERENCES

Alejandro A., Blanka L., 2011. Effect of potassium bicarbonate on fungal growth and sclerotia of *Sclerotinia cepivorum* and its interaction with *Trichoderma*. *Revisia Mexicana de Micologica* **33**: 53-61.

Attanayake R.N., Carter P.A., Jiang D., Del Ro-Mendoza L., Chen W., 2013. *Sclerotinia sclerotiorum* populations infecting canola from China and the United States are genetically and phenotypically distinct. *Phytopathology* **103**: 750-761.

Bell D.K., Well H.D., Markham C.R., 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology* **72**: 379-382.

Bolton D.M., Thomma J.H.P.B., Nelson D.B., 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Molecular Plant Pathology* **7**: 1-16.

Boubekeur S.B., Mahiout D., Benzohra I.E., Benkada M.Y., 2012. Antagonism of three *Trichoderma* species against *Botrytis fabae* and *B. cinerea,* the causal agents of chocolate spot of faba bean (*Vicia faba* L.) in Algeria. *World Applied Science Journal* **17**: 278-283.

Carisse O., EL-Bassam S., Benhamou N., 2001. Effect of *Microsphaeropsis* sp. Stain P130 A on germination and production of sclerotia of *Rhizoctonia solani* and interaction between the antagonist and the pathogen. *Phytopathology* **103**: 782-791.

Charles R.H., James E.D., Richard H.G., William E.B., 1997. Field control of cotton seedling diseases with *Trichoderma virens* in combination with fungicide seed treatments. *The Journal of Cotton Science* **1**: 15-20.

Cherednichenko O.V., Zvereva E.A., Shumaev K.B., Kozlov I.P., Rabinovich M.L., 2002. Effect of antioxidants on regeneration of protoplasts in the mycelial fungus *Trichoderma reesei* 6/16. *Prikladnaia biokhimiia i mikrobiologiia.* **38**: 482-485.

Cherif S.S., Benhamou C.S., 1990. Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* spp., on *Fusarium oxysporum* f. sp. *radicans – Lycopersici*. *Phytopathology* **80**: 1406-1414.

Dennis C., Webster J., 1971. Antagonistic properties of species groups of *Trichoderma*: 1. Production of non–volatile antibiotics. *Transactions of the British Mycological Society* **57**: 25-39.

Duffy B.K., Ownley B.H., Weller D.M., 1997. Soil chemical and physical properties associated with suppression of take–all of wheat by *Trichoderma koningii*. *Phytopathology* **87**: 1118-1124.

El-Mougy N.S., Abdel-Kader M.M., Lashin M.S., 2014. Fungicides alternatives for controlling cantaloupe root rot incidence under plastic houses conditions. *IJEIT* **3**: 319-323.

Fagundes C., Pérez M.B., Monteiro A.R., Palou L., 2013. Antifungal activity of food additives in vitro and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria alternate* on cherry tomato fruit. *International Journal of Food Microbiology* **166**: 391-398.

Gadd G.M., 1993. Interaction of fungi with toxic metals. *New Phytologist* **124**: 25-60.

Garg H., Li H., Sivasithamparam K., Kuo J., Barbetti M.J., 2010. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a susceptible genotype of *Brassica napus*. *Annals of Botany* **106**: 897-908.

Georgiou C.D., 1997. Lipid peroxidation in *Sclerotium rolfsii*: a new look into the mechanism of sclerotial biogenesis in fungi. *Mycological Research* **101**: 460-464.

Georgiou C.D., Patsoukis N., Papapostolou I., Zervoudakis G., 2006. Sclerotial metamor-phosis in filamentous fungi is induced by oxidative stress. *Integrative and Comparative Biology* **46**: 691-712.

Hanlin R.T., 1998. Combined keys to illustrated genera of ascomycetes volumes I & II. APS Press, St. Paul, MN, USA.

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M., 2004. *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nature Review of Microbiology* **2**: 43.

Ivashechkin A.A., Sergeeva Y.E., Lunin V.V., Mysyakina E.P., 2015. Antioxidant effect on the growth and formation of lipids in the fungus *Lentinus tigrinus* growing on medium with lignosulfonate. *Applied Biochemistry and Microbiology* **51**: 350-354.

Keswani C., Mishra S., Sarma B.K., Singh S.P., Singh H.B., 2014. Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. *Applied Microbiology and Biotechnology* **98**: 533-544.

Li C.X., Liu S.Y., Sivasithamparam K., Barbetti M.J., 2008. New sources of resistance to sclerotinia stem rot caused by *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *Brassica juncea* germplasm screened under Western Australian conditions. *Australian Plant Pathology* **38**: 149-152.

Morid M., Zafari D., 2013. Evaluation of micronutrients effects on production and activity of chinase enzyme of some *Trichoderma* pp. *Iranian Journal of Plant Pathology* **49**: 105-107.

Nesci A., Rodriguez M., Etcheverry M., 2003. Control of *Aspergillus* growth and aflatoxin production using antioxidants at different conditions of water activity and pH. *Journal of Applied Microbiology* **95**: 279-287.

Olivier C., Halseth D.E., Mizubuti E., Loria R., 1998. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. *Plant Disease* **82**: 213-217.

Park M., Seo G.S., Lee K.H., Bae K.S., Yu S.H., 2005. Characterization of *Trichoderma* spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of DNA. *Plant Pathology Journal* **21**: 229-236.

Patsoukis N., Georgiou D.C., 2008. Differentiation of *Sclerotinia minor* dependson thiol redox state and oxidative stress. *Canadian Journal of Microbiology* **54**: 28-36.

Peciulyte D., Lugauskas A., 2000. On research of the antifungal activity of copper containing compounds. In: Metspalu L., Mitt S. (ed.). *Development of environmentally friendly plant protection in the Baltic region: proceedings of the International Conference, Tartu, Estonia, September 28-29*, 2000, pp. 153-156.

Reverberi M., Fabbri A.A., Zjalic S., Ricelli A., Punelli F., Fanelli C., 2005. Antioxidant enzymes stimulation in *Aspergillus parasiticus* by *Lentinul aedodes* in hibits aflatoxin production. *Applied Microbiology and Biotechnology* **69**: 207-215.

Rodriguez-Navarro A., 2000. Potassium transport in fungi and plants. *Biochimica an Biophysica Acta* **1469**: 1-30.

Sanjeev K.K., Eswara A., 2008. Efficacy of micro nutrients on *Banana fusarium* wilt. (*Fusarium oxysporum* f. sp. *cubense*) and it's synergistic action with *Trichoderma viride. Notulae Botanicae Horti Agrobotanici* **36**: 52-54.

Sundravadana S., Alice D., 2006. Antifungal activity of zinc sulphate on *Macrophomina phaseolina* (Tassi) Goid and its synergistic action with *T. viride*. *Journal of Biological Control* **20**: 213-218.

Upadhyay R.S., Rai B., 1979. Ecological survey of Indian soil fungi with special reference to *Aspergilli, Penicillia and Trichoderma*. *Revue de Ecologie et Biologie du Sol* **16**: 39-49.

Yousef S.A.M., El-Metwally M.M., Gabr S.A., Al-Ghadir A.H., 2013. New strategy for managing damping-off and root rot disease of cucumber caused by *Rhizoctonia solani* by seed soaking in formula of antioxidant with micronutrients. *Journal of Plant Pathology and Microbiology.* Available at: http://dx.doi.org/10.4172/2157-7471.1000196.

Yousef S.A.M., Ezzat S.M., El-Ghareeb Naglaa R., 2015. Innovate combinations between *Trichodema harzianum* and fungicides alternatives against Sclerotinia white mold disease of bean. *Journal of Biological Chemistry and Environmental Sciences* **10**: 203-214.

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