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PESTS AND DISEASES MANAGEMENT USING COMPATIBLE BIOCONTROL BACTERIA AND ENTOMOPATHOGENIC FUNGAL STRAINS

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Abstract

Pest and disease management using biocontrol microbial strains is a request of the organic agriculture or a phytosanitary alternative that can decrease chemical inputs in the integrated agricultural systems. Biocontrol bacteria of Bacillus spp. proved to suppress soil-borne phytopathogenic fungi. RDIPP selected strains of Bacillus amyloliquefaciens, B. licheniformis and B. subtilis provided to be useful in plant protection and formulated them as bioproducts for seed and soil treatments. For pest biological control, entomopathogenic fungi such as Beauveria bassiana, B. brongniartii, Isaria farinosa, Metarhizium anisopliae and Verticillium lecanii are known as efficient.
For this reason, the aim of our work was to select compatible microbial strains of biocontrol bacteria and entomopathogenic fungi that could be applied together, as simultaneously treatments, for suppressing diseases and pests attack. Results revealed in vitro compatibility of Bacillus licheniformis 77.1s biocontrol strain with Beauveria spp. entomopathogenic fungi. These biological control microorganisms could be used in combination to prevent in the same time pests and diseases.
As a requirement for environmental safety, the selected microbial strains were ecotoxicologicaly tested according to the GLP principles (Good Laboratory Practices) and OECD guidelines. Results proved that the selected strains were non-toxic for non-target species of the aquatic and soil macrofauna: Daphnia magna (crustacean) and Eisenia foetida (earth worm) respectively.

Keywords: *biocontrol bacteria, entomopathogenic fungi, microbial compatibility*

INTRODUCTION

Pest and disease management using biological control methods could be sustained with microbiological techniques. The bacterial and fungal biocontrol agents possess different complex and efficient mechanisms of action useful enough to suppress pest and disease negative impact on crops (Mishra et al., 2013). Biocontrol bacterial strains have a wide range of beneficial traits such as antagonism by antibiotics production, lytic enzymes or bioactive volatile compounds, competition for nutrients and niches, induction of systemic resistance in plants or increase plant growth promotion (Ownley and Windham, 2007; Constantinescu et al., 2010). The entomopathogenic fungi could manifest pest parasitism or produce different lytic enzymes (such as proteases and peptidases, chitinases and lipases), certain metabolic acids, or it could secret toxins with inhibitory action against insects (Khan et al., 2012).

Naturally occurring biocontrol results mostly from a mixture of antagonists (as in suppressive soils) rather than from high population of a single antagonist (Mishra et al., 2011; Mishra et al., 2013). Previous studies on combining different biocontrol agents for pests and diseases management included fungal antagonist mixtures (Datnoff et al., 1995, Núñez del Prado et al., 2008), bacteria and fungi mixtures (Koppenhöfer and Kaya, 1997; Koppenhöfer et al., 1999; Hassan et al., 1997; Mishra et al., 2013) and bacteria mixtures (Raupach and Kloepper, 1998; Stockwell et al., 2011). However, most of the studies refer to combining microorganism with same target activity either for pest control (Koppenhöfer and Kaya, 1997; Koppenhöfer et al., 1999; Thurston et al., 1993, 1994), or for disease

suppression (Guetsky et al., 2002; Stockwell et al., 2011). Co-inoculation of biocontrol microbial agents can lead to an incompatibility between the microorganisms by inhibiting each other (Mishra et al., 2013). Therefore, it is necessary and important to analyze microbial compatibility prior to apply or formulate biocontrol microbial mixtures for plant protection. Considering these, we analysed *in vitro* compatibility between different strains of biocontrol *Bacillus* spp. with several entomopathogenic fungi in order to reveal a compatible microbial mixture for biological pest and disease control.

Since biological control methods aim to reduce negative impacts on the environment, we subjected the microbial strains studied to different ecotoxicological evaluations.

MATERIALS AND METHODS

Microbial strains

Six bacterial biocontrol strains, *Bacillus subtilis* B49b, 98a and Us.a2, *Bacillus licheniformis* 77.1s, *Bacillus pumilus* OS15 and *Bacillus amyloliquefaciens* OS17 were used in this study. These strains were previously selected in the frame of PN-09-40-02-01 project as bacterial biocontrol agents (BCA), for their beneficial characteristic in plant protection. Routinely, these strains were grown on Luria Bertani agar medium at 28ºC, for 48 h.

The enthomopahogenic fungi (EPF) used in the study were *Beauveria bassiana* DSM62075 (IMB 7389), *B. brongniartii* DSM6651 (E 1246/91), *Metarhizium anisopliae* DSM1490 (OSU Ma Re 4SS), *Verticillium lecanii* (sin. *V. hemileiae*) DSM63098 (IMB 11471) and *Isaria farinosa* (sin. *Paecilomyces farinosus*) IHEM 2526, purchased from DSMZ (Germany) and BCCM/IHEM (Belgium) international collections. These fungal strains were routinely grown on potato-dextrose-agar (PDA) medium, for 14 days at 26ºC.

Microbial compatibility assessment

Microbial compatibility between the BCA and EPF strains was performed *in vitro*, using the dual culture technique. Tests were carried out on PDA plates. The fungal inoculum consisted in mycelia plugs of 1 cm in diameter, placed in the middle of the plate. The bacterial strains were streaked at 2 cm from the fungal colony, on both sides. Plates were than incubated at 28^{o}C for 14 days.

After co-cultivation, the fungal growth was evaluated using a modified version of Islam *et al.* (2009) calculation, to reveal the bacterial influence on the mycelia development:

$$Fungal\_inhibition(\%) = \frac{R_{control} - R_{interaction}}{R_{control}} \times 100$$

where: $R_{control}$ = the radius of mycelial development in control plate, $R_{interaction}$= radius of mycelial development in co-cultivation with BCA.

Bacterial growth inhibition was evaluated according to Manka and Manka algorithm (1992).

Ecotoxicological evaluation of entomopathogenic fungi

All five EPF strains were subjected to ecotoxicological evaluation on *Daphnia magna* crustacean and *Eisenia foetida* earthworm. The studies were performed according to the GLP principles, using the C2 and C8 method for acute toxicity on Daphnia and earthworm, respectively; both included in the Regulation (EC) no.440/2008. The entomopatogenic fungi were tested in different concentration, depending on the species: *Beauveria bassiana* at $3.6x10^{7}$cfu/ml, *Beauveria brongniartii* at $1.9x10^{8}$ cfu/ml, *Metarhizium anisopliae* at $7.0x10^{7}$cfu/ml, *Verticillium lecanii* at $1.7x10^{9}$cfu/ml and *Isaria farinosa* at $1.4x10^{8}$cfu/ml. These concentrations are the one recommended and used for pest biocontrol.

The acute toxicity test with daphnid crustaceans was performed according to "immobility" test using the static method, certified as Good Laboratory Practice (GLP). The "immobility" criterion is approved by several international organizations, such as ISO and OECD, and represents the inability of the test organisms to resume swimming within 15 seconds after gentle agitation (Persoone et al., 2009). During the trial, the *Daphnia* were not fed, except of 2 ml testing solution administered to each of them. The experiment included a control group exposed to the same experimental conditions, such as period of analysis, water quality, oxygen concentration, pH of the tested solutions, parameters that are

compulsory by the European Standard. The *Daphnia* were examined every 24 and 48 hours, according with the OECD guidelines (2004), in order to establish the degree of *Daphnia* immobilization. Experimental parameters of the water, as the concentration of dissolved oxygen and water temperature and pH, were also registered at the beginning and in the end of the test.

For the toxicological evaluation on *Eisenia foetida*, the fungal strains were prepared as aqueous suspensions at the mentioned concentrations, using fungal spores from PDA cultures. The chloracetamide was used as a toxic reference standard. Suspensions to be tested were applied to an artificial soil in which adult *Eisenia foetida* earthworms were placed. For the ecotoxicological studies, the *Eisenia foetida* must be at least two months old, with fully developed clitellum and individual body weight between 300-600 mg. The artificial soil used as substrate was prepared according to the standard procedure, and consisted in peat (10%), kaolin (20%) and industrial quartz sand (70%). For each experimental variant, 2500g of artificial soil with 20% humidity was moisturized to 35% humidity by adding 375ml fungal spores suspension. Each experimental variant had for replicates. Tests were performed in glass vessels of 1L capacity, covered with a fine mesh for ventilation. The test conditions were 20±2°C temperature and continuous light of 400 to 800 lux in intensity; requirements provided according to the standard procedure. All operations performed were recorded in data tables according to the Annexes from the technical procedure. Results were recorded after 7 and 14 days, were the ecotoxicological effect of the tested fungi on *Eisenia foetida* was examined by counting the adult earthworms that survived to the tested concentrations.

The BCA strains of *Bacillus subtilis* Us.a2, *B. pumilus* OS15 and *B. amyloliquefaciens* OS17 were also ecotoxicologicaly evaluated on *Eisenia foetida* earthworms. Tests were performed as mentioned for EPF. The bacterial strains were prepared as aqueous suspensions of 10^8 cfu/ml.

RESULTS AND DISCUSSIONS

In vitro compatibility between biocontrol bacteria and entomopathogenic fungal strains

In vitro co-inoculation assay between the EPF *Beauveria bassiana* and tested *Bacillus* biocontrol strains revealed a strong mycelia growth inhibition. Likewise, *Bacillus subtilis* 98a and Us.a2, *B. pumilus* OS15 and *B. amyloliquefaciens* OS17 were influenced by the presence of *B.bassiana*. This fungal strain inhibited bacterial ability to colonize the surface of the PDA medium in the proximity of the fungi, therefore the bacterial growth avoided to near the *B.bassiana* mycelia. The bacterial strains whose growth was not influenced by *B. bassiana* were *Bacillus subtilis* B49b and *B.licheniformis* 77.1s.

The compatibility studies between the biocontrol bacteria and *Beauveria brongniartii* revealed a slight growth inhibition to each interaction partner. Results obtained after 7 days of co-inoculation are included in table 1.

Table 1. Co-cultivation of *Beauveria brongniartii* DSM6651 with different bacterial bio-control strains (after 7 days of incubation)

Experimental variants	Radial growth of the EPF (cm)	Fungal inhibition
Beauveria brongniartii DSM6651 fungal control	1.2	-
Bacillus subtilis B49b	1	16.7%
B. subtilis 98a	0.9	25%
B. subtilis Us.a2	0.9	25%
B. pumilus OS15	1.1	8.3%
B. amyloliquefaciens OS17	0.9	25%
Bacillus licheniformis 77.1s	1.1	8.3%

The growth inhibition of *Beauveria brongniartii* was inhibited with 25% by *B. subtilis* Us.a2, 98a and *B. amyloliquefaciens* OS17 bacterial strains. *Bacillus subtilis* B49b inhibited the mycelial growth of *B.brongniartii* with 16,7%, while *B.licheniformis* 77.1s and B. pumilus OS15 inhibited fungal growth with 8,3% after 7 days of cultivation. *Beauveria brongniartii* manifested also a slight limitation of growth on *Bacillus subtilis* Us.a2 and 98a strains. Therefore, these two bacterial trains could not colonize the surface of the PDA

medium, as would normally do, and maintained a distance of 5mm (figure 1) and 1mm respectively, from the fungus. Results revealed that *Bacillus licheniformis* 77.1s, *Bacillus subtilis* B49b, *Bacillus pumilus* OS15 and *Bacillus amyloliquefaciens* OS17 strains were not visibly affected by this entomopathogen (figures 1 and 2).

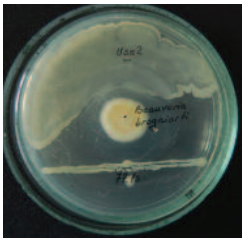

Figure 1. Co-cultivation of *Beauveria brongniartii* with *Bacillus subtilis* Us.a2 and *B.licheniformis* 77.1s

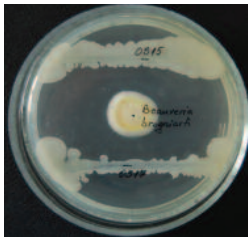

Figure 2. Co-cultivation of *Beauveria brongniartii* with *Bacillus pumilus* OS15 and *B.amyloliquefaciens* OS17

Regarding *Metarhizium anisopliae*, by co-cultivation with biocontrol bacteria, the fungal growth was inhibited with 28.6 to 57.1% after 7 days of incubation (table 2). Likewise, *Bacillus subtilis* Us.a2 and 98a and *B.amyloliquefaciens* OS17 were also inhibited by this fungus. Their colonising ability was limited in the proximity of *M.anisopliae* (figures 3 and 4). *Bacillus licheniformis* 77.1s growth was not influenced by this fungus, and *Bacillus subtilis* B49b (figure 3) and *Bacillus pumilus* OS15 (figure 4) showed only a slight inhibition after 14 days of incubation with this *Metarhizium anisopliae* strain.

Table 2. Co-cultivation of *Metarhizium anisopliae* DSM1490 with different bacterial bio-control strains (after 7 days of incubation)

Experimental variants	Radial growth of the EPF (cm)	Fungal inhibition
M.anisopliae DSM1490 fungal control	1.4	-
Bacillus subtilis B49b	1	28.6%
B. subtilis 98a	0.8	42.8%
B. subtilis Us.a2	0.8	42.8%
B. pumilus OS15	0.8	42.8%
B. amyloliquefaciens OS17	0.6	57.1%
Bacillus licheniformis 77.1s	0.8	42.8%

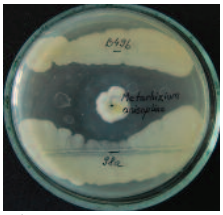

Figure 3. Co-cultivation of *M.anisopliae* with *Bacillus subtilis* B49b and 98a

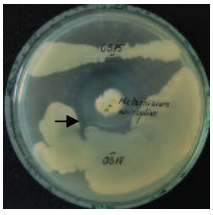

Figure 4. Co-cultivation of *M.anisopliae* with *B.pumilus* OS15 and *B.amylolique-faciens* OS17

The EPF inhibition by some BCA strains is more obvious after 14 days of incubation (table 3).

Table 3. Mycelia inhibition of some entomopathogenic fungi in co-cultivation with different bio-control bacterial strains (after 14 days of incubation)

EPF strains	Cultural characteristics	Fungal control	Biocontrol bacterial strains					
			B49b	98a	Us.a2	OS15	OS17	77.1s
Beauveria bassiana DSM62075	Mycelia radius (cm)	2.3	0.5	0.7	0.5	0.6	0.5	1.3
	Fungal growth inhibition	-	78.3%	69.6%	78.3%	73.9%	78.3%	43.5%
Beauveria brongniartii DSM6651	Mycelia radius (cm)	3.7	0.5	0.7	0,1	0.6	0.5	n.a.
	Fungal growth inhibition	-	78.3%	69.6%	66.7%	73.9%	78.3%	11.1%
Metarhizium anisopliae DSM1490	Mycelia radius (cm)	3.8	0.7	0.8	0.9	0.9	0.8	2.3
	Fungal growth inhibition	-	81.6%	78.9%	76.3%	76.3%	78.9%	39.5%
Verticillium lecanii DSM63098	Mycelia radius (cm)	1.5	0.5	0.5	0.5	0.8	0.8	0.8
	Fungal growth inhibition	-	66.7%	66.7%	66.7%	46.7%	46.7%	46.7%
Isaria farinose IHEM 2526	Mycelia radius (cm)	1.9	0.5	0.5	n.a.	0.5	0.5	n.a.
	Fungal growth inhibition	-	73.7%	73.7%	n.a.	73.7%	73.7%	n.a.

Regarding BCA co-cultivation with *Verticillium lecanii*, the bacterial strains *B.licheniformis* 77.1s, *B.pumilus* OS15 and *B.amyloliquefaciens* OS17 inhibited fungal growth with 46.7%, and *B.subtilis* Us.a2, B49b and 98a strains inhibited this fungus with 66.7% after 2 weeks of co-cultivation, comparing with the control fungal growth. No inhibition was shown on the fungal growth in co-cultivation of *Isaria farinosa* with *B. subtilis*

Us.a2 and *B. licheniformis* 77.1s strains. Only *B. pumilus* OS15, *B.amyloliquefaciens* OS17 ans *B. subtilis* B49b and 98a strains inhibited *I. farinosa* growth.

During the co-inoculation interactions between tested biocontrol *Bacillus* strains and some of the EPF strains, a precipitation compound was observed in the area of fungal growth inhibition. Similar aspects were also described by Cornea et al. (2009), Machado et al. (2010) and Sicuia et al. (2013) on lectin producing filamentous fungi. Kossowska et al (1999) mentioned that *B.bassiana* is capable to produce lectins. Other lectin producing EPF, described in the literature, are *Conidiobolus obscurus* (Latgé et al., 1988) and *Paecilomyces japonica* (Park et al., 2004). In our studies, we noticed this characteristic at *Beuveria bassiana* co-inoculated with *Bacillus subtilis* and *B.amyloliquefaciens* strains, at *Beuveria brongniartii* co-inoculated with *Bacillus subtilis* B49b and Us.a2 strains, and to *Metarhizium anisopliae* alone or co-inoculated with bacteria. An interesting phenomenon was noticed in co-inoculation of *M .anisopliae* with *B.amyloliquefaciens* OS17, the EPF precipitation halo was diminish and a more intense precipitation line was seen when bacterial colony became closer. In addition, the OS17 bacterial colony created a slight line of precipitation at its proximity due to *M.anisopliae* presence (figure 4).

As Sicuia et al. (2013) previously reported, the presence of precipitation line is strictly dependent on both fungal and bacterial strains interactions. These aspects are observed only in co-cultivation of lectin producing fungi with certain bacterial strains. Same authors attributed these aspects to the specific sugar binding of bacterial glycoconjugates by the lectin or lectin-like compounds secreted by the fungi.

Regarding the influence of the EPF tested on the growth of BCA strains, only B. licheniformis 77.1s strain was not influenced by any of the fungi tested (table 4).

Table 4. Bacterial growth inhibition of different bio-control bacterial strains in co-cultivation with some entomopathogenic fungi (after 14 days of incubation)

Bacterial strains	Entomopathogenic fungi				
	Beauveria bassiana DSM62075	*Beauveria brongniartii* DSM6651	*Metarhizium anisopliae* DSM1490	*Verticillium lecanii* DSM63098	*Isaria farinosa* IHEM 2526
Bacillus subtilis B49b	–	+ / –	+	–	–
Bacillus subtilis 98a	+ +	+	+ +	+ / –	–
Bacillus subtilis Us.a2	+ +	+	+ +	+ / –	–
Bacillus pumilus OS15	+ +	+ +	+	–	–
B. amyloliquefaciens OS17	+ +	+ +	+ +	+ / –	–
Bacillus licheniformis 77.1s	–	–	–	–	–

Legend: + + + + = very strong inhibition of the fungal growth; + + + = strong inhibition of the fungal growth; + + = moderate inhibition of the fungal growth; + = slight inhibition of the fungal growth; – = no inhibition of the fungal growth.

The ecotoxicological evaluation of the entomopathogenic fungi

The ecotoxicological evaluation of the EPF *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Isaria farinosa* at the standard concentration used for pest biocontrol revealed no toxic effects on *Daphnia magna*. There were no immobilization or adverse reaction recorded. However, *Verticillium lecanii, Metarhizium anisopliae* and *Isaria farinosa* revealed a stimulatory action on the reproduction cycle of *Daphnia*. The study has been validated, since the quality criteria of the C2 method from the Regulation (EC) no. 440/2008 were fulfilled. There was no mortality registered in the control, and the concentration of oxygen in water was between 6.0 to 6.5 mg/L (≥ 3 mg/L) at the end of the test, in both control and test variants.

The ecotoxicological evaluation of the EPF towards *Eisenia foetida* earthworms revealed no toxicity after 14 days observation. All five EPF strains tested at the standard concentration used for pest biocontrol showed no mortality within the earthworm populations. In the untreated control, all 40 earthworms survived, as well.

The BCA strains *Bacillus subtilis* Us.a2, *B. pumilus* OS15 and *B. amyloliquefaciens* OS17 were also ecotoxicologicaly evaluated towards *Eisenia foetida* earthworms. After 7 days of incubation, no mortality was registered to any of the testing variants. However, after 14 days of incubation, a slight mortality percent was registered in each experimental variant, including the control (table 5).

Table 5. Ecotoxicological evaluation of some bio-control bacterial strains towards *Eisenia foetida earthworms* (after 14 days of incubation)

Experimental variants	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Total earthworms / experiment		Mortality average (%)
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	
Bacillus subtilis Us.a2	8	2	8	2	8	2	9	1	**33**	**7**	**17.5**
B. pumilus OS15	9	1	9	1	9	1	10	0	**37**	**3**	**7.5**
B. amyloliquefaciens OS17	10	0	10	0	8	2	8	2	**36**	**4**	**10.0**
Control	9	1	10	0	10	0	9	1	**38**	**2**	**5.0**

According to the standard procedure, since the mortality in the control was less than 10% at the end of the test, the ecotoxicological studies towards earthworms were considered valid.

CONCLUSIONS

The compatibility between the beneficial microorganisms such as biocontrol bacteria with the entomopathogenic fungi could allow simultaneous treatments for suppressing diseases and pests attack.

The lowest inhibitory activity was registered between *Beauveria brongniartii* DSM6651 and *Bacillus licheniformis* 77.1s, where the fungal inhibition was only 11.1%, and the bacterial growth was not influenced by the fungi.

The highest inhibitory activity was registered between *Metarhizium anisopliae* DSM1490 and *Bacillus subtilis* B49b, where the biocontrol bacteria suppressed the fungal growth to 81.6%, and the expansion of the bacterial growth was inhibited in the presence of the fungi.

The inhibitory activity of the biocontrol bacterial strains against some of the entomopathogenic fungi could be influenced by the slow growth of the fungi comparing with the high ability of some bacteria to colonize the culture media.

None of the EPF strains tested influenced the growth of *Bacillus licheniformis* 77.1s.

No toxic or negative effects towards *Daphnia magna* crustacean and *Eisenia foetida* earthworms were found in the ecotoxicological studies, when testing the entomopathogenic fungal strains at the standard concentration used for pest biocontrol.

The biocontrol bacterial strains, tested at 10^8cfu/ml towards *Eisenia foetida* earthworms showed reduced mortality percents of 7.5 to 17.5% in the ecotoxicological studies.

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REFERENCES

Constantinescu F., Sicuia O.A., Zamfiropol R., Dinu S., Oancea F., 2010. Beneficial characteristics of some bacterial strains isolated from rhizosphere. Romanian Journal of Plant Protection , 3: 6-11.

Cornea C.P., Pop A., Matei S., Ciucă M., Voaideş C., Matei M.A, Popa G., Voicu A., Ştefănescu M., 2009. Antifungal action of new Trichoderma spp. Romanian isolates on different plant pathogens. Biotechnology and Biotechnological Equipment, 23(2): 766-770.

Datnoff L.E., Nemec S., Pernezny K., 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Control, 5: 427-431.

Guetsky R., Shtienberg D., Elad Y., Fischer E., Dinoor A. 2002., Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology, 92: 976-985.

Hassan D.G., Zargar M., Beigh G.M., 1997. Biological control of Fusarium root rot of common bean (Phaseolus vulgaris L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Mol. Ecol., 34: 74-80.

Islam R., Jeong Y.T., Ryu Y.J., Song C.H., Lee Y.S., 2009. Isolation, identification and optimal culture

conditions of *Streptomyces albidoflavus C247* producing antifungal agents against *Rhizoctonia solani* AG2-2. Mycobiology, 37(2): 114-120.
Khan S., Guo L., Maimaiti Y., Mijit M., Qiu D., 2012. Entomopathogenic Fungi as Microbial Biocontrol Agent, Molecular Plant Breeding, 3 (7): 63-79.
Koppenhöfer A.M., Choo H.Y., Kaya H.K., Lee D.W., Gelernter W.D., 1999. Increased yield and greenhouse efficacy against scarab grubs with a combination of an entomopathogenic nematode and Bacillus thuringiensis. Biol. Control., 14: 37–44.
Koppenhöfer A.M., Kaya H.K., 1997. Additive and Synergistic interactions between entomopathogenic nematodes and Bacillus thuringiensis for scarab grub control. Biol. Control, 8: 131–137.
Machado A.P., Vivi V.K., Tavares J.R., Filho F.J.G., Fischman O., 2010. Antibiosis and dark-pigments secretion by the phytopathogenic and environmental fungal species after interaction *in vitro* with a *Bacillus subtilis* isolate. Braz. Arch. Biol. Technol., 53(5): 997-1004.
Manka K., Manka M, 1992. A new method for evaluating interaction between soil inhabiting fungi and plant pathogens. New approaches in biological control of soil-borne diseases, Buletin OILB/SORP. XV, 1: 73-75.
Mishra D.S., Gupta A.K., Prajapati C.R., Singh U.S., 2011. Combination of fungal and bacterial antagonists for management of root and stem rot disease of soybean. Pack. J. Bot., 43**:** 2569-2574.
Mishra D.S., Kumar A., Prajapati C.R., Singh A.K., Sharma S.D., 2013. Identification of compatible bacterial and fungal isolate and their effectiveness against plant disease. Journal of Environmental Biology, 34: 183-189.
Núñez del Prado E., Iannacone J., Gómez H., 2008. Effect of two entomopathogenic fungi in controlling *Aleurodicus cocois* (Curtis, 1846) (*Hemiptera*: *Aleyrodidae*). Chilean Journal of Agricultural Research, 68(1): 21-30.
Ownley B.H., Windham M., 2007. Biological control of plant pathogens. In: Trigiano RN, Windham MT, Windham AS, (Eds.), Plant Pathology: Concepts and Laboratory Exercises, 2nd ed. CRC Press, Boca Raton, Florida, pp.423-436.
Persoone G., Baudo R., Cotman M., Blaise C., Thompson K.Cl., Moreira-Santos M., Vollat B., Törökne A., Han T., 2009. Review on the acute *Daphnia magna* toxicity test evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowledge and Management of Aquatic Ecosystems, 393:01, 29p
Raupach G.S., Kloepper J.W., 1998. Mixture of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology, 88: 1158-1164.
Sicuia O.A., Cornea C.P., Pop A., 2013. Fungal and bacterial glycoconjugats interactions: Specific mechanism between lectin producing fungi and *Bacillus* biocontrol strains? AgroLife Scientific Journal, 2(1):127-132.
Stockwell V.O., Johnson K.B., Sugar D., Loper J.E., 2011. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. Phytopathology, 101:113-123.
Thurston G.S., Kaya H.K., Gaugler R., 1994. Characterizing the enhanced susceptibility of milky disease-infected scarabaeid grubs to entomopathogenic nematodes. Biol. Control, 4: 67–73.
Thurston G.S., Kaya H.K., Burlando T.M., Harrison RF., 1993. Milky disease bacterium as a stressor to increase susceptibility of scarabaeid larvae to an entomopathogenic nematode. J. Invertebr. Pathol, 61: 167–172.